Benefit of a 600-mg Loading Dose of Clopidogrel on Platelet Reactivity and Clinical Outcomes in Patients With Non–ST-Segment Elevation Acute Coronary Syndrome Undergoing Coronary Stenting

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OBJECTIVES We analyzed the benefit of a 600-mg clopidogrel loading dose on platelet reactivity and clinical outcomes after stenting for non–ST-segment elevation acute coronary syndrome (NSTE ACS).

BACKGROUND High post-treatment platelet reactivity (HPPR = adenosine diphosphate 10 μmol·l⁻¹ [ADP]-induced platelet aggregation >70%) is a marker for low responders to dual antiplatelet therapy with increased risk of recurrent cardiovascular (CV) events after stenting for NSTE ACS.

METHODS A total of 292 consecutive NSTE ACS patients undergoing coronary stenting were included and randomly received a 300-mg (n = 146) or 600-mg (n = 146) loading dose of clopidogrel at least 12 h before percutaneous coronary intervention. A single post-treatment blood sample was obtained before percutaneous coronary intervention to analyze maximal intensity of ADP-induced platelet aggregation and platelet surface expression of P-selectin. One-month follow-up CV events were recorded.

RESULTS The ADP-induced platelet aggregation and expression of P-selectin were significantly lower in patients receiving 600 mg than in those receiving 300 mg (mean ± SD: 50 ± 19% vs. 61 ± 16%, p < 0.0001 and 0.38 ± 0.24 arbitrary units vs. 0.60 ± 0.40 arbitrary units; p < 0.0001 respectively). Persistence of HPPR was less common in patients receiving 600 mg than in those receiving 300 mg (15 vs. 25%, p = 0.03). During the 1-month follow-up, 18 CV events (12%) occurred in the 300-mg group versus 7 (5%) in the 600-mg group (p = 0.02); this difference was not affected by adjustment for conventional CV risk factors (p = 0.035).

CONCLUSIONS In NSTE ACS patients undergoing coronary stenting, a 600-mg loading dose of clopidogrel shows its benefit on platelet reactivity and clinical prognosis. (J Am Coll Cardiol 2006;48: 1339–45) © 2006 by the American College of Cardiology Foundation

Platelet reactivity plays a key role in the pathogenesis of ischemic complications after stenting for non–ST-segment elevation acute coronary syndrome (NSTE ACS). Accordingly, platelet inhibition with a thienopyridine (clopidogrel) has significantly reduced recurrent ischemic events in this clinical setting, and in combination with aspirin, clopidogrel is now the treatment of choice for preventing stent thrombosis (1) and reducing major adverse cardiovascular (CV) events after NSTE ACS (2,3). Nevertheless, ischemic events still occur. These recurrences involve a complex and multifactorial process, and low response to antiplatelet therapy could be a major factor. Indeed numerous biological studies, essentially based on adenosine diphosphate (ADP)-induced platelet aggregometry, have reported interindividual variability in platelet response to clopidogrel (4–8); these studies identified patients who did not achieve platelet inhibition as low responders or “resistants.” However, antiplatelet effect monitoring would be of limited relevance in the absence of clinical implication. Precisely, as has been shown for aspirin (9), a relationship between clopidogrel resistance and recurrence of clinical outcomes has been reported in several studies (7,10–13). We previously showed that in NSTE ACS patients undergoing coronary stenting, the high post-treatment platelet reactivity (HPPR) (maximal intensity of ADP 10 μmol·l⁻¹-induced platelet aggregation >70%) identified low responders to dual antiplatelet therapy (aspirin and clopidogrel) at higher risk of CV events during a 1-month follow-up (14). According to these results, other therapeutic approaches should be considered for these low-responder patients, such as higher doses or alternative treatments. Currently, the 300-mg loading dose of clopidogrel given at least 6 h before the procedure represents the conventional antiplatelet regimen before percutaneous coronary intervention (PCI). A higher loading dose of clopidogrel has already shown its capacity to improve platelet reactivity after treatment in healthy volun-
teers and patients (15–21) with an earlier and stronger inhibition of ADP-induced platelet activation than the 300-mg loading regimen, and its efficiency for the prevention of peri-procedural infarction in stented patients (22). However, to the best of our knowledge, no study has ever tested both platelet parameters and clinical outcomes after increasing the loading dose of clopidogrel, especially in high-risk groups such as stented NSTE ACS patients. Therefore, we conducted a randomized and prospective study to evaluate the benefit of a higher loading dose of clopidogrel on platelet aggregation and recurrent ischemic events for NSTE ACS patients undergoing coronary stenting.

METHODS

Study population. Consecutive patients admitted for NSTE ACS to the Department of Cardiology of the Timone hospital between June 2004 and October 2005 were eligible for this prospective study if they had undergone successful coronary stenting. We defined NSTE ACS as clinical symptoms compatible with acute myocardial ischemia within 12 h before admission and at least 1 of the following: a new finding of ST-segment depression >0.05 mV, transient (<20 min) ST-segment elevation >0.1 mV, T-wave inversion >0.3 mV in at least 2 leads, elevated levels of cardiac markers or coronary disease as documented by a history of catheterization, revascularization, or myocardial infarction. The exclusion criteria were a history of bleeding diathesis, persistent ST-segment elevation ACS, New York Heart Association functional class IV, PCI or coronary bypass grafting <3 months, contraindications to antiplatelet therapy, platelet count <100 g·l⁻¹, creatinine clearance <25 ml·min⁻¹, and use of glycoprotein IIB/IIIa inhibitors before the procedure. Patients were randomly assigned to receive a 300-mg or 600-mg loading dose of clopidogrel at least 12 h before stenting. All patients received aspirin 160 mg daily after a loading dose of 250 mg administered at least 12 h before stenting. The PCI was performed within 48 h after admission. For all patients, anticoagulation was begun before PCI in the intensive care unit and performed with low-molecular-weight heparin (enoxaparin), or unfractionated heparin in patients over 75 years old or with renal insufficiency. The study protocol was approved by the institutional ethics committee of our institution, and patients gave written informed consent for participation.

Blood samples. Blood samples for testing platelet reactivity were drawn in the catheterization laboratory from a 6-F arterial sheath before the PCI at least 12 h after the loading dose of clopidogrel and aspirin, and before administration of tirofiban if needed. The initial first millimeters of blood were discarded to avoid platelet activation induced by needle puncture, and blood was immediately collected in Vacutainer tube containing 3.8% trisodium citrate, filled to capacity, and then inverted 3 to 5 times for gentle mixing and drawn immediately to the hemostasis laboratory.

Platelet aggregation. The blood–citrate mixture was centrifuged at 120 g for 5 min. The resulting platelet-rich plasma (PRP) was kept at room temperature for use within 1 h. The platelet count was determined in the PRP sample and adjusted to 2.5 × 10⁸ ml⁻¹ with homologous platelet-poor plasma. Platelets were stimulated with ADP (10 μmol·l⁻¹), and aggregation was assessed with a PAP4 Aggregometer (Biodata Corporation, Wellcome, Paris, France). Aggregation was expressed as the maximal percentage change in light transmittance from baseline with platelet-poor plasma as a reference. Here we report data on maximal intensity of platelet aggregation with ADP concentrations. The coefficient of variation of maximal intensity of platelet aggregation with ADP was 6.5%.

Flow cytometry. The surface expression of the internal alpha-granule membrane protein P-selectin expressed on the surface of ADP activated platelets was determined by flow cytometry with monoclonal antibody. The following antibodies were used: anti-CD62P (Beckman Coulter, Fullerton, California) and antimouse goat immunoglobulin (Ig)G FITC (antimouse goat IgG labeled with fluorescein isothiocyanate; Beckman Coulter). Briefly, PRP was diluted 1:10 in tyrode albumin buffer (0.25 10⁸ ml⁻¹) and gently mixed. Both antibody anti-CD62P (10 μl of dilution 1:10) and ADP (final concentration 10 μmol·ml⁻¹) or tyrode albumin buffer were added to 10 μl of diluted PRP. After incubation in the dark at room temperature for 15 min, 10 μl of antimouse goat IgG FITC (diluted 1:10) were added and again incubated in the dark at room temperature for 15 min. Scatter signals and fluorescence intensity were analyzed on a EPICS XL-MCL flow cytometer (Beckman Coultronic, Margency, France). The light-scattering properties projected on a scattergram identified the platelet cluster. Fluorescence intensity was expressed on individual cytohistograms, with the region of interest limited to the platelet cluster. The mean channel fluorescence intensity was used as an index of antibody binding and P-selectin surface expression.

Platelet function end point. We analyzed the mean maximal intensity of ADP-induced platelet aggregation in both groups, receiving 300 mg or 600 mg of clopidogrel. We also reported the rate of persistence of HPPR, defined as
ADP-induced platelet aggregation >70%, in both groups. Finally, we studied the expression of P-selectin to evaluate platelet activation.

**Clinical end point.** The clinical end point included the following CV events: CV death, acute or subacute stent thrombosis, recurrent ACS, and stroke. Follow-up events were prospectively assessed by clinical visit. An ACS was defined by the presence of symptoms compatible with recurrent ischemia needing new hospitalization and coronary angiography, ischemic stroke was defined as a new focal neurologic deficit without bleeding on tomodensitometry and confirmed by a neurologist that as occurring within 1 month of PCI. Drug therapy compliance was assessed. Occurrence of major bleeding defined as intracranial bleeding or clinically overt bleeding associated with a decrease in hemoglobin of 5 g·dl⁻¹, according to the Thrombolysis in Myocardial Infarction criteria (23), were reported. The treating physician and the investigators who evaluated the clinical end points were blinded to the results of the platelet testing.

**Statistical analysis.** Statistical analysis was performed with the SAS Software (version 9.01; SAS Institute Inc., Cary, North Carolina). Continuous variables are expressed as mean ± SD. Categorical variables are expressed as frequencies and percentages. For continuous variables, mean levels were compared between individuals with 300 mg and 600 mg of by analysis of variance, and for categorical variables, proportions were compared by chi-square test. Variables with a skewed distribution were log- or square root-transformed before analysis. The square root of the loading dose of clopidogrel on CV event was performed using logistic regression analysis. Three models were considered successively: Model 1: after adjustment for age and gender; Model 2: additionally adjusted for potential confounders such as conventional CV risk factors, treatment, and inflammatory parameters; Model 3: additionally adjusted for HPPR.

**RESULTS**

**Patient characteristics.** From June 2004 to October 2005, a total of 387 NSTE ACS patients who fulfilled the enrollment criteria were randomized to a 300-mg (n = 192) or 600-mg (n = 195) loading dose of clopidogrel. After coronary angiography, 95 patients who did not receive successful angioplasty were excluded from the study (72 were treated medically, 20 with elective bypass surgery and 3 with failed PCI). Of the 292 study patients with successful coronary stenting, 146 were randomized to a 300-mg and 146 to a 600-mg loading dose of clopidogrel. Demographic data of the studied population are summarized in Table 1. The demographic, clinical, and therapeutic parameters were similar in the 300-mg and 600-mg groups. The mean time between the loading dose of clopidogrel and blood sampling was 18.4 ± 2.6 h and 19.2 ± 2.7 h, respectively, for patients receiving 300 mg or 600 mg (p = 0.56). All patients received 75 mg clopidogrel and 160 mg aspirin during the 1-month follow-up period.

**Platelet parameters.** **PLATELET AGGREGATION.** We analyzed post-treatment platelet reactivity using the maximal intensity of ADP-induced platelet aggregation. The distribution of this response was consistent with a normal

| Table 1. Baseline Characteristics of the Patients According to the Loading Dose of Clopidogrel |
|---------------------------------------------|---------------------------------------------|----------------|
| Characteristics                           | 300 mg (n = 146)                           | 600 mg (n = 146) |
| **Age, yrs**                               | 64.2 ± 10.3                               | 65.2 ± 12.0     | 0.48 |
| **Men, n (%)**                             | 114 (79)                                  | 108 (73)        | 0.34 |
| **Hypertension, n (%)**                    | 84 (58)                                   | 81 (56)         | 0.67 |
| **Diabetes mellitus, n (%)**               | 42 (29)                                   | 48 (33)         | 0.45 |
| **Current smoker, n (%)**                  | 59 (40)                                   | 76 (52)         | 0.06 |
| **Dyslipidemia, n (%)**                    | 81 (56)                                   | 79 (55)         | 0.81 |
| **Previous ACS, n (%)**                    | 65 (44)                                   | 66 (45)         | 0.90 |
| **Familial history, n (%)**                | 49 (34)                                   | 53 (36)         | 0.62 |
| **Body mass index, kg/m²**                 | 26.6 ± 3.6                                | 27.3 ± 7.2      | 0.10 |
| **Medications**                            |                                           |                 |     |
| **Statins, n (%)**                         | 87 (60)                                   | 88 (61)         | 0.90 |
| **Beta-blocker, n (%)**                    | 71 (49)                                   | 62 (42)         | 0.24 |
| **Diuretics, n (%)**                       | 24 (16)                                   | 24 (16)         | 1.00 |
| **Troponin, n (%)**                        | 38 (26)                                   | 30 (21)         | 0.27 |
| **ST-segment changes, n (%)**              | 32 (22)                                   | 36 (25)         | 0.58 |
| **Left ventricular ejection fraction, %**  | 56 ± 9                                    | 55 ± 11         | 0.54 |
| **Creatinine, μmol·l⁻¹**                   | 95 ± 21                                   | 113 ± 89        | 0.06 |
| **Procedural features**                    |                                           |                 |     |
| **Total stent length, mm**                 | 18 ± 4                                    | 17 ± 3          | 0.74 |
| **Tirofiban, n (%)**                       | 51 (35)                                   | 48 (33)         | 0.75 |
| **Anticoagulation**                        |                                           |                 |     |
| **LWMH**                                   | 95 (65)                                   | 99 (68)         | 0.78 |
| **UFH**                                    | 51 (35)                                   | 47 (32)         | 0.81 |

Values are mean ± SD for quantitative variables and n (%) for qualitative variables. For skewed variables, tests were performed on log-transformed values.

ACS = acute coronary syndrome; LMWH = low molecular weight heparin; UFH = unfractionated heparin.
bell-shaped distribution. Maximal intensity of ADP-induced platelet aggregation was significantly lower in patients who received a 600-mg loading dose of clopidogrel than in those who received 300 mg (mean ± SD: 50 ± 19\% vs. 61 ± 16\%, p < 0.0001) (Fig. 1).

The persistence of HPPR was less common in patients receiving a 600-mg loading dose of clopidogrel (n = 22, 15\%) than in those receiving 300 mg (n = 36, 25\%, p = 0.04).

**Expression of P-Selectin.** The mean expression of P-selectin was significantly lower in patients receiving a 600-mg loading dose of clopidogrel than in those receiving 300 mg (0.38 ± 0.24 arbitrary units vs. 0.60 ± 0.40 arbitrary units; p < 0.0001) (Fig. 2).

**Clinical Outcomes.** One-month follow up was completed in all patients. Twenty-five CV events (8.5\%) occurred in the whole population during follow-up. Recurrent ischemic events occurred more frequently in the 300-mg group than in the 600-mg group: 18 (12\%) and 7 (5\%), respectively (p = 0.02). This difference was not affected by adjustment for potential confounders such as age, gender, CV risk factors, troponin elevation, ST-segment changes, left ventricular ejection fraction, or tirofiban use (p = 0.035). Distribution of the CV events was 15 hospitalizations for recurrent ACS (2 acute stent thrombosis), 2 strokes, and 1 CV death for the 300-mg group; and 6 hospitalizations for recurrent ACS (1 acute stent thrombosis) and 1 stroke for the 600-mg group (Figs. 3 and 4).

**Discussion**

Compared with the 300-mg clopidogrel loading dose, our study shows that a 600-mg bolus allows a superior antiplatelet effect and improves clinical outcomes in patients undergoing coronary stenting for NSTE ACS. Clopidogrel is an antiplatelet agent inhibiting the ADP receptor. Because of its safety profile and the results of clinical trials, clopidogrel has become the standard treatment for ACS and stenting (1–3). Two large studies in patients with ACS have shown that pretreatment with clopidogrel may have beneficial effects. This pretreatment was given a mean of 6 days before intervention in the observational PCI-CURE (Percutaneous Coronary Intervention—Clopidogrel in Unstable An-
Moreover, recent studies (14, 26) proposed the ADP-clopidogrel (4–8). Mechanisms involved are not yet clear; ability of in vitro platelet response to standard dose of aggregation, which emphasized high interindividual variability in intestinal absorption and the hepatic cytochrome P450 3A4 activity were reported as important factors (9). Moreover, several factors could modulate clopidogrel antplatelet effectiveness. Indeed, a recent study by Saraff et al. (25) showed that the benefit of dual antiplatelet therapy with aspirin and clopidogrel was especially pronounced in smokers, probably because smoking increases platelet reactivity and smokers have poor outcomes after PCI compared with nonsmokers. In the present study, there was a trend toward an increased prevalence of smokers in the 600-mg group. It suggests that the benefit of a higher loading dose could have been greater without this difference between the 300-mg and 600-mg groups. The clinical relevance of the clopidogrel resistance has already been reported in several retrospective studies showing a correlation between platelet parameters and stent thrombosis (7, 10–12). Only 2 prospective studies evaluated the relationship between clopidogrel responsiveness and recurrent ischemic events after ACS (13, 14). Nevertheless, the lack of a standard definition of resistance as well as the lack of a standard diagnostic modality has hampered the field in identifying and treating this clinical entity.

Consequently, some investigators suggested to study post-treatment platelet reactivity rather than antplatelet responsiveness as a risk marker of clinical outcomes (14). Moreover, recent studies (14, 26) proposed the ADP-induced post-treatment platelet aggregation as a means of evaluating the response both to clopidogrel and to aspirin. To determine a useful measurement of platelet reactivity in daily clinical practice, we use one single blood sample testing ADP-induced post-treatment platelet aggregation. We identified HPPR (ADP-induced platelet aggregation >70%), in spite of dual antiplatelet therapy with a 300-mg loading dose of clopidogrel as a risk marker of recurrent ischemic events (14). Therefore, the results of the present study confirmed that the HPPR have a predictive value for recurrent ischemic events in both the 300-mg and the 600-mg groups. Moreover, the multivariable analysis showed that the HPPR was a better predictor of clinical outcomes than the 300-mg or 600-mg loading dose, suggesting that the benefit of higher clopidogrel loading dose on clinical prognosis is directly linked to the decreasing persistence of HPPR. These supplementary data seem to validate the use of this marker in daily clinical practice to identify “high-risk” patients with persistent HPPR.

The dose of 300 mg of clopidogrel derives from dose-finding data on healthy volunteers; however, patients with coronary artery disease may have enhanced platelet reactivity as compared with healthy individuals, and so probably require more aggressive platelet inhibition. Indeed, in an environment of high thrombin activity, it has been shown that clopidogrel might not reduce enough platelet activity because it did not inhibit the platelet aggregation and degranulation after stimulation with the thrombin-related activating peptide (27). Moreover, a study by Soffer et al. (28) has correlated the level of angina class with platelet inhibition by clopidogrel and found that patients with a higher Braunwald angina class had lower inhibition of platelet aggregation. The standard dose of 300 mg is probably not as efficient during ACS as it has been shown in healthy subjects. Plasma concentration of the active drug influences platelet aggregation after first administration in a dose-dependent manner (29). Thus, a more rapid and intense platelet suppression represents the rationale for pretreatment with a 600-mg loading dose of clopidogrel. Several studies have shown a benefit of higher clopidogrel loading on platelet aggregation with a decreased rate of nonresponders and platelet reactivity (15–21), and a previous study showed a benefit of 600 mg of clopidogrel compared with the standard 300-mg regimen on the incidence of periprocedural elevation of markers of myocardial necrosis (22). Even more recently, a dose of 900 mg was tested, although this did not seem to produce any more active metabolite or inhibition of platelet function than the 600-mg dose (20). In the present study, we showed both a biological and a clinical benefit of a higher loading dose of clopidogrel. Moreover, in our study, a higher loading dose of clopidogrel was not associated with important bleeding complications. Elsewhere, other antithrombotic strategies may be useful for these “antiplatelet nonresponders” at high risk of recurrent ischemic events. First, use in the periprocedural period of GPIIb/IIIa antagonist infusion would be beneficial in these patients. Indeed, a recent study has shown the lower clinical event rate after administration of abciximab in higher-risk NSTE ACS patients in spite of a 600-mg loading dose of clopidogrel (30). Secondly, the emergence of new ADP-antagonists such as prasugrel would probably be helpful in this clinical setting. Clinical studies are required to validate these hypotheses.

Study limitations. It should be mentioned that the sample size of the present study is relatively small, and therefore does not allow for definitive conclusions. In our study, timing between the loading dose of clopidogrel and PCI was 12 to 2 h. However, a recent study by Steinuhbl et al. (31) from the CREDO study showed that a 300-mg loading dose had no effect if administrated <15 h before PCI, and
that the effect was not optimal until approximately 24 h before PCI. Moreover, in the ISAR REACT (Intracorony Stenting and Antithrombotic Regimen Rapid Early Action for Coronary Treatment) study with a 600-mg loading dose, no relationship was found between the duration of pretreatment (2 to 24 h) and clinical events (32). The design of our study could partially explain the benefit of a higher loading dose for patients pretreated with a 300-mg loading between 12 to 24 h before PCI. Finally, the clinical combined end point chosen in our study is obviously debatable, especially because the usually used periprocedural infarction, defined with elevation of creatine kinase-Mb or troponin, was not included.

**Conclusions.** Our study showed that pretreatment with a 600-mg loading dose of clopidogrel given before PCI for NSTE ACS is safe and, as compared with the 300-mg dose, improves periprocedural platelet inhibition and reduces recurrent ischemic events. The low risk and efficiency of this pharmacological regimen may support its routine use in ACS patients before PCI, as recently evoked in the European Society of Cardiology (33) and American College of Cardiology/American Heart association guidelines (34), but larger clinical studies are required.

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