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A Randomized Comparison of High Clopidogrel Loading Doses in Patients With Non–ST-Segment Elevation Acute Coronary Syndromes

The ALBION (Assessment of the Best Loading Dose of Clopidogrel to Blunt Platelet Activation, Inflammation and Ongoing Necrosis) Trial

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OBJECTIVES We sought to compare the antiplatelet effects of three clopidogrel loading doses (LDs).

BACKGROUND Administration of a 300-mg clopidogrel LD is beneficial in situations requiring rapid platelet inhibition. Whether higher LDs can provide further benefits remains unknown.

METHODS Patients (n = 103) with non–ST-segment elevation acute coronary syndromes were randomized to receive a 300-mg, 600-mg, or 900-mg clopidogrel LD, given on top of other standard therapy (including acetylsalicylic acid). The main outcome measure was inhibition of adenosine diphosphate-induced inhibition of platelet aggregation (IPA); inhibition of platelet activation, inflammatory markers, troponin I release, and major adverse cardiac events also were evaluated; all measures were blindly evaluated.

RESULTS Compared with the 300-mg LD, greater doses were associated with significantly greater platelet inhibition, with dose-effect relationships observed for onset of action, maximal plateau, 24-h areas under the curves of IPA, and rates of low IPA (<10% at 6 h), using 20 μmol/l major adverse cardiac events. A significant dose-response was also observed for the vasodilator-stimulated phosphoprotein index, a measure of $P2Y_{12}$ receptor inhibition. Similar but nonsignificant trends were observed for troponin release and major adverse cardiac events. Bleeding rates were similar in each group.

CONCLUSIONS In low-to-moderate risk patients with non–ST-elevation acute coronary syndromes, clopidogrel LDs >300 mg provide a faster onset of action, a higher IPA plateau, and greater reductions in platelet activation during the first 24 h. A 900-mg LD may induce a greater antiplatelet effect than 600 mg, when compared with the standard 300-mg regimen. These findings require further clinical confirmation. (J Am Coll Cardiol 2006;48:931–8) © 2006 by the American College of Cardiology Foundation

Optimal and rapid inhibition of platelet function is an important therapeutic goal for the management of patients with acute coronary syndromes and in those undergoing percutaneous coronary intervention (PCI). In the case of antiplatelet therapy with clopidogrel, a $P2Y_{12}$ adenosine diphosphate (ADP)-receptor antagonist, this has traditionally been achieved by initiating treatment with a 300-mg oral loading dose (LD) to reduce the time to onset of action from a few days to a few hours (1), with a significant clinical benefit in addition to acetylsalicylic acid (ASA), in various situations (2–10).

However, the slow onset of action still remains a question in the urgent care setting and/or PCI and has led several groups to conduct small randomized studies evaluating greater LDs (i.e., 400 to 600 mg) of clopidogrel. Most of these studies have suggested that greater LDs reduce the time to achieve optimal inhibition of platelet aggregation (IPA) (11–13), although divergent results have also been published (14,15). Recent data also have suggested an incremental benefit of a 600-mg LD (compared with the standard 300-mg LD) on the release of cardiac markers after PCI (13,16). These studies typically have been single center and open label, with a limited number of sampling time points; all have used clopidogrel LDs ≤600 mg.

To address the question of the optimal clopidogrel LD rigorously, we performed a randomized, multicenter,

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Abbreviations and Acronyms

ADP	= adenosine diphosphate
ASA	= acetylsalicylic acid
AUC	= area under the inhibition of platelet aggregation curve
GP	= glycoprotein
IPA	= inhibition of platelet aggregation
LD	= loading dose
LMWH	= low molecular weight heparin
MACE	= major adverse cardiac events
MFI	= median fluorescence intensity
NSTE-ACS	= non–ST-segment elevation acute coronary syndrome
PA	= platelet aggregation
PAI	= plasminogen activator inhibitor
PCI	= percutaneous coronary intervention
PGE_1	= prostaglandin E1
sCD40L	= soluble CD40 ligand
VASP	= vasodilator-stimulated phosphoprotein
vWF Ag	= von Willebrand factor antigen

parallel-group evaluation of the effects of three different clopidogrel LDs, testing a dose as high as 900 mg. In this dose-ranging study, multiple sampling time points were used to precisely determine the onset of action and the timing of the maximal effect on inhibition of platelet function with the different LDs. Although evaluation of platelet aggregation (PA) was the primary objective of the study, platelet activation and markers of inflammation and necrosis also were evaluated during the first 24 h. To avoid any interaction of PCI on the kinetics of all these markers, it was decided to study patients with non–ST-segment elevation acute coronary syndrome (NSTE-ACS) before they presented to the catheterization laboratory. Finally, ischemic and bleeding events were closely monitored during a month of follow-up.

METHODS

Study design. The ALBION (Assessment of the best Loading dose of clopidogrel to Blunt platelet activation, Inflammation and Ongoing Necrosis) trial was a randomized, parallel-group study of patients hospitalized with NSTE-ACS with a blinded evaluation of the primary end point and all biological secondary end points. The study was conducted at seven cardiology centers in Paris, France, according to the principles of the Helsinki Declaration and the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines for Good Clinical Practice. Approval was obtained from the Pitié-Salpêtrière University Hospital Ethics Committee, and patients provided written informed consent.

Major inclusion criteria were: 1) age >18 and ≤85 years; 2) ischemic symptoms (onset <48 h) and at least one of the following: electrocardiogram ST-segment or T-wave changes or positive troponin; 3) treatment at hospital admission with 250 to 500 mg of oral or intravenous ASA and low molecular weight heparin (LMWH); and 4) an assignment for clopidogrel treatment.

Major exclusion criteria were: 1) catheterization performed before randomization or scheduled to be performed <24 h after randomization; 2) contraindication to LMWH, clopidogrel, or ASA; 3) severe hypertension; 4) platelet count $<100{,}000/mm^3$; 5) neutrophil count $<1800/mm^3$; 6) increased risk of bleeding; and 7) recent (within 10 days) or planned use of nonpermitted concomitant medications (any antiplatelet agent other than ASA, oral anticoagulants, hirudin, nonsteroidal anti-inflammatory drugs). In case of emergent PCI or glycoprotein (GP) IIb/IIIa inhibitor use during the 24-h study period, the patient was excluded from the analysis.

Treatment and follow-up. Using central randomization, patients were allocated to receive a 300-, 600-, or 900-mg oral clopidogrel LD on the morning of day 1. Twenty-four hours after the LD, all patients were started on a regimen of clopidogrel 75 mg/day and ASA ≤100 mg/day. Low molecular weight heparin was administered twice daily. All study medication was administered on an open-label basis. Patients were followed up at 30 (±7) days to record clinical outcome and adverse event reporting.

Processing of samples. To ensure prompt sample transfer and assaying, patients could only be randomized if the baseline blood sample was to be taken between 7 AM and 11 AM between Monday and Friday. The blood samples were transferred by the delivery service to the central laboratory, arriving within 1 h of venipuncture. Platelet aggregometry and flow cytometry tests were blindly performed immediately upon receipt.

Assay methods. Aggregometry, flow cytometry, measurements of inflammatory biomarkers, and markers of myonecrosis at all time points during the 24 h were processed in the core laboratory that was blinded to the treatment received.

ADENOSINE DIPHOSPHATE-INDUCED PLATELET AGGREGATION. Platelet-rich plasma was obtained by centrifugation of citrated whole blood at 100 *g* for 15 min at room temperature. Platelet-poor plasma was obtained by further centrifugation at 1000 *g* for 20 min. In vitro PA in platelet-rich plasma was measured at 37°C in an aggregometer (Model 490-4D, Chrono-Log Corporation, Kordia, the Netherlands) following the optical aggregometry method of Born (17). Platelet aggregation was induced by the addition of ADP (Chrono-Par, Kordia, the Netherlands) at final concentrations of 5 μmol/l and 20 μmol/l, and PA parameters were measured on samples obtained at baseline, 0.5 h, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, and 24 h. Inhibition of platelet aggregation (%) at time Tx was (intensity of aggregation at T baseline) − (intensity of aggregation at Tx)/(intensity of aggregation at T baseline).

FLOW CYTOMETRY. Flow cytometry measurements were performed at the same nine time points. All flow cytometric studies were conducted on a FACScalibur flow cytometer

(Becton Dickinson, Franklin Lakes, New Jersey) using CellQuest software (Becton Dickinson) for data acquisition and analysis. Analyses were performed on citrated whole blood diluted 1:4 in phosphate-buffered saline incubated with either PAC1 fluorescein isothiocyanate-conjugated monoclonal antibody (Becton Dickinson), anti-P-selectin phycoerythrin-conjugated monoclonal antibody (Becton Dickinson), or anti-fibrinogen fluorescein isothiocyanate-conjugated polyclonal antibody (Dako, Trappes, France). Platelet activation markers were measured in unstimulated platelets and after stimulation of platelets with ADP (5 μmol/l final concentration). In addition, the phosphorylation of vasodilator-stimulated phosphoprotein (VASP) was measured at 6 and 24 h by quantitative flow cytometry using a commercially available kit from Diagnostica Stago/Biocytex (Marseille, France). The VASP index was calculated from the median fluorescence intensity (MFI) of samples incubated with prostaglandin E1 (PGE_1) and ADP according to the formula: VASP index = $[(MFI_{(PGE1)} - MFI_{(PGE1 + ADP)})/MFI_{(PGE1)}] \times 100$.

INFLAMMATORY MARKERS. Determinations were performed on samples obtained at baseline, 6 h, and 24 h using commercially available enzyme-linked immunosorbent assays. High-sensitivity C-reactive protein in serum was measured using a highly sensitive kit (Dade-Behring, Paris la Défense, France). von Willebrand factor antigen (vWF Ag) and plasminogen activator inhibitor (PAI)-1 antigen were measured in citrated plasma (Asserachrom, Stago, Asnières, France). Soluble CD40 ligand (sCD40L) was measured in serum (R&D Systems, Lille, France).

TROPONIN I. Troponin I measurements were performed on samples taken at baseline, 6 h, and 24 h, with serum concentrations measured by immunoassay using an AXSYM analyzer (Abbott, Rungis, France). Patients with an increasing troponin were defined as either patients with a negative troponin on admission becoming positive over the course of 24 h or patients with a positive troponin on admission increasing by ≥30%, during the first 24 h.

END POINTS. The primary objective of the study was to evaluate changes in PA (IPA, %). The kinetic profile of clopidogrel-mediated IPA induced by 5 μmol/l ADP was compared across the three groups with respect to change from baseline to each time point and maximum effect. The same criteria were applied to the kinetic profile of inhibition of 20 μmol/l ADP-induced PA.

Among the secondary objectives of the study were the kinetics of inhibition of platelet activation (activated GP IIb/IIIa receptor complexes, P-selectin expression, fibrinogen binding, P-VASP); the frequency of low response (defined as IPA <10% at 6 h); the impact on markers of inflammation (C-reactive protein, vWF Ag, PAI-1 antigen, sCD40L); the effect on troponin I (a marker of myonecrosis); the incidence of death, myocardial infarction, or ischemic recurrences leading to revascularization and/or rehospitalization; and the safety profile using the GUSTO (Global Utilization of Streptokinase and TPA for Occluded Arteries) (18) definitions of bleeding.

STATISTICAL ANALYSES. The time course of the change from baseline to the maximum intensity of PA induced by ADP was compared through an analysis of variance of the changes from baseline at each time point. A Student *t* test, using the common residual variance from the global analysis of variance, was used to make pairwise comparisons between the 300-mg group and each of the higher LDs at each time point. No adjustment for multiple comparisons was made. The following comparisons were performed: 300 mg versus 600 mg and 300 mg versus 900 mg. The area under the IPA curve (AUC) was calculated and compared across dosages using an analysis of variance. This AUC was a combination of the level of inhibition with the duration of the inhibition. The platelet activation parameters were analyzed using the same model of analysis of variance but after log transformation of the number of epitopes. Qualitative variables were compared using the Pearson chi-square test or the Fisher exact test when the calculated sample size of at least 1 cell of a table was <5.

RESULTS

A total of 103 patients with NSTE-ACS were enrolled into the study. Baseline demographic characteristics are listed in Table 1. Overall, there were no statistically significant differences between the treatment groups.

Inhibition of platelet aggregation. In the 300-mg LD group, maximal inhibition of 5 μmol/l ADP-induced PA was achieved by 6 h after the initiation of clopidogrel. Greater LDs were associated with a significantly faster onset of inhibition (within the first 6 h: $p < 0.05$ at 3 time points with both 600 mg and 900 mg vs. 300 mg) (Table 2). In addition, the greater clopidogrel LDs produced significantly greater maximal IPA compared with the 300-mg LD. For each of the higher LDs, this significant increment in platelet inhibition was still observed at 24 h (Fig. 1A, Table 2).

Using 20 μmol/l ADP, the kinetics of IPA were faster with 900 mg, which produced already significantly greater IPA at 1 h after the administration of clopidogrel than with 300 mg (Fig. 1B). Within 2 h, the level of IPA obtained with 900 mg was greater than that obtained at the plateau of IPA with 300 mg. The onset of action of the 600-mg dose was intermediate between the 300- and 900-mg dose groups. A similar dose-effect relationship was found for the maximal plateau of IPA. Significantly higher inhibition was found at all time points from 1 to 24 h with 900 mg versus 300 mg. Intermediate levels were measured with 600 mg, which were only statistically different from 300 mg at the 4-h time point ($p = 0.04$) (Table 2).

Comparison of the 24-h AUCs for IPA suggested again a dose-response effect, with statistically significant higher AUCs in the 600-mg and 900-mg groups compared with the 300-mg group (Fig. 2).

Table 1. Baseline Demographics

	Clopidogrel, 300 mg (n = 35)	Clopidogrel, 600 mg (n = 34)	Clopidogrel, 900 mg (n = 34)
Age, mean (SD), yrs	59.7 (11.8)	62.8 (11.8)	63.5 (13.0)
Men, no. (%)	27 (77.1%)	26 (76.5%)	26 (76.5%)
Weight, mean (SD), kg	78.1 (17.5)	76.3 (15.3)	75.4 (13.2)
Symptoms onset, no. (%)			
<24 h	28/35 (80.0%)	25/33 (75.8%)	27/34 (79.4%)
≥24 h and <48 h	7/35 (20.0%)	8/33 (24.2%)	7/34 (20.6%)
Risk factors, no. (%)			
Previous MI	4/35 (11.4%)	4/33 (12.1%)	6/34 (17.6%)
Previous PCI/CABG	8/35 (22.9%)	8/33 (24.2%)	14/34 (41.2%)
Hypertension	18/35 (51.4%)	19/33 (57.6%)	21/34 (61.8%)
Diabetes	10/35 (28.6%)	5/33 (15.2%)	7/34 (20.6%)
Hypercholesterolemia	17/35 (48.6%)	19/33 (57.6%)	19/34 (55.9%)
Statins within 10 days before randomization			
CYP3A4-metabolized	20 (57.1%)	13 (38.2%)	13 (38.2%)
Non–CYP3A4-metabolized	2 (5.7%)	6 (17.6%)	7 (20.5%)
No/unknown	13 (37.1%)	15 (44.1%)	14 (41.2%)
Current smoker (>5 cigarettes/day)	11/35 (31.4%)	8/33 (24.2%)	11/34 (32.4%)
Admission ECG, no. (%)			
ST-segment elevation (transient)	2/35 (5.7%)	3/33 (9.1%)	2/34 (5.9%)
ST-segment depression	8/35 (22.9%)	10/33 (29.4%)	15/34 (44.1%)
Left bundle branch block	0	0	2/34 (5.9%)
Positive troponin I on admission, no. (%)	10 (28.6)	11/34 (32.4)	8/34 (23.5)

CABG = coronary artery bypass grafting; CYP3A4 = cytochrome P450 3A4 isoenzyme; ECG = electrocardiogram; MI = myocardial infarction; PCI = percutaneous coronary intervention.

Interestingly, although maximal IPA increased with increasing doses, it was obtained between 5 and 6 h in all three groups with both 5 and 20 μmol/l ADP-induced PA. Patients with less than 10% of IPA at 6 h, a time point when maximal IPA was reached in all 3 groups, were specifically analyzed. When using 5 μmol/l, the percentages of patients with a low response (IPA <10% at 6 h) were 28.6%, 17.2%, and 7.1% with 300, 600, and 900 mg, respectively ($p = 0.20$). When using 20 μmol/l, these rates were 46.4%, 20.7%, and 10.7% with 300, 600, and 900 mg, respectively ($p = 0.007$).

Platelet activation. All clopidogrel LDs reduced platelet membrane expression of PAC-1, P-selectin, and fibrinogen after stimulation. Peak reductions in these markers occurred at 5 to 6 h after administration of each clopidogrel LD. At 6 h, stimulated PAC-1 expression was reduced significantly in both the 600- and 900-mg groups as compared to the 300-mg group (absolute changes: −2280, −3250, and −3540 standardized sites with 300, 600, and 900 mg, respectively); at 6 h, stimulated P-selectin was reduced significantly in the 900-mg group only as compared with the

Table 2. p Values for Comparisons of Effects of Different Loading Doses on Inhibition of Platelet Aggregation

	5 μmol/l ADP		20 μmol/l ADP	
Sampling Time (h)	600 mg vs. 300 mg	900 mg vs. 300 mg	600 mg vs. 300 mg	900 mg vs. 300 mg
0.5	0.196	0.905	0.241	0.881
1.0	0.399	0.196	0.269	0.038
2.0	0.049	0.039	0.325	0.030
3.0	0.002	0.009	0.054	0.017
4.0	0.002	0.012	0.040	0.014
5.0	0.093	0.063	0.120	0.015
6.0	0.163	0.061	0.129	0.012
24.0	0.023	0.017	0.179	0.011

ADP = adenosine diphosphate.

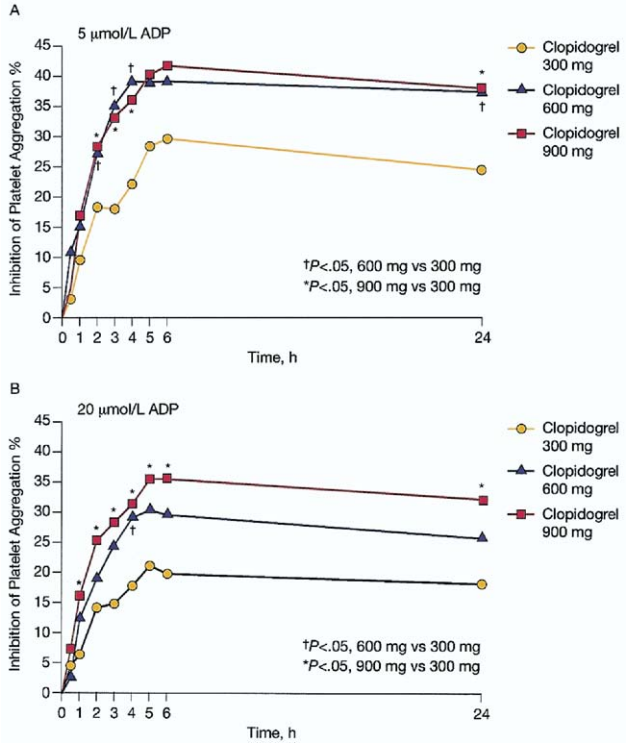

Figure 1. (A) Percentage inhibition of platelet aggregation after stimulation with 5 μmol/l adenosine diphosphate (ADP). **(B)** Percentage inhibition of platelet aggregation after stimulation with 20 μmol/l ADP.

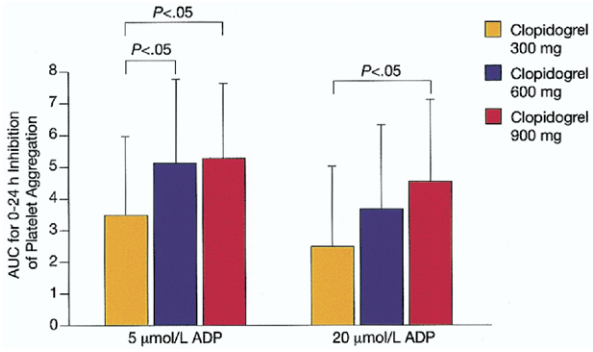

Figure 2. Areas under the curves for inhibition of adenosine diphosphate (ADP)-induced platelet aggregation between 0 and 24 h. Error bars indicate standard deviations of the mean. AUC = area under the IPA curve.

300-mg group (absolute changes: −5180, −5370, and −5470 standardized sites with 300, 600, and 900 mg, respectively); at 6 h, stimulated fibrinogen binding to the platelet membrane decreased in all three study groups (−405, −490, and −489 fluorescence count in the 300-, 600-, and 900-mg groups, respectively, without significant differences between groups).

The absolute platelet content of phosphorylated VASP, as measured after the dual effect of PGE_1 and ADP, increased after ADP inhibition of the adenylate cyclase pathway. This increase was dose dependent at 6 h (Fig. 3A), and similar results were observed at 24 h. The VASP index, which calculates the relative ADP-dependent inhibition of the platelet adenylate cyclase pathway, also exhibited a dose-response effect that favored the 900-mg group (Fig. 3B), a difference that was still present at 24 h.

Kinetics of markers of inflammation and myonecrosis. Temporal changes in markers of inflammation are shown in Table 3. At 24 h, there was a trend toward an increasing reduction from baseline in high-sensitivity C-reactive protein with greater clopidogrel LDs, although this effect was neither significant at 24 h nor apparent at 6 h. Levels of PAI-1, sCD40L, and vWF Ag were not significantly affected during the first 24 h by the administration of increasing LDs of clopidogrel.

Average troponin I or creatine kinase levels did not differ between groups at 6 h or 24 h, but this analysis is impaired by the important interindividual variability and a lack of sensitivity. However, on day 2, a trend was observed toward a lower incidence of increasing troponin I levels during the first 24 h with increasing clopidogrel LD (50.0% vs. 42.9% vs. 34.6% for 300-, 600-, and 900-mg treatment groups, respectively, p = NS) (Fig. 4).

Major adverse cardiac events (MACE). No deaths occurred during the study. Compared with the standard 300-mg LD, the incidence of MACE was numerically lower in the higher LD groups (300 mg, 4 patients [1 nonfatal myocardial infarction, 1 nonplanned PCI, 2 hospitalizations for recurrent ischemia]; 600 mg, 2 patients [2 nonfatal myocardial infarctions); 900 mg, 0 patients) but these differences were not statistically significant.

Safety. There were no episodes of severe bleeding during the trial (Table 4). The incidence of mild bleeding was comparable in the 3 treatment groups and mainly due to bleeding at puncture sites (e.g., oozing, hematoma, subcutaneous bleeding).

DISCUSSION

To our knowledge, the ALBION trial is the largest randomized, direct pharmacodynamic comparison of 3 different clopidogrel LDs. Furthermore, it is the only trial to evaluate multiple time points during the first 24 h after dosing and perform blinded evaluation of biological end points in a core laboratory. The results of the ALBION trial demonstrate that clopidogrel doses >300 mg can provide faster onset of action and greater levels of IPA in patients presenting with NSTE-ACS. A dose-response effect across the three LDs was found for IPA using 20 μmol/l ADP and inhibition of platelet activation. Compared with a standard 300-mg LD, a 900-mg LD provides greater platelet inhibition within 1 h, an effect that persisted for at least 24 h. In addition, there was a trend to lower occurrence of myocardial necrosis with the higher LDs.

Clopidogrel-induced platelet inhibition is dose and time dependent. In healthy subjects, maximum inhibition of ADP-induced PA was obtained within 5 h after the administration of a first dose of 400-mg LD of clopidogrel, with no further inhibition observed with a 600-mg LD (11). Similar results were reported in coronary patients receiving clopidogrel pretreatment before PCI, with no difference in IPA detected between 450- and 600-mg LDs of clopidogrel (14). More intriguing was the absence of a difference in IPA between 300 and 600 mg in a recent study, despite greater inhibition of ADP-induced platelet activation (as assessed by flow cytometry) with 600 mg of clopidogrel (15). These data are in contrast to other convincing studies demonstrating a higher IPA after the administration of clopidogrel 600 mg compared with 300 mg (12,13), and evidence of reduced myocardial necrosis and/or periprocedural events with the greater LD (13,16).

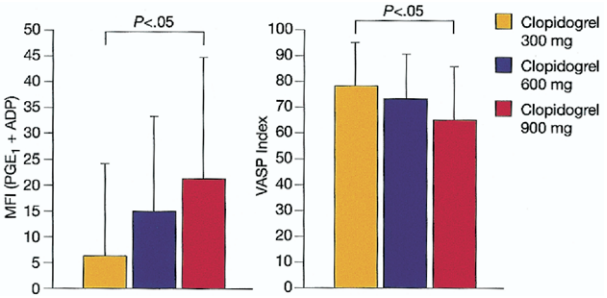

Figure 3. (Left) Mean fluorescence intensity (MFI) after the coadministration of PGE_1 and adenosine diphosphate (ADP) at 6 h after dosing. **(Right)** Vasodilator-stimulated phosphoprotein (VASP) index at 6 h after dosing. **Error bars** indicate standard deviations of the mean.

Table 3. Changes in Levels of Inflammatory Markers and Troponin I From Baseline by Treatment Group

	Clopidogrel, 300 mg	Clopidogrel, 600 mg	Clopidogrel, 900 mg	p Value
Δ hsCRP mean (SD), ng/ml				
6 h (n)	0.6 ± 1.8 (30)	0.1 ± 3.5 (26)	−0.8 ± 7.7 (28)	NS
24 h (n)	−0.1 ± 5.0 (29)	−1.2 ± 8.3 (28)	−2.0 ± 14.4 (29)	NS
Δ PAI-1 mean (SD), ng/ml				
6 h (n)	−9.2 ± 14.7 (29)	−15.0 ± 17.3 (29)	−4.8 ± 20.4 (29)	NS
24 h (n)	−5.8 ± 17.3 (29)	−3.1 ± 35.7 (30)	−8.0 ± 14.5 (29)	NS
Δ sCD40L mean (SD), pg/ml				
6 h (n)	1.6 ± 2.7 (29)	1.5 ± 3.1 (25)	2.9 ± 3.7 (28)	NS
24 h (n)	1.0 ± 1.8 (29)	0.7 ± 2.3 (23)	1.8 ± 2.8 (29)	NS
Δ vWF Ag mean (SD), %				
6 h (n)	2.2 ± 36.3 (29)	4.8 ± 45.2 (29)	3.3 ± 33.5 (29)	NS
24 h (n)	5.9 ± 37.3 (29)	13.8 ± 42.1 (30)	15.1 ± 25.1 (29)	NS

hsCRP = high-sensitivity C-reactive protein; PAI-1 = plasminogen activator inhibitor-1; sCD40L = soluble CD40 ligand; vWF Ag = von Willebrand factor antigen.

Although never evaluated versus clopidogrel 300 mg in an adequately sized clinical trial, the 600-mg LD has been used as standard therapy in placebo-controlled trials designed to assess the incremental benefit of GP IIb/IIIa inhibitors (19–21), and recent European guidelines have considered 600 mg to be an acceptable LD strategy in PCI (22). Moreover, there has been no dose-ranging study conducted so far and it was still unknown whether 600 mg was the optimal strategy to reach the maximal antiplatelet effect as early as possible. Our results suggest a gradual benefit of 600 mg and 900 mg to achieve more complete $P2Y_{12}$ receptor antagonism than 300 mg, translating into more inhibition of both platelet activation and aggregation. The ADP concentrations used to induce aggregation influence the degree of IPA, and low ADP concentrations can sometimes elicit only the primary and reversible phase of aggregation. It has been suggested that this concentration may artificially blunt the drug or dose effect, reduce the number of poor responders, and may lack clinical relevance (23–25). On the other hand, greater ADP concentrations can recruit more $P2Y_{12}$ receptors, recruit the platelet $P2Y_1$ receptor, and may be more representative of highly thrombogenic clinical situations. From the ADP content of platelets, it is possible to calculate that a local ADP concentration of 20 μmol/l can be easily achieved in the context of in vivo platelet activation (26). Indeed, 20 μmol/l ADP was more effective than 5 μmol/l in unmasking the dose-effect relationship in the present study. Although we acknowledge the numerous factors contributing to the variability of PA measurements, the multiplicity of sampling times with consistent differences at all time points suggest strongly that 900 mg provides the most complete $P2Y_{12}$ receptor occupancy. That dose impacts not only on the rapidity of action but also on the level of maximum IPA. Although it was reasonable to predict a more rapid onset of action with increasing doses, a higher maximal IPA during the first 24 h of observation with increasing doses, persisting beyond the 6-h timepoint, is quite interesting. The maximal plateau IPA was reached between 5 and 6 h in all three groups, and the curves remained parallel during the course of the 24-h sampling period, with a persistent advantage for the 900-mg group at 24 h. The impact of higher doses also clearly reduced the number of patients with a lower response in a dose-related manner. In these patients, 900 mg was associated with a 4-fold decrease in low IPA response compared with 300 mg, whatever the concentration of ADP. Our data underscore the importance of both the dose and sampling time when evaluating the degree of platelet response to clopidogrel.

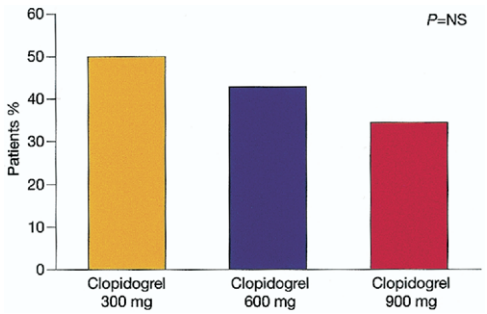

Figure 4. Proportion of patients with increased troponin over the course of 24 h.

These aggregation findings are reinforced by data on platelet activation. Measurements of activated GP IIb/IIIa receptor, fibrinogen binding, and P-selectin expression indicated less activation with the 2 higher doses of clopidogrel. The VASP phosphorylation assay, which is the most rational evaluation of ADP-induced platelet $P2Y_{12}$ receptor activation and, as a consequence, a specific marker of the clopidogrel effect, demonstrated a dose-effect relationship across the 3 doses of clopidogrel. The mean of the absolute fluorescence intensity measured on activated platelets (PGE_1 + ADP), which increases with the degree of platelet inhibition via the $P2Y_{12}$ receptors, was substantially increased in a dose-related fashion with increasing doses of clopidogrel. Calculation of the VASP index, a measure of $P2Y_{12}$-dependent platelet reactivity, confirmed the greater inhibition obtained with increasing doses of clopidogrel.

An additional supportive line of evidence was the observation that troponin I release was blunted in a dose-related manner with increasing doses of clopidogrel. This trend, which was measured during the 24-h period of medical treatment preceding catheterization, was nonsignificant but

Table 4. Incidence of Bleeding Complications by Treatment Group, Day 1 to Hospital Discharge*

	Patients, No. (%)			
	Clopidogrel, 300 mg (n = 35)	Clopidogrel, 600 mg (n = 34)	Clopidogrel, 900 mg (n = 34)	p Value
Severe bleeding	0	0	0	NS†
Moderate bleeding	1 (2.9)	0	1 (2.9)	NS‡
Mild bleeding	10 (28.6)	10 (29.4)	13 (38.2)	NS‡
Total	11 (31.4)	10 (29.4)	14 (41.2)	NS‡

*Bleeding complications were categorized according to the GUSTO classification (18). A patient may appear in more than 1 bleeding category. †Fisher exact test. ‡Pearson chi-square.

seems to confirm similar findings seen in prior PCI studies (13,16,27).

At 30 days, the incidence of MACE was asymmetrically split between the three LD groups, with a trend favoring the highest LD of clopidogrel. There was no excess of severe or moderate bleeding with the higher doses. A larger trial with adequate statistical power would be required to confirm these findings.

Finally, and in contrast to previous reports suggesting a potential anti-inflammatory effect of clopidogrel, we did not observe significant reductions in markers of inflammation. At 24 h, there was a nonsignificant trend toward increasing reduction from baseline in high-sensitivity C-reactive protein with greater clopidogrel LDs, but no evidence from the other inflammatory markers tested. This result is likely to be related to the short period of observation as well as to the absence of PCI (28,29).

The recent ISAR-CHOICE (Intracoronary Stenting and Antithrombotic Regimen: Choose Between 3 High Oral Doses for Immediate Clopidogrel Effect) study also compared the antiplatelet effects of the same 3 LDs (30). In this trial, which enrolled 60 stable patients, platelet aggregometry measurements were restricted to baseline and 4 h after administration of the LD. Incremental benefit was observed across the 3 doses, with statistical significance limited to the comparison between 600 and 300 mg with similar findings for the levels of the active clopidogrel metabolite. However, with only a single post-treatment measurement of PA, the ISAR-CHOICE investigators had little possibility of detecting differences in the onset of action according to the LD. In contrast, the present study used eight consecutive blood samples during the first 6 h, which demonstrated a dose-effect relationship for the onset of IPA as well as for platelet activation as measured by VASP, a direct measure of the action of the active clopidogrel metabolite. These 2 studies should stimulate further investigations to determine the optimal LD of clopidogrel.

Study limitations. Although our study is the largest pharmacodynamic evaluation of 3 different LDs of clopidogrel, it remains small in size and, therefore, low power may be the explanation for lack of significance for some of the comparisons; moreover, our sample size does not allow interpretation of clinical events, which have been provided here only for information. The unblinded nature of the study is another limitation, although the primary end point (IPA) and all biological endpoints were blindly and centrally evaluated.

Conclusions. The present study has shown that in moderate-risk patients with non–ST-segment elevation acute coronary syndromes, LDs of clopidogrel >300 mg provide a more rapid onset of action, a greater IPA plateau, less-frequent low response, and greater reductions in platelet activation during the first 24 h, which all are suggestive of a dose-effect relationship. Favorable trends also were seen on troponin release and ischemic events with higher doses, with no apparent adverse effects on safety. Our study suggests that 900 mg might induce a greater antiplatelet effect than 600 mg, when compared with the standard 300-mg regimen. These findings require further clinical evaluation in a larger, randomized, clinical-end point trial, ideally using a double-blind design.

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