The Pattern of Platelet Response to Clopidogrel in Iranian Patients After Percutaneous Coronary Intervention

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Despite certain clinical benefit in using clopidogrel in patients undergoing percutaneous coronary intervention (PCI), some patients do not attain adequate antiplatelet effects. In this study, the authors investigated the response to clopidogrel in Iranian patients after PCI. Patients who were candidates for elective PCI were enrolled in this study. All patients had received aspirin 80 to 325 mg daily for ≥1 week before PCI. Blood samples were taken from patients at baseline, 2 hours after taking a 600-mg loading dose of clopidogrel, and 24 hours and 30 days after stenting. Platelet aggregation was measured by light transmittance aggregometry with adenosine diphosphate (5 and 20 μM) and arachidonic acid (500 and 5000 μg/mL). One hundred twelve patients were included (79 men, 33 women). Maximal and minimal clopidogrel nonresponsiveness occurred at 2 hours (26%) and 48 hours (13%) after taking 600 mg clopidogrel, respectively. Pretreatment platelet reactivity had no effects on posttreatment platelet reactivity. Moreover, clopidogrel responsiveness did not correlate with pretreatment reactivity. Patients' demographic and procedural characteristics had no significant effect on clopidogrel responsiveness. The frequency of clopidogrel nonresponsiveness in this study was similar to other studies. However, clopidogrel required more than 2 hours for induction of its maximal antiplatelet effect in this study.

Keywords: *PCI; LTA; platelet response; clopidogrel resistance; ASA responsiveness; clopidogrel responsiveness*

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Platelets play a significant role in the pathway of thrombosis after plaque rupture.[1] Plaque rupture spontaneously occurs in patients with acute coronary syndromes (ACS) or may be induced in patients undergoing percutaneous coronary interventions (PCI). Different mediators are involved in platelet activation; however, adenosine diphosphate (ADP) plays an important role. ADP binds to several receptors on the platelet membrane.[2] Thienopyridines are irreversible inhibitors of the ADP P2Y12 receptor. Clopidogrel is currently the antiplatelet treatment of choice for prevention of stent thrombosis.[3] In addition, prolonged dual antiplatelet therapy with clopidogrel and aspirin in patients undergoing PCI has been associated with better long-term clinical outcomes.[4,5]

Despite the certain clinical advantage achieved with the administration of clopidogrel in patients undergoing PCI, previous studies, using light transmission aggregometry, have indicated that 4% to 30% of patients do not achieve adequate antiplatelet effects.[6,7] This has been ascribed to the fact that some patients may have poor clopidogrel-induced antiplatelet effects. Despite treatment with clopidogrel, these patients still show enhanced platelet reactivity, which is essential for the development of atherothrombotic complications.[1] The mechanisms leading to variability in clopidogrel responsiveness are unclear. Individual variability is largely determined by environmental and genetic factors.[8] Previous studies indicated that

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increased baseline platelet reactivity may be more commonly observed in patients with ACS, increased body mass index (BMI), and diabetes mellitus.[9,10] There is also an interracial difference of clopidogrel effectiveness. One study in Indian patients indicated that Asians have higher platelet reactivity than do whites,[11] whereas other studies showed that stent thrombosis occurs less frequently in Asians than in white patients.[12,13] Therefore, patient characteristics may predict the prevalence of poor clopidogrel responders.

In this study, we investigated the pattern of platelet response to clopidogrel and the influence of demographic and procedural characteristics on clopidogrel responsiveness in patients undergoing elective coronary artery stenting by measuring platelet aggregation. Although there are studies about clinical response to clopidogrel in different populations,[14-16] this survey is the first study about clopidogrel effectiveness in an Iranian population.

PATIENTS AND METHODS

Patients

The patients who were admitted to Kowsar Hospital in Shiraz and scheduled for elective PCI between September 2007 and October 2008 were enrolled in this study. PCI was performed for all patients with a drug-eluting stent. All patients gave written informed consent. This cross-sectional study was approved by the ethics committee of Shiraz University of Medical Sciences. All patients had received aspirin 80 to 325 mg daily for ≥1 week before PCI and had not received thienopyridine derivatives in the week prior to enrollment. Patients were older than 18 years old. Exclusion criteria were acute myocardial infarction (AMI) within 1 week, any contraindications to aspirin or clopidogrel, thrombocytopenia (platelet $<100 \times 10^3$ cells/mm^3), anemia (hemoglobin <10 g/dL), and renal failure (serum creatinine >2.5 mg/dL). Demographic and procedural characteristics, lab data, and clinical information of the patients were recorded from their files or face-to-face interview. These factors included age, sex, smoking, diabetes mellitus, hypertension (blood pressure [BP] ≥140/90 mm Hg), hyperlipidemia (low-density lipoprotein cholesterol [LDL-C] ≥100 mg/dL), BMI, white blood cell (WBC) count, platelet (PLT) count, multivessel disease (involving 2 or more coronary arteries), multivessel intervention (intervention involving 2 or more coronary arteries), left ventricular ejection fraction <45%, length, and diameter of stent.

Medications

All patients received clopidogrel (Plavix; Sanofi-aventis, Bridgewater, New Jersey) loading dose 600 mg at least 24 hours before PCI, followed by 150 mg/d clopidogrel for 2 weeks and then 75 mg/d for 12 months after PCI. Aspirin was prescribed as 325 mg/d for 1 week and then 80 mg/d for an indefinite time after PCI. Unfractionated heparin (50-70 IU/kg) was administrated as a bolus to all patients in the catheterization laboratory immediately before stenting. Patients receiving other drugs that affect the platelet activity or had a major effect on clopidogrel metabolism rate (ie, abciximab, dipyridamole, warfarin, phenytoin, phenobarbital, and omeprazole)[17] were excluded.

Blood Sampling

Blood samples were collected in tubes containing 3.8% Na-citrate. Samples were obtained before coronary intervention (baseline sample, patients only on aspirin), 2 hours after taking clopidogrel loading dose 600 mg, and 24 hours and 30 days after stenting.

Laboratory procedures (platelet aggregation and hematology assay) were done within 2 to 3 hours after sampling to minimize environmental effects.

Platelet Aggregation

The blood-citrate mixture was centrifuged at 800 rpm for 8 minutes to recover platelet-rich plasma (PRP) and further subjected to centrifugation at 4000 rpm for 20 minutes to recover platelet-poor plasma (PPP). The PRP and PPP were stored at room temperature for using within 2 hours. The platelet count was determined in the PRP sample and adjusted to between 250×10^3/μL and 300×10^3/μL with PPP. Platelets in PRP were stimulated with a final concentration of 5 and 20 μM ADP and also 500 and 5000 μg/mL arachidonic acid (AA; Helena BioSciences Europe, Sunderland, UK), and platelet aggregation was evaluated by light transmittance aggregometry (LTA; Helena PACKS-4). Platelet aggregation was expressed as the maximal percent change in light transmittance from baseline, using PPP as a reference.

Definition of Clopidogrel Responsiveness

Responsiveness was defined as the relative platelet inhibition (RI) induced by the addition of clopidogrel. RI = [(pretreatment aggregation − posttreatment aggregation)/(pretreatment aggregation)] × 100.

For both 5 and 20 μM ADP-induced aggregation, nonresponders were defined as those patients with RI <10%, semi-responders as those with RI = 10% to 30%, and responders as those with RI >30%.[14,18]

Definition of Aspirin Insensitivity

Aspirin insensitivity was defined as 500 μg/mL AA-induced platelet aggregation ≥20% and 5 μmol/L ADP-induced platelet aggregation ≥70%[16,19] or 5000 μg/mL AA-induced aggregation ≥20% and 20 μM ADP-induced aggregation ≥90%.[18]

Statistical Analysis

Continuous variables are presented as mean values ± SD. Categorical variables are reported as counts (percentage). The patients were into nonresponders, semi-responders, and responders on the basis of RI. The Kolmogorov-Smirnov test was used to assess conformity with a normal distribution.

To assess the effect of pretreatment reactivity on drug response, we divided patients into high, moderate, and low baseline reactivity. For 5 μmol/L ADP-induced aggregation, high reactivity was defined as percent aggregation >70%; moderate, 60% to 70%; and low, <60%.[16,18] For 20 μmol/L ADP-induced aggregation, high reactivity was defined as percent aggregation >90%; moderate, 80% to 90%; and low, <80%.[18]

Comparisons were made between continuous variables and groups by 1-way analysis of variance (ANOVA). Pearson χ^2 and Fisher's exact test were used for comparisons between categorical variables and groups.

Mauchly's sphericity test was used to find an association between platelet aggregation and time at baseline and at 2 hours, 24 hours, and 30 days in each concentration of ADP and AA. The comparisons of differences between these measurements in each concentration of ADP were made by *t* test. Wilcoxon signed-rank test was used for the comparisons of differences between these measurements in each concentration of AA. The association between length and diameter of stent and clopidogrel responsiveness was found by the Mann-Whitney *U* test. Because the patients might have received more than 1 stent, length and diameter of stent were indicated as average of maximal stent length (or diameter), average of minimal stent length (or diameter), and also average of total stent length (or diameter). Pearson correlation was used to find the correlation between 5 and 20 μM ADP, as well as 500 and 5000 μg/mL AA.

$P < .05$ was considered significant. Analyses were performed using SPSS version 13 (SPSS, Inc, an IBM Company, Chicago, Illinois) statistical software.

To determine a cutoff point of platelet reactivity for estimating the clopidogrel responsiveness, receiver operating characteristic (ROC) curves were obtained by plotting sensitivity or true-positive rate against 1 – specificity or false-positive rate for RI at all times of sampling with 2 ADP concentrations (5 and 20 μM). Among all ROC curves drawn, the one with the highest area under the curve (AUC) that was closest to 1 was chosen. In this ROC curve, we selected the RI with the highest sensitivity and the lowest 1 – specificity (or the highest specificity) as the cutoff point for clopidogrel responsiveness.

RESULTS

Patient Characteristics

One-hundred twelve patients fulfilled the inclusion criteria. Demographic characteristics, clinical information, lab data, and procedural information of the patients are presented in Table I.

Pretreatment Platelet Reactivity

To assess the function of pretreatment platelet reactivity, we divided the patients into 3 groups based on platelet reactivity. Table II shows that 93% and 96% of our patients had a low platelet reactivity induced by 5 and 20 μM ADP, respectively.

All patients had low platelet reactivity after clopidogrel therapy using 5 and 20 μM ADP-induced platelet aggregation at all times of sampling (Table II). Therefore, pretreatment platelet reactivity had no effects on posttreatment platelet reactivity. There was also no significant difference between pretreatment platelet reactivity and clopidogrel responsiveness ($P > .05$).

Platelet Response to Clopidogrel

Platelet response to clopidogrel was analyzed using the maximal intensity of ADP-induced platelet aggregation. The distribution of this response was consistent with a normal distribution.

The results of platelet aggregation by 2 concentrations of ADP at baseline, 2 hours after taking 600 mg clopidogrel, and 24 hours and 30 days after stenting are shown in Table III.

At 2 hours after administration of clopidogrel loading dose, 26% of the patients met the definition

Table I Demographic Characteristics, Clinical Information, Lab Data, and Procedural Information of the Patients in This Study (N = 112)

Age, y, mean ± SD	**58 ± 11**
Sex, No. (%)	
Male	79 (70)
Female	33 (29)
Smoking, No. (%)	47 (42)
Diabetes mellitus, No. (%)	21 (19)
Hypertension (blood pressure ≥140/90 mm Hg), No. (%)	57 (51)
Hyperlipidemia (LDL-C ≥100 mg/dL), No. (%)	76 (68)
BMI, kg/m^2, mean ± SD	26.3 ± 5.0
Multivessel disease,[a] No. (%)	34 (30)
Multivessel intervention,[b] No. (%)	41 (37)
LVEF <45%, No. (%)	10 (9)
WBC (× 1000/μL), mean ± SD	7.2 ± 1.9
Platelets (× 1000/μL), mean ± SD	230 ± 64
Length of drug-eluting stent, mm, mean ± SD	
Average length	21.6 ± 7.2
Average smallest length	19.2 ± 8.2
Average largest length	24.0 ± 8.1
Diameter of drug-eluting stent, mm, mean ± SD	
Average diameter	3.1 ± 1.3
Average smallest diameter	2.9 ± 0.7
Average largest diameter	3.3 ± 2.7

BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; WBC, white blood cell.
a. Involving 2 or more coronary arteries.
b. Intervention involving 2 or more coronary arteries.

of nonresponder. At 24 hours and 30 days after PCI, the rate of nonresponders was 13% and 14%, respectively (measured by 5 μM ADP).

The response to 20 μM ADP showed a similar pattern. RI of less than 10% was present in 29% of the patients at 2 hours. At 24 hours and 30 days after PCI, 7% and 12% of the patients had RI <10%, respectively. Therefore, our results indicate that the maximum frequency of nonresponders was at 2 hours after administration of the 600 mg loading dose of clopidogrel in both concentrations of ADP.

Mauchly's sphericity test showed that there were significant differences between platelet aggregation and time at baseline, 2 hours, 24 hours, and 30 days in each concentration of ADP ($P < .001$).

Pearson correlation analysis showed that there was a significant correlation between the 5 and 20 μM ADP aggregation response ($P < .001$, $r = 0.88$). Therefore, we can use only 1 concentration of ADP for future studies or in clinical practice.

Platelet response to clopidogrel was also analyzed using the maximal intensity of AA-induced platelet aggregation. The distribution of this response was not consistent with a normal distribution. Baseline aggregation (mean ± SE) to 500 and 5000 μg/mL AA was 6.6% ± 0.5% and 12.4% ± 1.7%, respectively.

The mean ± SE of platelet aggregation by 500 μg/mL AA was 5.5% ± 0.2% at 2 hours after taking the 600 mg loading dose of clopidogrel, 5.9% ± 0.2% at 24 hours, and 5.6% ± 0.2% at 30 days after PCI. The mean ± SE of platelet aggregation by 5000 μg/mL AA was 10.8% ± 1.6% at 2 hours, 7.9% ± 0.9% at 24 hours, and 7.8% ± 0.7% at 30 days.

There was no correlation between the 500 and 5000 μg/mL AA aggregation response ($P = .35$, $r = 0.34$). When comparing the differences between platelet aggregation at baseline, 2 hours, 24 hours, and 30 days, only 5000 μg/mL AA ($P < .001$) was significant. However, in the comparison of paired samples in 5000 μg/mL AA, only the difference between platelet aggregation at baseline and 2 hours after taking the clopidogrel loading dose was significant ($P = .016$).

ROC CURVE ANALYSIS

According to the ROC curves, the best AUC belonged to RI at 48 hours after the 600 mg loading dose with 20 μM ADP (AUC = 0.9); therefore, the cutoff point was selected as 25.7% according to a sensitivity of 1 and 1 – specificity of 0.357.

Platelet Response to Aspirin

Aspirin insensitivity was determined for 2 concentrations of AA based on the definition of aspirin insensitivity mentioned previously. At baseline, aspirin insensitivity was observed in only 1 patient in both concentrations of AA. There were no aspirin-insensitive patients at other times.

Association Between Patient Information and Procedural Characteristics With the RI of Platelets After Taking Clopidogrel

There were no significant differences in patient characteristics among nonresponders, semi-responders, and responders ($P > .05$). The only patient characteristic that had a significant effect on clopidogrel

Table II Pretreatment Platelet Reactivity Measured by 5 and 20 μM ADP-Induced Aggregation (N = 112)

Agent	Low Reactivity, %,[a] Mean ± SD	Moderate Reactivity, %,[b] Mean ± SD	High Reactivity, %,[c] Mean ± SD
Aggregation, 5 μM ADP	31.5 ± 13.2 (n = 104)	65.9 ± 2.9 (n = 6)	99.8 ± 4.8 (n = 2)
Aggregation, 20 μM ADP	51.1 ± 15.6 (n = 108)	88.2 (n = 1)	101.5 ± 10.1 (n = 3)

ADP, adenosine diphosphate.
a. % Aggregation <60% (5 μM), % aggregation <80% (20 μM).
b. % Aggregation 60% to 70% (5 μM), % aggregation 80% to 90% (20 μM).
c. % Aggregation >70% (5 μM), % aggregation >90% (20 μM).

Table III Platelet Aggregation Measured by 5 and 20 μM ADP at Different Times of Sampling in Patients Undergoing Percutaneous Coronary Intervention (N = 112)

Agent	Baseline	2 Hours After 600-mg Clopidogrel Loading Dose	24 Hours After Stenting	30 Days After Stenting
Aggregation, 5 μM ADP, %, mean ± SD	34.5 ± 17.4	23.0 ± 13.0	15.8 ± 9.9	17.7 ± 8.8
Aggregation, 20 μM ADP, %, mean ± SD	52.8 ± 17.7	36.9 ± 17.5	23.3 ± 12.1	27.6 ± 13.9

ADP, adenosine diphosphate.

responsiveness at 24 hours after PCI (measured by 5 μM ADP-induced aggregation) was BMI (P = .001). The results showed no significant differences between procedural characteristics and clopidogrel responsiveness. The only procedural characteristic that had a significant effect on clopidogrel responsiveness at day 30 (measured by 5 μM ADP-induced aggregation) was the maximal stent length (P = .021).

DISCUSSION

Early identification of nonresponders to clopidogrel may be important in recognizing subgroups of patients who might be at risk for thrombotic events in the future. The present study illustrates the platelet inhibitory response to clopidogrel in Iranian patients. Although the frequency of clopidogrel nonresponsiveness in our patients is comparable to other studies (4%-30%),[6,7] there is a significant difference in the time of clopidogrel effectiveness and PCI between our survey and other studies. Previous studies indicated that inhibition of ADP-induced platelet aggregation was near maximal 2 hours after loading with 600 mg of clopidogrel, and PCI was conducted 2 hours after patients took the 600 mg loading dose of clopidogrel.[20,21] In our population, the maximum rate of clopidogrel nonresponsiveness was seen 2 hours after the 600 mg loading dose of clopidogrel, and PCI was performed 24 hours after patients received the 600 mg loading dose of clopidogrel. Interestingly, the minimum frequency of clopidogrel nonresponsiveness was observed 48 hours after patients took the clopidogrel loading dose. Therefore, clopidogrel required more than 2 hours for induction of its maximal antiplatelet effect, and stenting needed to be performed 2 hours after administration of the 600 mg clopidogrel loading dose in our patients. The study by Motovska et al[22] showed that the maximal inhibition of platelet activation was at 28 hours after the administration of the 600 mg loading dose of clopidogrel.

The presence of the maximum frequency of nonresponders at 2 hours after administration of the 600 mg clopidogrel loading dose could have been caused by the low blood level of the clopidogrel active metabolite during 2 hours in our population, although we could not determine the blood level of the active metabolite. Reduced intestinal absorption and CYP450 enzymatic activity of clopidogrel

metabolism could be 2 probable causative factors for this dilemma.[8] None of our patients received drugs that affected the rate of metabolism or intestinal absorption of clopidogrel, suggesting the role of genetic makeup in this variability.

In the studied population, most patients had low pretreatment platelet reactivity, as measured by ADP-induced aggregation (93%, ADP = 5 μM; 96%, ADP = 20 μM), as a result of our patients receiving aspirin before clopidogrel administration and PCI. The data analysis indicated that patients with low pretreatment reactivity would have low posttreatment reactivity, whether or not they were clopidogrel nonresponders. Other patients, with moderate and high pretreatment reactivity, had low reactivity after clopidogrel therapy at all times of sampling for both concentrations of ADP. Therefore, pretreatment platelet reactivity had no effect on posttreatment platelet reactivity. Moreover, pretreatment platelet reactivity had no significant effect on clopidogrel responsiveness, and it could not be a predictive factor for clopidogrel responsiveness.

The ROC curves demonstrated that if the patient's RI was less than 25.7% forty-eight hours after the clopidogrel loading dose, the patient would be hyporesponsive to clopidogrel (nonresponder or semi-responder) and should be monitored more for clopidogrel dosing and cardiovascular events. Gurbel et al[23] found that a cutoff value of 50% posttreatment reactivity, using LTA, was associated with the occurrence of thrombotic events during a 6 month follow-up. Another study demonstrated that a cutoff value of 50% posttreatment platelet reactivity using the vasodilator-stimulated phosphoprotein phosphorylation (VASP) index predicted 6 months of major adverse cardiovascular events.[24] Contrary to our results, Samara et al[18] indicated that the posttreatment reactivity was related to pretreatment reactivity. They showed that patients' posttreatment platelet reactivity was a more important risk factor for thrombosis than the responsiveness to clopidogrel, and responders with the greatest pretreatment reactivity would be at greatest risk of thrombosis. Although current studies[25-28] and our survey report that posttreatment platelet reactivity is a better predictor of a thrombotic event than baseline platelet reactivity, criteria for individual clopidogrel responsiveness have not been standardized. Obstacles to standardization include the diversity of assays available for detecting clopidogrel antiplatelet effects, differing techniques in which laboratories use these assays, and lack of a standard description for nonresponsiveness.[29]

In our study, the only demographic factor that had a positive effect on platelet aggregation 24 hours after PCI (induced by 5 μM ADP) was BMI. Statistical analysis showed that maximal stent length 30 days after PCI had a significant effect on platelet aggregation induced by 5 μM ADP. Because we could not obtain these results at other times and also in the concentration of 20 μM ADP, and the aim of this study was to find clinical and therapeutic protocols to control the factors that affect clopidogrel responsiveness, we cannot use these factors as prognostic factors for clopidogrel responsiveness, although they are significantly effective.

In a study by Lev et al[30] in a US population, there were no significant differences in patient and procedural characteristics between clopidogrel nonresponsive and clopidogrel-sensitive patients. Samara et al[18] also performed a study in a US population and showed no significant differences among nonresponders, semi-responders, and responders in patient demographic parameters. Furthermore, other studies performed in Japanese[31] and US[32] patients demonstrated that clinical and demographic characteristics of patients had no significant effect on clopidogrel responsiveness. These results are comparable to our findings, although some studies do not support our results. In 1 study in a US population, Gurbel et al[16] indicated that total stent length was the only contributing factor to clopidogrel responsiveness. Arméro et al[33] found that insulin-dependent diabetes mellitus caused a decreased response to clopidogrel in Franc patients undergoing PCI. One study in the United States reported that nonwhite race, female gender, proton pump inhibitors, and current smoking were independent predictors for high platelet reactivity after clopidogrel therapy.[34]

AA was used as well as ADP in our study for assessing aspirin response because all the patients received aspirin before and after PCI. To control the confounding effect of aspirin on the inhibitory effect of clopidogrel on platelet aggregation, LTA was conducted to evaluate aspirin insensitivity. Another reason was that, according to the study by Macchi et al,[35] the platelets from aspirin-resistant patients appear to have increased sensitivity to ADP. Furthermore, aspirin resistance has been associated with platelet hyperreactivity.[35,36] These hyperactive platelets may also be less responsive to inhibition by other antiplatelet drugs such as clopidogrel.[6] In our study, 111 patients were aspirin sensitive. Only 1 patient was aspirin insensitive while clopidogrel responsive. Therefore, aspirin insensitivity was not a confounding factor for clopidogrel responsiveness in this study.

CONCLUSION

In summary, we found that environmental factors had no significant effect on clopidogrel responsiveness. Measurement of platelet aggregation between 2 and 48 hours after taking the 600 mg loading dose of clopidogrel seems to be the best predictor for post-treatment clopidogrel responsiveness in our patients.

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