A Randomized Open Blind Control Study Assessing Clopidogrel Resistance in Iranian Patients Undergoing Percutaneous Coronary Intervention in Rajaie Heart Center

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Background

The prevalence of response variability to clopidogrel in Iranian patients undergoing elective percutaneous coronary intervention (PCI) with stenting, impleta has not been fully investigated. We evaluated clopidogrel antiplatelet effects of 300 mg versus a 450 mg in open blind randomized clinical trial in a referral teaching hospital in Iran.

Methods

Fifty patients scheduled for elective PCI were enrolled. One group received 300 mg and the other one received 450 mg before PCI procedure. Platelet aggregation analyzed by optical aggregometery at baseline and after loading dose prior to PCI.

Results

The ratios of responders (IPA ≥30%), hypo-responders (10%≤IPA<30%), and non-responders (IPA <10%) were 36%, 42%, and 22%, respectively. Significantly more patients achieved 95% IPA with the high-dose tirofiban regimen.

Conclusions

It can be concluded that 450mg of clopidogrel had approximately 35% and 300 mg of clopidogrel had 20% reduction in ADP-induced platelet aggregation, although there was still a broad inter-individual variation by increasing dose.

Introduction:

Dual antiplatelet therapy with aspirin and clopidogrel has become a standard approach in clinical practice guidelines to prevent future cardiovascular events in patients undergoing PCI (1, 2). Despite significant benefits reported with combined antiplatelet treatment in large clinical trials, some patients treated with dual antiplatelet still suffered from recurrent cardiovascular complications (3, 4). In the other words, uniformity of platelets inhibition and inter individual variability in antiplatelet response of clopidogrel may predispose patients to adverse ischemic events (5, 6). Available data showed that approximately 5-56% of patients treated with conventional clopidogrel doses; do not display adequate platelet response (7). Whereas low responsiveness to clopidogrel has been associated with poor clinical outcomes, there has not been a distinct practical guideline for reduction in ischemic events rates (6). Indeed adjustment treatment based on platelet response to antiplatelet agents remains to be controversy in clinical trials. A variety of techniques have been employed to quantify the platelet inhibition associated with clopidogrel therapy (7). However, no

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standard method has consensually been recommended (8).
In this trial we evaluate the prevalence of clopidogrel resistance and compare platelet function profiles in patients receiving two different clopidogrel regimens in Rajaie Heart Center; a tertiary care cardiac hospital in Iran.

Methods

This was a prospective, randomized, open-blind study. The study protocol was approved by the local ethics committees of our institution. All patients gave written informed consent before inclusion.
All 50 patients between 25-75 years of old who were supposed to undergo PCI were eligible for inclusion in this study. Exclusion criteria were pregnancy, breast feeding, having a history of stroke within a 3-month period prior to the commencement of the study, malignancy, active and diathesis bleeding, receiving GP IIb/IIIa inhibitors, history of using clopidogrel, serum creatinine > 2 mg/dl , hematocrit> 25% or platelet count < 100×109/L.
The first group of patients (n=25) received 300 mg loading dose of clopidogrel approximately 24 hours before PCI. In the second group(n=25) the regimen was initiated with the 300mg loading dose in the hospitalized day and continued by 75 mg daily in the two other days. The procedure was done on the third day after hospitalization. Therefore 450mg clopidogrel was received before the procedure. A daily maintenance dose (75 mg) was administered the morning after the procedure and continued thereafter for all patients. Also, ASA was administered at a daily dose of 81–100 mg thereafter.

Blood samples

Blood samples were collected for platelet aggregometry at baseline and after loading dose prior to PCI. The first 2 mL of blood was discarded to avoid measuring platelet activation induced by needle puncture. Peripheral venous blood was collected in 3.8% citrate vacutainer (6ml BD vacutainer, ph=5.2) The Vacutainer was inverted 3 to 5 times for gentle mixing and sent immediately to the hemostasis laboratory
Analysis of Platelet Aggregation
Platelet aggregation was evaluated by optical aggregometry in platelet-rich plasma (PRP). PRP was prepared by centrifugation at 1000 r.p.m at room temperature for 10 min. and platelet-poor plasma was prepared by centrifugation at 3000 r.p.m at room temperature for 10 min. Aggregation was measured with a optical Aggregometer (Helena bioscience Europe), hence The PRPs were stimulated by 20 µmol/L ADP (adenosine diphosphate), 500µg/ml Arachidonic acid, 10µg/ml Collagen and 1500 µg/ml Ristocetin. The degree of light transmission of the PRP was defined as 0% of the aggregation rate and the cognitive platelet-poor plasma as 100%.The degree of light transmission was monitored for 10 min after agonist stimulation and platelet aggregation was evaluated. All the procedures were completed within 60 min of blood sampling. All analyses were performed by a single highly trained and experienced technician.
Definition of Clopidogrel Responsiveness and End Point
Classification of clopidogrel effectiveness was based on the definition from a previous report: (9) Inhibition of platelet aggregation (IPA) <10% (clopidogrel non-responders); 10%≤ IPA <30% (hypo-responders); IPA ≥30% (responders).The primary end point of this study was the percent inhibition of Aggpeak at the time of angiography. Patients were screened for the possible occurrence of composite thrombotic events, including cardiovascular death, myocardial infarction, or vessel revascularization for 1 month after coronary stenting.

Statistical analysis

Statistical analysis was performed using SPSS 15 software (SPSS Inc., Chicago, Illinois). Data are presented as mean±SD for continuous variables and percentages for categorical variables. Comparison between two groups was performed using the t-test,chi-square test or Fisher exact test. P-values <0.05 were considered statistically significant.

Results

The baseline characteristics of the 50 enrolled patients are shown in Table 1. Mean age was 60.68 ±9.25, 59.52 ±8.78 years respectively in 300 mg and 450 mg clopidogrel loading dose. Demographic data and body mass index were similar in the 2 groups. Concomitant medications with β-blockers, ACEI, CCB, diuretics, nitrates and statins are shown in Table 2. No significant differences were observed. Demographic data and body mass index were similar in the 2 groups. The 2 groups did not differ in left ventricular ejection fraction (p = 0.13). The PCI data were also similar including the number of drug-eluting stents and number of stenosis (p=0.2 and 0.5 respectively).

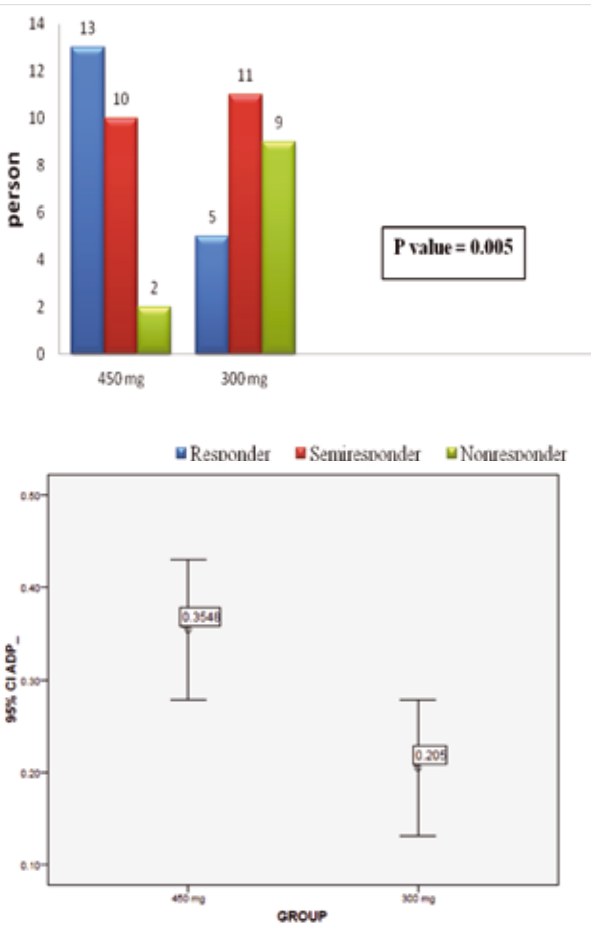
Table 1 Baseline Characteristics

	Group 1 (LD1: 300 mg)	Group 2 (LD:[1] 450 mg)	P value
Age	60.68 ±9.25	59.52 ±8.78	0.98
Sex			
Female	32% (8)	56% (14)	0.87
Men	68% (17)	44% (11)	
BMI[2](kg/m^2)	27.77±3.06	27.78±4.72	0.095
Drug treatment			
statins	68% (17)	60(15)	0.769
B blocker	48% (12)	80 (20)	0.018
ACEI[3]	44% (11)	48% (12)	0.777
ARBI[4]	12% (3)	12% (3)	1.00
CCB[5]	28% (7)	24% (6)	0.747
Nitrate	80%(20)	64% (16)	0.208
Diuretics	16%(4)	24% (6)	0.480

1: Loding dose clopidogrel,2: Body Mass Index, 3: Angiotensin-convertingEenzyme Inhibitor;
4: Angiotensin II-Receptor Blocker, 5: Calcium Channel blocker

According to definition the proportion of responders, semiresponders, and resistances was 36%, 42%, 22% respectively. Diagram 1 showed the different responds in 2 groups. It was indicated that there was a significant difference between the 2 groups in response to ADP. (P< 0.05) Clopidogrel didn't significantly affect other agonists- induced platelet aggregation.
According to diagram 2 it can be concluded that 450mg of clopidogrel had approximately 35% and 300mg of clopidogrel had 20% reduction in ADP-induced platelet aggregation. Role of sex, age, underlying disease and drugs used were evaluated. No significant differences were observed except with β-blockers (p=0.03).
Patients were screened for the possible occurrence of composite thrombotic events, including cardiovascular death, myocardial infarction, or vessel revascularization for 1 month after coronary stenting. 66% of patients were completely healthy and there were no significant problems. 24% of patients had given chest or left hand pain. Restenosis and MI occurred in 6% and 4% of patients' respectively.

Diagram 2: comparison percentage of reduction for ADP-induced platelet aggregation in 2 groups



Conclusion

In this study, we evaluated the antiplatelet effect of clopidogrel under low-dose ASA therapy in Iranian patients scheduled for PCI, and found that there was a significant difference between two loading dose and a wide inter-individual variation in each group.
The rates of patients with so called 'clopidogrel resistance' ranged between 5% and 56%, although the definitions of clopidogrel resistance varied (7).
This discrepancy has been mainly due to different methods used to monitor clopidogrel action, concentrations of the agonist used to induce platelet aggregation, type of anticoagulant used to preserve the assayed samples, criteria and cut off values used to define clopidogrel resistance and times chosen to measure post-clopidogrel platelet reactivity (7).
As shown in diagram 1, we also detected (22%) non-responders, 42% clopidogrel semiresponder and only 36%

responders. These data suggest that there is also a wide variety of responses to clopidogrel in the Iranian patients. Consequently, the degree of platelet inhibition in this study obtained with a similar regimen of clopidogrel, in which a 300-mg loading dose were administered under ASA therapy, might be less than the other studies (10,11).

Considering the higher dose of clopidogrel and response to this drug, most of the articles achieved this thesis that the higher dose of clopidogrel has higher effect on platelet aggregation and shows lower rate of resistance (12). A study in 2008 reported that clopidogrel 600-mg double loading achieves greater platelet inhibition than conventional single loading doses (13). According to a new guideline for patients undergoing PCI procedure a 600 mg loading dose of clopidogrel recommended (2). Importantly, as clopidogrel dose is increased, it can get more degree of platelet inhibition, but the point is, clopidogrel non responder was still remaining.

Our study also shows that 300 mg loading dose of clopidogrel has high degree of platelet aggregation and also higher rate of clopidogrel resistance.

The mechanisms responsible for clopidogrel response variability and resistance have not been completely defined. Differences in intestinal absorption, hepatic conversion to the active metabolite through cytochrome P450 isoenzymes (CYP) activity, and platelet receptor polymorphisms have been reported (14, 15).

Therefore the increasing loading dose may not completely resolve the clopidogrel resistance.

Further study is essential to link the effectiveness of clopidogrel to the clinical outcomes in Iranian patients and compare antiplatelet response by 600 mg clopidogrel as a loading dose. Determination of mechanisms involved in clopidogrel resistance in Iranian people can help to overcome this problem any better. Acquired and genetic factors have been suggested as causes of resistance. So adding cilostazol to the dual antiplatelet therapy of ASA and clopidogrel, substitute clopidogrel with other members of P2Y12 adenosine diphosphate (ADP) receptor blocker family may be some options. In conclusion, administration of a 450 mg oral of clopidogrel during 2 days before PCI results in a more intense inhibition of ADP-induced platelet aggregation than administration of the currently recommended 300mg loading dose. However, our study was small-scale, so further study with a larger number of patients and evaluation of continuous clopidogrel effectiveness throughout treatment period is essential.

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