COMPARISON OF LOADING DOSE OF 300mg VERSUS 600mg OF CLOPIDOGREL ON PLATELET AGGREGTION IN PATIENTS WITH CORONARY ARTERY DISEASE

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SUMMARY

Objective: To study the effects of loading dose of 300mg versus 600mg of clopidogrel on platelet aggregation in patients with coronary artery disease (CAD).

Material and Methods: This study was conducted on patients with CAD, not already taking any anti-platelet drugs, admitted in cardiology department Lady Reading Hospital from 1st June to 15th July 2005. Patients were randomized into two groups. Baseline platelet aggregation was measured followed by oral clopidogrel 300mg (group A) or 600mg (group B). Platelet aggregation was measured serially at 2, 4 and 6 hours.
Results: Total number of patients was 52 (16 males & 36 females). In group A, the baseline mean platelet aggregation was 2.36±1.5 ohms, there was significant difference at 2 hours i.e. 1.24 ±1.2 ohms (P<0.001), 4 hours i.e. 1.05 ± 1.05 ohms (P<0.001) and at 6 hours i.e. 0.71± 0.72 ohms (P< 0.001) as compared to baseline. In group B, the baseline mean platelet aggregation was 2.32±1.98 ohms. There was significant difference in platelet aggregation time at 2 hours i.e. 1.37±1.39 ohms (P< 0.001) 4 hours i.e. 0.92± 0.97 ohms (P<0.001) and at 6 hours i.e. 0.35±0.61 ohms (P< 0.001) as compared to baseline. No significant difference was observed on platelet aggregation in-group "A" and group "B" at 2 hours (P=0.479), 4 hours (P=0.858) and 6 hours (P=0.756).

Conclusion: Clopidogrel 300mg as a loading dose is as effective as clopidogrel 600 mg in inhibiting platelet aggregation in patients with coronary artery disease.

Key words: Coronary Artery Disease, Copidogrel, Platelet Aggregation.

INTRODUCTION

Platelet activation and aggregation play a key role in initiating and propagating coronary artery thrombosis. Clopidogrel is an adenosine diphosphate receptor antagonist, a class of oral anti-platelet agent that blocks the P2Y12 component of the adenosine diphosprate receptor and thus inhibit the activation and aggregation of platelets[1,2]. Clopidogrel has been shown to prevent death and ischaemic complications in patients with symptomatic atherosclerotic disease, patients who have undergone percutaneous coronary intervention, and patients with unstable angina or non-ST elevation myocardial infarction[3-4]. Arterial thrombi that are rich in platelets are relatively resistant to fibrinolysis and prove to induce reocclusion after reperfusion theraphy[5]. Despite the inhibition of cylooxygenase by aspirin, platelet activation can still occur through thromboxane A2 independent pathway leading to aggregation of platelets and the formation of thrombus[6]. Clopidogrel is a potent anti-platelet agent that has a synergistic anti-platelet effect when combined with aspirin[7]. Trials studying Clopidogrel alone or in combination with aspirin in different doses have demonstrated mortality and morbidity benefits. These benefits reflect the efficacy of clopidogrel on platelet inhibition[4].

The aim of this study was to evaluate the efficacy of loading of 600 mg versus 300 mg of clopidogrel in terms of platelet inhibition in our own setup.

MATERIAL AND METHODS

The study was conducted in cardiology department of Lady Reading Hospital, Peshawar, from 1st June to

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15th July 2005.

Inclusion criteria: Patients with coronary artery disease who were not already taking any anti-platelet
Exclusion criteria: Patients with acute myocardial infarction, acute coronary syndrome, hepatic insufficiency, history of significant bleeding disorder, abnormal platelets counts and patients already taking anti-platelet and anticoagulant therapy.

Procedure: Patients with coronary artery disease who were not taking any anti-platelet drug were admitted in cardiology department Lady Reading. All patients were randomized by coin tossing method to two groups i.e "A" or "B". Patients in group "A" received 300 mg of clopidogrel (17 patients) and those in group "B" received 600 mg of copidogrel (35patients). Baseline platelet aggregation was measured of every patient in each group. After giving loading doses, platelet aggregation was measured at 2,4 and 6 hours in patients of each group.

The trial was conducted from 1st June to 15th July 2005 at Cardiology department Lady Reading Hospital Peshawar.

The supplies needed for performing platelet aggregation test was aggaregometer & included reagents, curettes, stir bars, micropipettes, isotonic saline, vacuette tubes and blood collecting adaptor. After taking the baseline blood samples, 300 mg (4 tablets) of drug was given to each patient in group "A" and 600 mg (8 tablets) of drug were given to each patient in group "B". Concomitant medications needed for the treatment of underlying diseases were continued.

Electrical impedance aggregometry technique measures aggregation as an increase in the electrical impedance across two precious metal wires resulting from the accumulation of platelets in response to an agonist[8]. This impedance aggregation can be completed 30 minutes after a blood sample is obtained and the method provides accurate results up to 3 hours[9,10].

Blood samples were collected by direct venipuncture using vacuette tubes. After collection, the blood tubes were gently inverted several times to ensure complete mixing with the sodium citrate anticoagulant present in the vacuette tube.

Electrical impedance aggregation measurements were performed on the Chronolog whole-blood aggregometer model 591. The instrument has received approval from the Food and Drug Administration,USA[11].

An aliquot of whole blood (0.5 ml) was diluted with an equivalent volume of isotonic saline and incubated for 5 minutes at 370C. The impedance of each sample was monitored at sequential 1-minute intervals until a stable baseline established. After this the agonist ADP (20 µmol/L) was then added to the sample and aggregation was monitored for 6 minutes. The final increase in ohms over this period was displayed as a numeric LED readout. In addition, a graphical printout (i.e. chart tracing) of each electrical impedance aggregometry was also obtained. For each sample, the percent of baseline aggregation was determined by the maximum change in ohms of test sample divided by the maximum change in ohms of the baseline sample. Finally, the product of the above calculation was multiplied by 100[10].

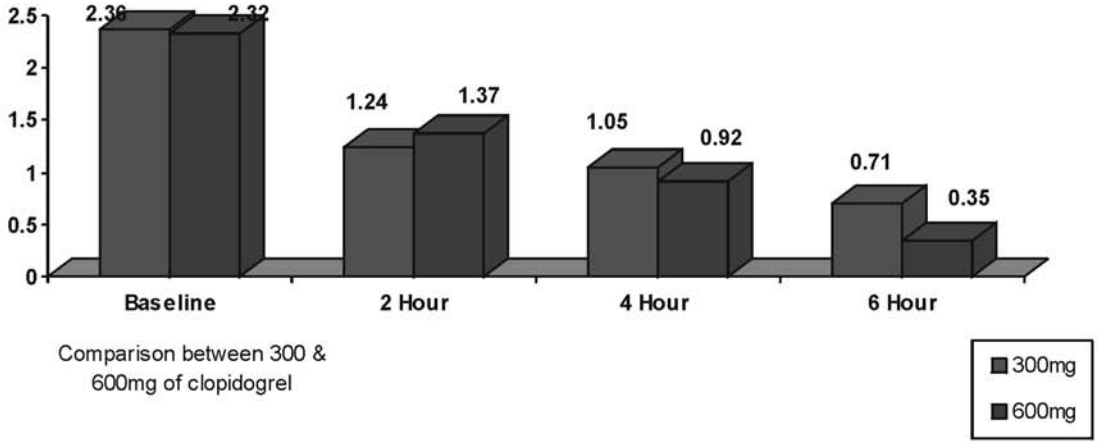
Statistical Evaluation
Data results were analyzed by SPSS version 10. Paired T-test was used to detect the difference at base line, and at 2,4 and 6 hours after taking clopridogrel 300 mg and 600 mg in each group. Result were expressed as mean ± SD.A p value of < 0.05 was considered significant.

RESULTS:

Total number of patients was 52, male were 16, and female were 35. Mean age of these patients was 52±12 years. Patients were divided into two groups. Out of 52 patients, 17 (32.7%) were randomly assigned to group "A". The baseline mean platelet aggregation of these patients was 2.36±1.5 ohms, there was significant difference at 2 hours i.e. 1.24 ±1.2 ohms ($P<0.001$), 4 hours i.e. 1.05 ± 1.05 ohms ($P<0.001$) and at 6 hours i.e. 0.71± 0.72 ohms ($P< 0.001$) as compared to baseline (Table 1). Thirty five (67.3%) atients were randomized to group "B" who received clopridogrel i.e. 600 mg as loading dose; the baseline mean platelet aggregation was 2.32±1.98 ohms. There was significant difference in platelet aggregation time at 2 hours i.e. 1.37±1.39 ohms (P<

Table 1: Comparison of platelet aggregation in patients getting 300mg Clopidogrel and 600mg Clopidogrel

	Clopidogrel		Clopidogrel 600 mg		
Time	**Platelet aggregation (Mean ± SD) Ohms**	**p value among Clopidogrel 300 mg as compared to base line**	**Platelet aggregation (Mean ± SD) Ohms**	**p value among Clopiodorel 600mg as compared to base line**	**p value between 300 and 600 mg Clopidogrel**
Baseline	2.36±1.5	----------	2.32± 1.98.	----------	0.802
2 hours	1.24±1.2	<0.001	1.37 ±1.39	<0.001	0.479
4 hours	1.05 ± 1.05	<0.001	0.92± 0.97	<0.001	0.858
6 hours	0.71± 0. 72	<0.001	0.35± 0.61	<0.001	0.756



0.001) 4 hours i.e. 0.92± 0.97 ohms (P<0.001) and at 6 hours i.e. 0.35±0.61 ohms (P< 0.001) as compared to baseline.

No significant difference was observed on platelet aggregation in-group "A" and group "B" of clopidogrel 300 mg and 600 mg respectively at 2 hours (P=0.479), 4 hours (P=0.858) and 6 hours (P=0.756) { Graph 1}.

DISCUSSION

The major cause of death worldwide is cardiovascular and cerebrovascular diseases, which is due to atherothrombotic events, highlighting the significance of anti-platelet agents. Large clinical trials done on clopidogrel like CAPRIE,[3] CLASSICS,[12] CURE[13] (including PCI-CURE)[14] CREDO,[8] COMMIT/CCS-2[16] and CLARITY-TIMI 2817 have shown that clopidogrel is effective in decreasing the mortality and morbidity in patients with atherothrombotic diseases. In CARESS Trial[18] the clopidogrel in combination with aspirin resulted, reduction of emboli in symptomatic carotid artery stenosis. In a study done on sample volume of 12562 patients, Salim Yousaf et al concluded that in acute coronary syndrome patients, clopidogrel reduces the risk of ischemic vascular events, with the benefits emerging within 24 hours of initiation of treatment and continuing throughout the 12 months (mean 9 months)of the study[19].

The CAPRIE trial[3], done on sample volume of 19,185 patients concluded that a true benefit is seen with clopidogrel over aspirin, and it is as safe as regular strength aspirin. PCI-CURE[14] included 2658 patients concluded that in patients with acute coronary syndrome receiving aspirin ,a strategy of clopidogrel pretreatment followed by long term therapy is beneficial in reducing major cardiovascular events, compared with placebo. CLASSIC[12] trial included 1020 patients concluded that the safety/tolerability of clopidogrel (plus aspirin) is superior to that of ticlopidine (plus aspirin).The 300 mg loading dose was well tolerated, notably with no increased risk of bleeding. This trial also concluded that clopidogrel and ticlopidine have comparable efficacy with regard to cardiac events after successful stenting.

In patients undergoing percutaneous coronary intervention (PCI), the recommended loading dose of clopidogrel range from 300 to 600 mg. A number of studies have used loading dose of Clopidogrel up to 600 mg, showing rapid and pronounced inhibition of ADP induced platelet aggregation inhibition[20,21]. However studies (like ISAR-CHOICE) have shown equal effects of loading doses of 300mg ,600 and 900 mg[22].

Muller et al[23] reported that 600 mg loading dose is more effective in suppressing platelet aggregation at 4 hours after drug administration than 300 mg loading of clopidogrel. Similarly Nicolas von Benckerath et al[22] reported that loading doses of clopidogrel 900 mg has no additional benefit over 600 mg of clopidogrel because of limited clopidogrel absorption, but 600 mg loading dose is more effective than 300mg.

Our study was aimed to investigate the anti-platelet effect of 300 and 600mg clopidogrel in Pakistani patients with stable coronary artery disease. We found that the baseline mean dose dependent inhibition of platelet aggregation achieved in patients receiving the 600 mg loading dose was well tolerated, and the same response was shown by 300 mg dose. The study data on whole-blood aggregometry provides direct evidence of decreased platelet aggregation, and confirming that 600 mg and 300 mg are equally effective and comparable.

CONCLUSION

This study concludes that there is no significant difference in loading dose of 600 mg or 300 mg of clopidogrel on platelet aggregation in patients with stable coronary artery disease.

Limitations of study:
The number of patients studied is relatively small and selection of coin tossing method resulted in uneven sample size in both groups. However, as each patient served its own control, therefore we conclude that any bias due to large inter-patient variation in values is minimized. Platelet aggregation as measured by Electrical Impedance measurement was the surrogate end point and not the clinical events; the results therefore, do not conclusively prove the clinical efficacy of these dosages. The clinical efficacy of clopidogrel has already been amply demonstrated and the methodology employed is standard and validated worldwide. We suggest that this study merit a follow up with larger randomized and well designed clinical trials.

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REFERENCES

1. Sabatine MS, McCabe CH, Gibson CM, Cannon CP. Design and rationale of Clopidogrel as Adjunctive Reperfusion Therapy (CLARITY)-thrombolysis in Myocardial Infarction (TIMI) 28 trial. Am Heart J 2005; 149:227-33.

2. Quinn MJ, Fitzgerald DJ. Ticlopidine and clopidogrel. Circulation 1999;100:1667-72.

3. CAPRIE Steering Committee. A randomized, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). Lancet 1996;348:1329-39.

4. Peters RJ, Mehta SR, Fox KA, Lewis BS, Copland I, Betrand ME, et al. Observation from the Clopidogrel in Unstable Angina to Prevent Recurrent Ischemic Events (CURE study). Effects of clopidogrel alone and in addition to aspirin in patients with acute coronary syndromes without ST segment elevation. Circulation 2003;108:1682-3.

5. Yasuda T, Gold HK, Leinbach RC. Lysis of plasminogen activator-resistant platelet-rich coronary artery thrombus with combined bolus injection of recombinant tissue-type plasminogen activator and anti platelet GPIIb/IIIa antibody. J Am Coll Cardiol 1190;16:1728-35.

6. Patrono C, Coller B, FitzGerald GA, Hirsh J, Roth G. Platelet-active drugs: the relationships among dose, effectiveness, and side effects: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. Chest 2004;126:234S-64S.

7. Hevert JM, Dol F, Bernat A, Falotico R, Lale A, Savi P. The antiaggregating and antithrombotic activity of clopidogrel is potentiated by aspirin in several experimental models in the rabbit. Thromb Haemost 1998;80:512-8.

8. Riess H, Braun G, Hiller E. Critical evaluation of platelet aggregation in whole blood. Am J Clin Pathol 1986;85:50-6.

9. Sweeny JD, Hoernig LA, Michnik BS, Fitzpatrick JE. Whole blood aggregometry: influence of sample collection and delay in study performance on test results. J Clin Pathol 1989;92:676-9.

10. Ashraf T, Ahmad M, Talpur MS, Kundi A, Masood A, Faruqui et al. Competency profile of locally manufactured clopidogrel Lowplat and foreign Manufactured Plavix in patient suspected of Ischemic heart disease. J Pak Med Assoc 2005;55:445-8.

11. Mary A, Seth W, Nicholas JV. Rapid assessment of platelet function with a modified whole-blood aggregation in percutaneous transluminal coronary angioplasty patients receiving anti-GP IIb/IIIa therapy. Circulation 1997;96:3860-6.

12. Bertrand ME, Rupprecht HJ, Urbain P. Double-blind study of the safety of clopidogrel with and without a loading dose in combination with aspirin compared with ticlopidine in combination with aspirin after coronary stenting: the clopidogrel aspirin stent international cooperative study (CLASSICS) Circulation 2000;102:624-9.

13. Yusf S, Zhao , Mehta SR. For the Clopidogrel in Unstable Angina to prevent Recurrent Events Trial Investigations. Effects of clopidogrel in addition to aspirin in patients acute coronary syndromes without ST-segment elevation. N Eng J Med 2001;345:494-502.

14. Mehta SR, Yusuf S, Peters RJG. For the Clopidogrel in Unstable angina to prevent Recurrent Events trial (CURE) Investigations. Effects of pretreatment with clopidogrel and aspirin followed by long term therapy in patients undergoing percutaneous coronary intervention: the PCI-CURE study. Lancet 2001;358:527-33

15. Steven R, Steinhubl, Peter B. For the CREDO Investigations Early and Sustained Dual Oral Antiplatelet Therapy Following Percutaneious Coronary Intervention: A Randomized Controlled Trial. JAMA 2002;288:2411-20.

16. COMMIT/CCS-2 (Clopidogrel and Metoprolol in Myocardial Infarction Trial), ACC's 54 Annual scientific sessions in Orlando, Florida, 9 March 2005.

17. Marc SS, Chirstopher PC, Michael GC.CLARITY-TIMI Clopidogrel as Adjunctive Reperfusion Therapy-Thrombolysis In Myocardial Infarction Study 28. N Engl J Med 2005;352:12.

18. Markus HS,Droste DW,Kaps M,Larrue V,Kennedy R,Siebler M,et al.Dual antiplatelet therapy with clopidogrel and aspirin in symptomatic carotid stenosis evaluated using Doppler embolic signal detection.Circulation2005;111:2233-40.

19. Yusuf S, Mehta SR, Zauf F, Fox KA, Lewis BS, Betrand ME, et al. Observation from the Clopidogrel in Unstable Angina to Prevent Recurrent Ischemic Events(CURE study). Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST segment elevation. Circulation 2003;25:996-72.

20. Iris M, Melchior S, Silja R. Effect of High Loading Dose of Clopidogrel on Platelet Functions in Patient's undergoing Coronary Stent Placement. Heart 2001;85:92-3.

21. Iris M, Felicitas B, Christan S. Prevalence of clopidogrel non-responders among patients with stable angina pectoris schedule for elective coronary stent placement. Thromb Haemost 2003;89:78-7.

22. Von Benckerath N, Taubert D, Pogatsa-Murray G, Schomig E, Kastrati A, Schomig A. Absorption. Metabolism and antiplatelet effect of 300, 600 and 900mg loading doses of Clopidogrel. Result of the ISAR-CHOICE. Circulation 2005;112:2946-50.

23. Muller I, Seyfarth M, Rudiger S, Wolf B, Pogasta-murray G, Schomig A, et al. Effect of a high loading dose of clopidogrel on platelet function in patients undergoing coronary stent placement. Heart 2001;85:92-3.