Original Article

Competency Profile of Locally Manufactured Clopidogrel Lowplat and Foreign Manufactured Clopidogrel Plavix in Patients of Suspected Ischemic Heart Disease (CLAP-IHD)

Tariq Ashraf[1], Munir Ahmed[1], M. Saeed Talpur[1], Asadullah Kundi[1], Azhar Masood A. Faruqui[1], Abdul Hafeez Jaffery[1], Aslam Fareed[2]

National Institute of Cardiovascular Diseases[1] and Aga Khan University[2], Karachi, Pakistan.

Abstract

Objective: The primary objective of this study was to test the hypothesis that the antiplatelet effects of loading dose of locally manufactured clopidogrel Lowplat referred as drug (B) 600 mg (8 tablets) given once is comparable to the antiplatelet effects of loading dose of foreign manufactured clopidogrel Plavix referred as drug (A) 600 mg (8 tablets) given once in patients with suspected ischemic heart disease.

Methods: This was a double blind, randomized, cross over, study, to compare the safety and efficacy of study drug (B) versus (A) in adult subjects suffering from suspected ischemic heart disease presented at National Institute of Cardiovascular Disease (NICVD), Karachi.

Results: Mean platelet aggregation inhibition by drug (B) was 60.7% ($p<0.001$), while with drug (A) it was 57.8% ($p<0.001$), using 20 µmol/L ADP, which is statistically significant and comparable. Clopidogrel 600 mg as loading dose was well tolerated.

Conclusion: Both drugs were equally effective in reducing the platelet aggregation. CLAP-IHD confirmed that drug (B) and (A) are equally effective and comparable antithrombotics in Pakistani population. The cost benefit of drug (B) should be made beneficial to the patients (JPMA 55:443;2005).

Introduction

Platelets provide the initial haemostatic plug at sites of vascular injury. The first step in the formation of this temporary clot begins with platelet adhesion. After adhesion and recruitment of additional platelets to the site of injury, activated platelets undergo a number of changes that result in platelet aggregation, a process that allows platelet, to adhere together and form a plug at the site of injury.[1-3]

The thienopyridine derivatives, ticlopidine and clopidogrel, are antiplatelet agents that inhibit platelet aggregation induced by adenosine diphosphate, thereby reducing ischemic events.[4]

There have been several randomized trials of antiplatelet drug in patients with disorders in which platelet activation was involved.[5] Their purpose was to determine the extent of reduction in various subsequent risks; in particular, risks of ischemic stroke, myocardial infarction, and death from vascular disease (vascular death). Patients at increased risk of such outcomes included those with atherothrombotic disease such as transient ischemic attacks or mild stroke, moderate or severe stroke, unstable angina, acute and remote myocardial infarction and atherosclerotic peripheral arterial disease, also include POBA and PCI as acquired causes of vascular trauma, predisposing to platelet aggregation and thrombosis.[6]

Aspirin and NSAIDs inhibit platelet cyclooxygenase, thereby generating blockade in the formation of thromboxane A2. These medications produce a systemic bleeding tendency by impairing thromboxane dependent platelet aggregation and consequently prolonging bleeding time.[7]

Clopidogrel is an inhibitor of platelet aggregation selectively inhibits the binding of adenosine diphosphate (ADP) to its platelet receptor and the subsequent ADP-mediated activation of the glycoprotein GPIIb/IIIa complex, thereby inhibiting platelet aggregation. Clopidogrel acts by irreversibly modifying the platelet ADP receptor. Consequently, platelets exposed to clopidogrel are affected for the remainder of their lifespan.

Dose dependent inhibition of platelet aggregation can be seen 2 hours after single oral doses of Clopidogrel 75 mg. Repeated doses of 75 mg Clopidogrel per day inhibit ADP-induced platelet aggregation on the first day, and inhibition reaches steady state level between Day 3 and Day 7.[8] Number of studies use loading dose of Clopidogrel up to 600 mg, resulting in rapid and pronounced inhibition of ADP induced platelet aggregation inhibition.[9,10]

Clopidogrel has been shown to effectively inhibit platelet aggregation and is at least as effective as aspirin in preventing cardiac events in patients with atherosclerosis.

Combined aspirin with clopidogrel therapy is superior to aspirin alone in reducing thrombotic events after stent placement.[11]

Clopidogrel is available in the international market with the brand name of drug (A), and is being widely used and has high cost (more then 10 times as compared to local brands). Recently many companies have launched local manufactured clopidogrel at economical price.

Many studies have shown substantial reduction in the thrombotic complications particularly acute and subacute stent thrombosis using aspirin and clopidogrel combination. Thus it would be pertinent to evaluate the safety and efficacy of this new brand in Pakistani population and observe the cost effective comparison. It was interesting to know that in Pakistan there were no such tests and/or facilities available to assess the aggregation inhibition by antiplatelet drugs. For this study state of the art equipments and/or reagents were imported from United States of America, and one of the local manufactured Clopidogrel, drug (B) was compared with foreign manufactured Clopidogrel drug (A).

Patients and Methods

This was a double blind, randomized, cross over, comparative study, to compare the safety and efficacy of drug (B), Lowplat, versus drug (A) Plavix, in adult subjects suffering from suspected ischemic heart disease presenting at National Institute of Cardiovascular Disease (NICVD), Karachi.

Thirty five subjects were enrolled in this study. Subjects who met the entry criteria were cases with suspected ischemic heart disease (IHD), stable cases of IHD who had stopped treatment and were willing to be included in the study and gave written consent to participate. All patients were randomized to Group A or B.

Exclusion criteria included patients with acute coronary syndrome, uncontrolled hypertension, severe renal or hepatic insufficiency, a history of bleeding disorder, or abnormal white or red blood cell, platelets and haemoglobin counts, also patients not taking antiplatelet and anticoagulant therapy.

In group A, patients took drug A in treatment period 1 and drug B in treatment Period 2, while in group B, patients took Drug B in treatment period 1 and drug A in treatment period 2.

Patients were subjected to routine laboratory screening tests e.g., ECG, CP, LFTs, Urea, Creatinine, electrolytes.

Those who met the entry criteria were randomized and baseline platelet aggregation study of each patient was performed on Chronolog aggregometer. Thereafter each patient received a loading dose of 600 mg (8 tablets) of either drug (B) or (A) (according to the randomization group) to be swallowed in the presence of study coordinator. After 24 hours, a 2nd blood sample was collected to determine the antiplatelet effect of the study drug and other laboratory tests mentioned earlier were also carried out. A two weeks washout period[12] was given to the patients before repeating the same procedure with the second drug in treatment period 2.

The trial was conducted from September to October, 2004 at NICVD.

Table. Baseline characteristic of the patients at time of enrolment.

Baseline Characteristics	Values
Total no of Patients	35
No of patients completed the trial	30
Mean Age	49 Years
Men / Women	21 / 9
Hypertension	11
Diabetes Mellitus	3
Smokers	3
MI	2
Stroke	1

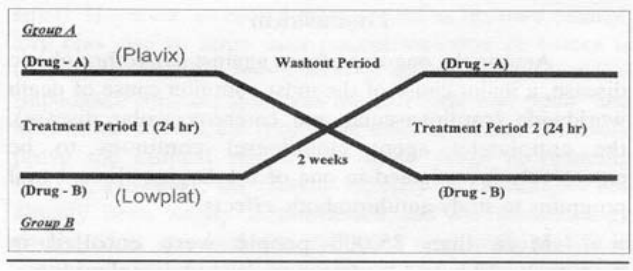


Figure 1. Crossover study design.

Equipment and Reagents

The whole-blood platelet aggregometer and supplies needed for performing whole-blood aggregation (i.e. reagents, cuvettes, stir bars, micropipettes, tips etc) were purchased from Chrono-Log Corp. Isotonic saline was from Otsuka and Vacuette tubes, blood collecting adaptor, multi sample needles were acquired from Greiner bio-one.

For the study commercially available tablets of drug (B) and drug (A) were directly purchased from the local pharmacy. After taking the baseline blood samples the study coordinator dispensed 600 mg (8 tablets) of drug (B) or (A) depending on the randomization group in the treatment period 1, patients took study medication orally in the presence of study coordinator, and after washout period the same procedure was repeated in the treatment period 2 with the second drug.

Concomitant medication needed for the treatment of underlying diseases or conditions were continued with the exception of antiplatelets/anticoagulants.

Platelet Aggregation Test

Currently available methods for assessing platelet function (e.g. light transmittance aggregometry) were developed primarily to detect inherited and acquired platelet abnormalities and are not readily adaptable to a point-of-care setting. Major limitations of the current light transmittance (turbidimetric) platelet aggregation assays are the multicomponent equipment requirements, the relatively long time required to perform these analysis, and the need for technologists experienced in preparation of PRP and cell counting techniques. In contrast, electrical impedance aggregometry requires no cell separation and minimal preparation time (only 1:1 dilution of blood with saline and a 5-minute incubation before the initiation of the assay) and is an FDA approved clinical method for evaluating platelet function.[13-15] The 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.[16]

Impedance aggregation can be completed 30 minutes after a blood sample is obtained, and the method provides accurate results up to 3 hours.[17,18] Comparison of turbidimetric and impedance aggregation responses on blood samples from healthy donors show a good correlation between the two techniques.[19]

Electrical Impedance Aggregation Assessment Study

Blood samples were collected immediately before administration of study drug on day 1 of treatment period 1 and 2 and on day 2 (24 hours after the administration of study drug) of treatment period 1 and 2, blood was drawn 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.

Platelet Aggregation Measurements

Impedance Method

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.[20]

An aliquot of whole blood (0.5 ml) was diluted with an equivalent volume of isotonic saline and incubated for 5 minutes at 37^{0}C. The impedance of each sample was monitored at sequential 1-minute intervals until a stable baseline established. After a stable baseline was established, 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 be multiplied by 100.[18]

The study was conducted in accordance with the ethical principles stated in the most recent version of the Declaration of Helsinki and the applicable guidelines on good clinical practice.

Statistical Evaluation

Paired T -test was used to detect the difference between pre and post treatment for both drugs

Both drugs were given to all patients in group A and Group B in following sequence:

Group A - Drug A followed by Drug B after the wash out period of 2 weeks.

Group B - Drug B followed by Drug A after the wash out period of 2 weeks.

Mean reduction in platelet aggregation by drug (A) was 57.8% (P<0.001) and standard deviation was 31.4%. Mean reduction in platelet aggregation by drug (B) was 60.7% (P<0.001) and standard deviation was 30.8%.

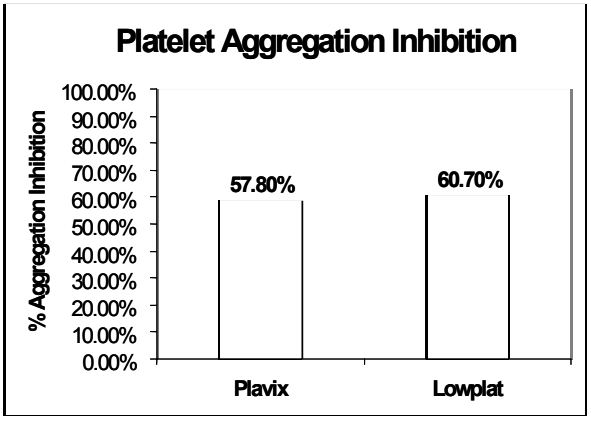
The Mean difference in platelet aggregation by both drugs was only 2.9% which was statistically insignificant (p=0.715). The 95% confidence interval for the difference between both drugs was 2.9% ± 15.5%

This showed that both drugs were equally effective in reducing the platelet aggregation in all patients.

Results

In this cross over study design the patients were divided into 2 groups i.e. Group "A" and Group "B" and were given Drug A + B according to the study protocol.

The mean reduction in platelet aggregation by drug (A) was 57.8% (P<0.001) and by drug (B) was 60.7% (P<0.001). This showed that both drugs were equally effective in reducing the platelet aggregation in all patients.



igure 2. Platelet Aggregation Inhibition.

A total of 35 patients were enrolled in this randomized cross over trial. One patient was dropped from the study due to low platelet count, while 4 patients did not return for follow-up visits. Thirty patients completed the study (n=30).

Only four patients complained of minor adverse reactions like headache, dizziness, burning sensation, weakness, pain, sense of warmth, vertigo, sweating and fatigue. The 600 mg loading dose of clopidogrel was well tolerated, notably without any increased incidence of bleeding.

There was no significant difference observed in the values of routine lab tests performed pre and post therapy for both groups.

Discussion

Among the ongoing efforts against atherothrombotic disease, a major cause of the most common cause of death worldwide (cardiovascular and cerebrovascular disease), the antiplatelet agent clopidogrel continues to be extensively investigated in one of the largest clinical trial programs to study antithrombotic effects.

More than 85,000 people were enrolled in previously published landmark trials studying clopidogrel alone or in combination with aspirin, such as CAPRIE[4], CLASSICS[21], CURE[22] (including PCI-CURE[23]), CREDO[24], COMMIT/CCS-2[25], and CLARITY-TIMI 28.[26] These studies have enabled the international medical community to better understand the potential role of clopidogrel alone and in combination with aspirin in reducing events (heart attack and stroke) caused by atherothrombotic disease, although aspirin is the treatment of choice in atherothrombotic disease, however it is given in combination with clopidogrel in patients with post PCI, post CABG, unstable angina/NSTEMI. The ongoing clopidogrel clinical trials are designed to further evaluate the role of clopidogrel in reducing these

manifestations and the potential benefits derived from continued clopidogrel therapy.

The present study sought to investigate the antiplatelet effect of 2 brands of clopidogrel namely drug (B) and (A) in Pakistani patients with suspected IHD. Compared to baseline mean dose dependent inhibition of platelet aggregation achieved by approximately 57-60% ($p<0.001$) in patients receiving the 600 mg loading dose and was well tolerated. Comparing our results with those of other randomized studies on clopidogrel confirm the outcome of the study.[8-10]

The study data on whole-blood aggregometry provides direct evidence of decreased platelet aggregation, while confirming drug (B) and (A) as equally effective and comparable antithrombotics in Pakistani population.

Antiplatelet therapy has immense importance particularly post PCI and CABG. In most instances outcome of intervention depends on regular intake of prescribed drugs over a long period of time. To enhance compliance of our patients while considering their socio-economic condition, it is advisable to prefer where possible locally manufactured quality drugs over expensive foreign brands, as at a cost of 3 tablets of drug (A) our patients whole months need could be fulfilled by locally manufactured drug (B).

While the study fulfils the objectives set by the study protocol for this project there remain limitations. Being a pilot study, the number of patients studied is relatively small. However, as each patient served as his own control, 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 clinical events, the results therefore do not conclusively prove the clinical efficacy of these drugs in reducing MACE. However, the clinical efficacy of clopidogrel has already been amply demonstrated and the methodology employed is standard and validated worldwide and is in routine clinical practice and research usage. The purpose of this study was only to compare the efficacy of two different brands of the same drug with vast difference in price. The result of this pilot study therefore are of definite relevance and merit a follow-up with larger clinical trials the matter of cost and quality of product are both critical in a developing country like Pakistan. The study supports the conclusion that despite very large difference in the cost of the two products, the efficacy of platelet aggregation appears to be no different.

Conclusion

Mean dose dependent inhibition of platelets aggregation achieved by Clopidogrel drug (B and A) is approximately 57-60% ($p<0.001$) in patients received Clopidogrel 600 mg as loading dose and was well tolerated. Study results are in accordance with other randomized studies on Clopidogrel confirmed the out come of this study.

CLAP-IHD confirms that drug (B) and (A) as equally effective and comparable antithrombotics in Pakistani Population.

The equivalent clinical benefit demonstrated by drug (B) and (A) in CLAP-IHD study suggest that; drug (B) is as safe and as effective as drug (A) and can be used with full confidence in patients who require antiplatelet therapy.

Acknowledgements

The authors are indebted to Prof. Hasina Thawerani, Mr. Jalaluddin Qureshi and Ms. Masooma Bano of NICVD for providing invaluable support of laboratory services.

We appreciate the substantial assistance of Dr. Asim Awan in the study and PharmEvo (Pvt) Ltd, for sponsoring the study.

References

1. George CM, kayser Sr, Adler DS. Thromboembolic disease in; Herfindal ET, Hirshman, jl, Eds. Clinical pharmacy and therapeutics.6th edition. Baltimore. MD; Williams and Wilkins 1996, pp. 959-72.
2. Diaz Linares M, Rodvold KA, Friedenberg WR. Coagulation disorders. In; Dipiro jt, Talbert RT, Eds Pharmacotherapy, a Pathophysiology Approach 4th ed. Stamford, ct; Appletion and Lange 1999, pp. 1549-72.
3. Easton JD. Evidence with Antiplatelet Therapy and ADP-Receptor Antagonist.Department of Neurology, Brown University, Providence, R.I., USA. Cerebrovasc Dis 2003;16 Suppl 1:20-6.
4. CAPRIE steering committee. A randomized, blinded, trial of clopidogrel versus aspirin at risk of ischemic events (CAPRIE). Lancet 1996;348;1329-39.
5. Antiplatelet Trialist Collaboration. Collaborative overview of randomized trials of antiplatelet therapy, Prevention of death, myocardial infarction and stroke by prolonged antiplatelet therapy in various categories of patients. BMJ 1994;308:81-106.
6. The Clopidogrel in Unstable Angina to Prevent Recurrent Events Trial Investigators. Effects of clopidogrel in addition to aspirin in patients with Ischemic heart diseases without ST-segment elevation. N Engl J Med 2001; 345:494-502. [Erratum, N Engl J Med 2001;345:1506.
7. Born GVR. Quantitative investigations into the aggregation of blood platelets. J. Physiol Lond 1962;162:67-
8. Clopidogrel product monograph, May 2003.
9. 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.
10. Iris M, Felicitas B, Christian S. Prevalence of Clopidogrel Non-Responders Among Patients With Stable Angina Pectoris Schedule for Elective Coronary Stent Placement. Thromb Haemost 2003;89:78-7.
11. Yan BP, Clark DJ, Ajani AE. Oral antiplatelet therapy and percutaneous coronary intervention. Expert Opin Pharmacother 2005;6:3-12.
12. Harber LA, Bruno JB: Ticlopidine Mechanism of Action On Human Platelets. Has WK, Easter JD (Eds): Ticlopidine, Platelets and Vascular Disease, New York, Springer-Verlag 1993, pp. 41-59.
13. Sweeny JD, Hoernig LA, Michnik BS, Fitzpatrick JE. Whole blood platelet aggregation predicts in vitro and in vivo haemostatic function in the elderly. Arterioscler Thromb Vasc Biol 1995;15:748-53.
14. Ingerman-Wojenski CM, Silver MJ. Quick method for screening platelet dysfunctions using the whole blood lumi-aggregometer. Thromb Haemost. 1984;51:154-6.
15. Cardinal DC, Flower RJ. The electronic aggregometer: A novel device of assessing platelet behavior in blood. J Pharmacol Meth 1980;3:1350-8.

16. Riess H, Braun G, Hiller E. Critical evaluation of platelet aggregation in whole blood. Am J Clin Pathol 1986;85:50-6.
17. 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.
18. Chronolog user manual, 2004.
19. CERI, Health care product comparison system 1999;8:16.
20. Mary A, Seth W, Nicholas J. V. Rapid assessment of platelet function with a modified whole-blood aggregometer in percutaneous transluminal coronary angioplasty patients receiving anti-GP IIb/IIIa therapy. Circulation 1997;96:3860-6.
21. Bertrand ME, Rupprecht H-J, Urban 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.
22. Yusuf S, Zhao F, Mehta SR. For the Clopidogrel in Unstable Angina to Prevent Recurrent Events Trial Investigators. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. N Engl J Med 2001;345:494-502.
23. Mehta SR, Yusuf S, Peters RJG. For the Clopidogrel in Unstable angina to prevent Recurrent Events trial (CURE) Investigators. 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.
24. Steven R. Steinhubl, Peter B. For the CREDO Investigators Early and Sustained Dual Oral Antiplatelet Therapy Following Percutaneous Coronary Intervention: A Randomized Controlled Trial JAMA 2002;288:2411-20.
25. COMMIT/CCS-2 (ClOpidogrel and Metoprolol in Myocardial Infarction Trial), ACC's 54 Annual Scientific Sessions in Orlando, Florida, 9 March 2005.
26. Marc SS, Christopher PC, Michael GC. CLARITY - TIMI 28 (CLopidogrel as Adjunctive ReperfusIon TherapY - Thrombolysis In Myocardial Infarction Study 28). N Engl J Med 2005;352:12.