

## EVALUATION OF CLOPIDOGREL IN INHIBITION OF PLATELET AGGREGATION

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### ABSTRACT

**Objective:** Evaluation of In-vivo therapeutic effects of Clopidogrel 75mg (Lowplat) given once daily for seven days to the patients requiring antiplatelet therapy.

**Methodology:** This is an open label, multicenter study to determine the platelet aggregation inhibition of the study drug in adult subjects suffering from diseases requiring antiplatelet therapy i.e. coronary artery diseases (CAD), peripheral vascular diseases (PVD) and cerebro vascular accident (CVA), presented at different hospitals and clinics in Faisalabad.

**Results:** Mean platelet aggregation inhibited by (LP) was 66% ( $P < 0.001$ ) and standard deviation was  $\pm 10\%$ , which is statistically significant.

**Conclusion:** This study proves that the Lowplat (LP) is effective in reducing platelet aggregation significantly in Pakistani patients' who require antiplatelet therapy. The cost benefit of locally manufactured drug may be passed on to the patients.

**KEYWORDS:** Clopidogrel, Antiplatelet therapy, Platelet Aggregation inhibition.

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## INTRODUCTION

Platelets provide the initial hemostatic 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 at 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.<sup>1-3</sup> The thienopyridine derivatives, ticlopidine and clopidogrel are antiplatelet agents that inhibit the platelet aggregation induced by adenosine diphosphate, thereby reducing ischemic events.<sup>4</sup>

There have been several trials of antiplatelet drug in patients with disorders in which platelet activation was involved.<sup>5</sup> 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 Plain old balloon angioplasty (POBA) & Percutaneous coronary interventions (PCIs) as acquired causes of vascular trauma, predisposing to platelet aggregation & thrombosis.<sup>6</sup>

Aspirin and NSAIDs inhibit platelet cyclooxygenase, thereby generating blockade in the formation of thromboxane A<sub>2</sub>. These medications produce a systemic bleeding tendency by impairing thromboxane dependent platelet aggregation and consequently prolonging bleeding time.<sup>7</sup>

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 two hours after single oral doses of Clopidogrel 75mg, repeated doses of 75mg Clopidogrel per day inhibit ADP-induced platelet aggregation on the first day, and inhibition reaches steady state level between Day three and Day seven.<sup>8</sup> Clopidogrel is available in international market which has very high cost (more than 10 times as compared to local brands). Recently many companies in Pakistan have launched local brands, thus it would be pertinent to evaluate the platelet aggregation inhibition of this new brand in Pakistani population and observe the cost effective comparison. It was interesting to know that in the entire Pakistan there were no such tests and/or facilities available to assess the aggregation inhibition by antiplatelet drugs. For this study modern equipments were

imported from United States of America, and one of the locally manufactured Clopidogrel (LowPlate or LP), was evaluated.

## METHODOLOGY

In this study Lowplat (LP) brand of Clopidogrel was used. This is an open label, multicenter study to evaluate the platelet aggregation inhibition by LP in adult subjects suffering from diseases requiring antiplatelet therapy. Inclusion criteria was patients with coronary artery diseases (CAD), peripheral vascular diseases (PVD) & cerebrovascular accident (CVA), male or female of age 18 years or above who presented at different hospitals and clinics in Faisalabad.

Fourteen investigators specialized and experienced in cardiology, neurology and medicine participated in this trial. Subjects who met the inclusion criteria and gave consent to participate in the study, were referred to the central lab for the platelet aggregation study.

Exclusion criteria was patients with acute coronary syndrome, uncontrolled hypertension, severe renal or hepatic insufficiency, history of a bleeding disorder, antiplatelets or anticoagulants drugs, allergy or hypersensitivity with Clopidogrel. Pregnant females and those who are nursing were also excluded.

At baseline platelet aggregation study of each patient was performed on Chronolog aggregometer at central laboratory, thereafter each patient received commercially available tablets of (LP) 75mg/day for seven days. Study co-coordinator was responsible to dispense the study drug and record in the drug dispensing log and patient file.

A drug accountability log was maintained that contains documentation of the subject's dose, lot number, date of manufactured and expiry of the study drug. After the completion of the therapy all study medication containers/blisters that are used, partially used or unused was collected back from the patients for drug accountability and compliance. The study was conducted from February to April 2005. Baseline characteristic of the patients at time of enrolment are shown in Table-I.

Table-I: Baseline characteristic of the patients at time of enrolment

<i>Baseline characteristics</i>	<i>n</i>
Total no. of patients enrolled	106
No. of patients who completed the trial	57
Mean Age (Years)	54
Men / Women	33 / 24
Hypertension	40
DM	26
Smokers	16
CAD	11
Stroke	7

**Equipment and Reagents:** The whole-blood platelet aggregometer from Chrono-log Corp, USA was used in the study. All supplies needed for performing whole-blood aggregation (i.e. reagents, cuvettes, stir bars, micropipettes, tips etc) was from Chrono-Log Corp. Isotonic saline were from Otsuka. Vacuette tubes, blood collecting adaptor, multi sample needles were acquired from Greiner bio-one. 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 longer duration required to perform these analysis, and the need for experienced technologists in the 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.<sup>9-11</sup> This 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.<sup>12</sup>

Impedance aggregation can be completed 30 minutes after a blood sample is obtained, and the method provides accurate results up to three hours.<sup>13,14</sup> Comparison of turbidimetric and impedance aggregation responses on blood samples from healthy donors show a good correlation between the two techniques.<sup>15</sup>

**Electrical Impedance Aggregation Assessment Study:** Blood samples were collected immediately before administration of study drug on day one & on day eight after the treatment period, 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.<sup>19</sup>

An aliquot of whole blood (0.5ml) diluted with an equivalent volume of isotonic saline and incubated for five minutes at 37°C. The impedance of each sample was monitored at sequential one-minute intervals until a stable baseline established. After a stable baseline was established, the agonist ADP (20umol/L) was then added to the sample and aggregation was monitored for six 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 hundred.<sup>14</sup>

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:* The statistical analysis was carried out on SPSS, version 11. Paired T –test was applied to detect the significance of difference between pre and post treatment of the study drug. P value of less than 0.05 was considered to be significant.

## RESULTS

In this open label study 106 subjects who met the eligibility criteria were enrolled of which 57 completed the study. Most of the patients who dropped out were from outskirts of Faisalabad, they found it difficult to come back for post test evaluation. Mean reduction in platelet aggregation by Lowplat was 66%, mean  $\pm$  SD (56 – 76),  $P = < 0.001$ , which is statistically significant. There was no serious adverse event reported during the course of study.

## DISCUSSION

The present study sought to investigate the platelet aggregation effect of study drug in Pakistani patients requiring antiplatelet therapy. A repeated dose of LP 75mg/day for seven days inhibits the average platelet aggregation by 66%. Comparing our results with those of other international and national data on clopidogrel confirm the outcome of our study.<sup>8,17,18</sup> This study data on whole-blood aggregometry provide direct evidence of decreased platelet aggregation by LP, while confirming drug effectiveness as antithrombotics in Pakistani population.

Antiplatelet therapy has immense importance in CAD particularly post PCI, CABG and CVA, PVD. In most instances outcome of intervention depends on regular intake of prescribed drugs over long period of time, to enhance compliance of our patients while considering their socio-economic condition. As such it is advisable to prefer where possible

locally manufactured good quality generic drugs over expensive foreign brands.

*Limitations of the study:* Many patients were lost during follow-up. Observations of this study need to be further confirmed in studies in large number of patients.

## CONCLUSION

Mean dose dependent inhibition of platelets aggregation achieved by the study drug is 66%, mean  $\pm$  SD (56 – 76)  $p = < 0.001$  in patients who received Lowplat 75mg/ day and it was well tolerated. Study results are in accordance with other local and multinational studies on Clopidogrel which have confirmed the outcome of this study. It confirms that the drug used in this study clopidogrel (Lowplat) is an effective antithrombotic in Pakistani population. It further suggests that LP is safe and effective antiplatelet drug, which can be used with full confidence in patients who require antiplatelet therapy.

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