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Bioequivalence Study of Two Formulations of Clopidogrel Tablets under Fasting Conditions in Healthy Adult Subjects

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Abstract

Clopidogrel is an inhibitor of platelet activation and aggregation through the irreversible binding of its active metabolite to the P2Y12 class of Adenosine Diphosphate (ADP) receptors on platelets. This study compared the pharmacokinetics and safety profiles of a new generic formulation of clopidogrel tablets with those of the branded reference formulation in healthy subjects under fasting conditions. The bioequivalence study was conducted as an open-label, randomized, two-treatment, three sequence, three period, single dose, crossover, semi-replicate, bioequivalence study of Clopidogrel Coated Tablets, 75 mg of Caplin Point Laboratories Ltd., India and PLAVIX (Clopidogrel) Coated Tablets, 75 mg of Sanofi-Aventis de Chile S.A. in healthy, adult, human subjects under fasting conditions. An ultra-performance liquid chromatography method with mass spectrometric detection for the determination of Clopidogrel in K_2EDTA human plasma was developed and validated. The 90% confidence intervals for Ln-transformed pharmacokinetic parameters - C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were 90.09 - 125.12%, 85.23 - 111.62% and 82.93 - 104.30% respectively for Clopidogrel, where C_{max} is within the acceptable limit of widen range 73.18 – 136.65% as per the obtained Intrasubject Coefficient of Variation (ISCV) of reference formulation and AUC_{0-t} and $AUC_{0-\infty}$ are within the acceptance limit of 80.00 - 125.00%. Hence, based on the ln-transformed results of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$, it is concluded that the test product (Clopidogrel Tablets USP 75 mg of Caplin Point Laboratories Ltd., India) is bioequivalent to reference (PLAVIX [Clopidogrel Tablets 75 mg] of Sanofi-Aventis de Chile S.A.) in healthy, adult, subjects under fasting conditions.

Keywords: Clopidogrel, Bioequivalence, Fasting, Liquid Chromatography, Pharmacokinetics.

INTRODUCTION

Clopidogrel is a thienopyridine class inhibitor of P2Y12 ADP platelet receptors. Chemically it is methyl (+)-(S)-α-(2-chlorophenyl)-6,7-dihydrothieno [3,2-c]pyridine-5(4H)-acetate sulfate (1:1). The empirical formula of clopidogrel bisulfate is C16H16ClNO2S•H2SO4 and its molecular weight is 419.9. Clopidogrel is an inhibitor of platelet activation and aggregation through the irreversible binding of its active metabolite to the P2Y12 class of ADP receptors on platelets [1, 2]. Clopidogrel must be metabolized by CYP450 enzymes to produce the active metabolite that inhibits platelet aggregation. The active metabolite of clopidogrel selectively inhibits the binding of ADP to its platelet P2Y12 receptor and the subsequent ADP-mediated activation of the glycoprotein GPIIb/IIIa complex, thereby inhibiting platelet aggregation. This action is irreversible [3-5]. Clopidogrel is a prodrug and is metabolized to a pharmacologically active metabolite and inactive metabolites. The bioequivalence study was conducted as an open-label, randomized, two-treatment, three sequence, three period, single dose, crossover, semi-replicate, bioequivalence study of Clopidogrel Coated Tablets, 75 mg of Caplin Point Laboratories Ltd., India and PLAVIX (Clopidogrel) Coated Tablets, 75 mg of Sanofi-Aventis de Chile S.A. in healthy, adult, human subjects under fasting conditions. Study subjects were screened and enrolled in the study as per the Ethics Committee-approved protocol. A total of 42 subjects were enrolled, among them 40 subjects completed all the periods of the study. The concentrations of Clopidogrel in plasma samples were analyzed using a validated bio-analytical method. Healthy male literate volunteers of 18 to 45 years (both years inclusive) with BMI of 18.50 – 30.00 kg/m^2 and weight > 50 kg, who were found to be

healthy by medical history, vitals, and general clinical examination were included. The subjects were administered with a single oral dose of Test (T) or Reference product (R) with a washout period of 07 days. Safety was assessed from the screening period to the end of the study through clinical examinations, vital signs assessment, 12-lead Electrocardiogram (ECG), Chest X-ray, clinical laboratory parameters (e.g. Hematology, Biochemistry, Urine analysis, Serology tests), and monitoring subjects' well-being, symptoms and signs for adverse events.

The pharmacokinetic and statistical analysis of Clopidogrel was performed using the concentration data obtained from 40 subjects who completed all the periods of the study.

EXPERIMENTAL SECTION/MATERIAL AND METHODS

Numerous prior studies have assessed the bioequivalence of new generic clopidogrel compared to the reference product, Plavix tablets [6-9], or examined the antiplatelet effects of other salts [10, 11]. In this study, the bioequivalence of the test product, Clopidogrel Tablets USP 75 mg of Caplin Point Laboratories Ltd., India, and the reference product PLAVIX (Clopidogrel Tablets 75 mg), of Sanofi-Aventis de Chile S.A was compared in healthy, adult, male subjects under fasting conditions.

The following pharmacokinetic parameters were evaluated, Primary PK Parameters - C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$. Secondary PK Parameters - T_{max}, $T_{½}$, Kel, Vd, CL and ratio of AUC_{0-t}/ $AUC_{0-\infty}$.

The study was conducted in accordance with the Indian Council of Medical Research (ICMR) Ethical Guidelines for Biomedical Research on Human Subjects, The New Drugs and Clinical Trials Rules, International Council for Harmonisation - Good Clinical Practice (ICH – GCP) [12], and in compliance with the ethical principles enunciated in the Declaration of Helsinki. The study protocol was approved by an independent Ethics Committee. Informed consent was obtained from all the subjects before the study.

Study Objectives

The primary objective was to assess the bioequivalence between Clopidogrel Tablets USP 75 mg of Caplin Point Laboratories Ltd., India, and PLAVIX (Clopidogrel) Coated Tablets, 75 mg of Sanofi-Aventis de Chile S.A. in healthy, adult, human, male subjects under fasting conditions.

The secondary objective was to assess the safety and tolerability of the drug.

Overall Study Design and Plan – Description

The bioequivalence study is designed as an open-label, randomized, two-treatment, three-sequence, three-period, single dose, cross-over, semi-replicate, bioequivalence study.

A total of 42 subjects were selected, enrolled, and randomly assigned to one of the sequences of TRR, RTR, or RRT for study drug administration. All the protocol restrictions were followed by the subjects throughout the study.

The pharmacokinetic plasma samples were analyzed using a validated bio-analytical method. The drug concentration data of 40 subjects who completed all the periods were used for performing the pharmacokinetic and statistical analysis using Phoenix® WinNonlin® version 8.1 and SAS® version 9.4.

Study Design

This study was designed based on the known pharmacokinetic profile of the investigational product and generally accepted standards for the conduct of bio-equivalence study.

Alcohol breath analysis and Urine drugs screening were performed during check-in of each period.

42 subjects who met the eligibility criteria were enrolled and dosed with a single dose of test or reference product in sitting posture at a fixed time with 200 ± 02 ml of water at ambient temperature in each period. At least 12 hours pre-dose and 36 hours post-dose housing period was maintained for all subjects. Subjects were instructed to maintain the upright position for 04 hours post-dosing, except for the planned study activities and during natural exigencies. Subjects were restricted from consumption of water 01 hour before and 02 hours after dosing, as per the protocol, and were allowed to consume water ad libitum thereafter. Identical food was provided to the subjects at 04.00, 08.00, 12.00, 24.00, 28.00 and 32.00 hours post-dose. A washout period of 07 days was maintained between each treatment, to minimize any possibility of a carryover effect from preceding treatment. The blood samples were collected at pre-defined time intervals for the measurement of concentration and pharmacokinetic parameters of Clopidogrel in each period.

Data obtained from subjects, who completed the study were used for pharmacokinetic and statistical analysis of Clopidogrel as per the protocol. Bioequivalence was determined by statistical comparison of Ln-transformed data of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of the test and reference formulations.

Treatments Administered:

After an overnight fasting of 10 hours, subjects were administered with a single oral dose of either test product or reference product with 200 ± 2 ml of water as per the randomization schedule in sitting posture at ambient temperature in each period.

Test product (T): Clopidogrel Tablets USP 75 mg of Caplin Point Laboratories Ltd., India Reference product (R): PLAVIX (Clopidogrel Tablets 75 mg) of Sanofi-Aventis de Chile S.A.

Treatment Compliance

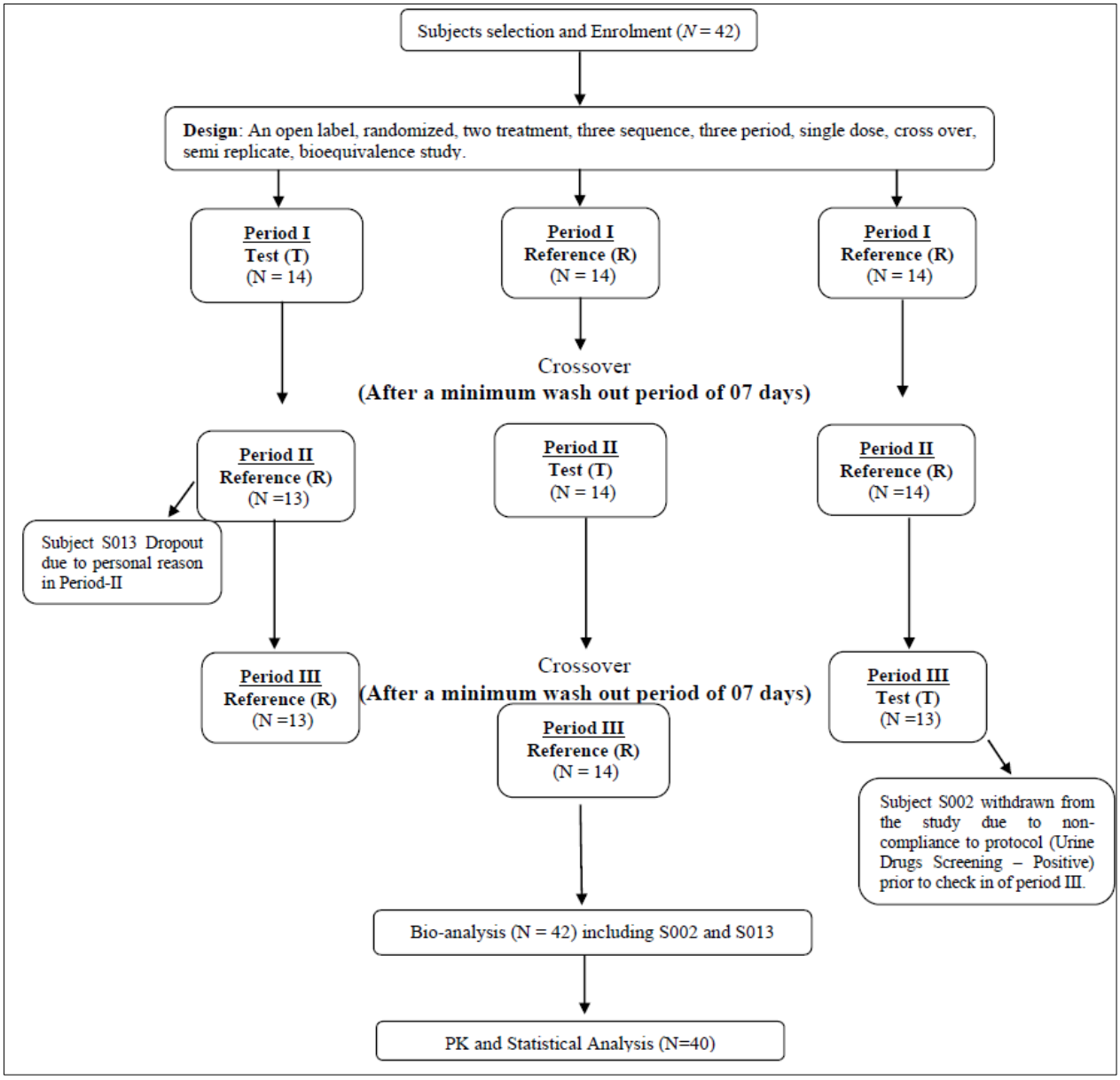
Dosing was performed by trained personnel according to the randomization schedule under the supervision of Investigators/Quality Control/Quality Assurance personnel in sitting posture at ambient temperature.

All the subjects maintained a sitting posture for 04 hours post-dosing except when required to change positions for planned study activities and natural exigencies. Water restriction of 01 hour prior to and 02 hour post dose was followed as per the protocol by all the subjects and were allowed to consume water ad libitum thereafter.

Drug Concentration Measurements

A total of 23 blood samples of 04 mL each at 00.00 (Pre-dose), 00.17, 00.33, 00.50, 00.67, 00.83, 01.00, 01.25, 01.50, 01.75, 02.00, 02.33, 02.67, 03.00, 03.50, 04.00, 05.00, 06.00, 08.00, 12.00, 16.00, 24.00 and 36.00 hours post-dose were collected for measurement of pharmacokinetic parameters in each period. All the samples were collected in the clinic.

Disposition of Subjects



RESULTS AND DISCUSSION

A total of 42 subjects were enrolled among them 40 subjects completed the study. Samples from subjects were analyzed for determining the concentrations of Clopidogrel. The drug concentration

data of 40 subjects who completed all the periods were included for pharmacokinetic analysis of Clopidogrel.

Demographic and Other Baseline Characteristics

The mean age, height, weight and Body Mass Index (BMI) of all the subjects who completed the study are presented in Table 1.

Table 1: Summarized Demographic Profile of subjects who completed the Study (N=40)

Parameter	Mean	SD	Minimum	Maximum
Age (Years)	33.2250	6.60803	21	44
Height (cm)	169.5375	5.67734	159.0	183.5
Weight (Kg)	70.8203	8.84756	52.30	88.22
BMI (Kg/m^2)	24.6445	2.89032	19.18	29.72

Pharmacokinetic Analysis

Pharmacokinetic parameters were calculated using Phoenix® WinNonlin® software (version 8.1). The mean, standard deviation, standard error, geometric mean, coefficient of variation, minimum, median, maximum and range were calculated for C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, T_{max}, K_{el}, Vd, CL, ratio of AUC_{0-t} / $AUC_{0-\infty}$, and $T_{1/2}$.

The summary of pharmacokinetic parameters for Clopidogrel of Reference (R) and Test (T) product is given below in Table 2, and Table 3 respectively.

Table 2: Summary of Pharmacokinetic Parameters for Clopidogrel of Reference Product (R)

Parameter	N	Reference (R) (Mean ± SD)
C_{max} (pg/mL)	80	2359.2337 ± 2920.17652
AUC_{0-t} (hr*pg/mL)	80	3818.2462 ± 4905.94381
$AUC_{0-\infty}$ (hr*pg/mL)	80	4046.9563 ± 4966.15766
$^{\#}T_{max}$ (hr)	80	0.8300 (0.330 - 5.000)
$T_{½}$ (hr)	80	10.2932 ± 32.71524
K_{el} (1/hr)	80	0.2050 ± 0.19770
CL (mL/hr)	80	48016452.6770 ± 45616309.03391
Vd (mL)	80	371800683.6906 ± 790584531.57063
Ratio of $AUC_{0-t}/AUC_{0-\infty}$	80	0.9300 ± 0.12696

#Expressed in terms of median (range)

Table 3: Summary of Pharmacokinetic Parameters for Clopidogrel of Test Product (T)

Parameter	N	Test (T) (Mean ± SD)
C_{max} (pg/mL)	40	2538.4478 ± 3780.21175
AUC_{0-t} (hr*pg/mL)	40	4222.4873 ± 6519.48659
$AUC_{0-\infty}$ (hr*pg/mL)	40	4365.5457 ± 6599.04766
$^{\#}T_{max}$ (hr)	40	0.9150 (0.500 - 3.000)
$T_{½}$ (hr)	40	4.6352 ± 2.99281
K_{el} (1/hr)	40	0.2484 ± 0.23476
CL (mL/hr)	40	55175116.4856 ± 57348611.72829
Vd (mL)	40	217824682.0609 ± 131942630.13581
Ratio of $AUC_{0-t}/AUC_{0-\infty}$	40	0.9520 ± 0.04663

#Expressed in terms of median (range)

Statistical Analysis

For Clopidogrel, analysis of variance (ANOVA) was performed on the Ln-transformed data of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ using PROC GLM of SAS® (version 9.4) software.

The analysis of variance model included sequence, period and treatment (formulation) as fixed effect. The sequence effect was tested at the 0.10 level of significance. All other main effects were tested at the 0.05 level of significance against the residual error (mean square error/MSE) from the ANOVA as the error term using 90% confidence interval approach.

Based on comparisons of the test and reference product for Ln-transformed C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ data, the ratio of the least square mean was calculated, as well as the 90% confidence intervals for Ln-transformed C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were determined.

Sequence effect was insignificant for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ with respect to p - value 0.4621, 0.3287 and 0.4236 respectively.

Period effect was insignificant for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ with respect to p -value 0.0598, 0.5430 and 0.9030 respectively.

Treatment (Formulation) effect was insignificant for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ with respect to p - value 0.5458, 0.7589 and 0.2956 respectively.

All fixed effects in the ANOVA model were statistically insignificant (i.e. p - value > 0.05 for the period effect and treatment effect and > 0.10 for sequence effect) for Clopidogrel.

The ANOVA was performed on the Ln-transformed C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ parameters. The least - square mean ratios, 90% confidence intervals and intra-subject CVs were also determined for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of Clopidogrel for test and reference products.

The statistical results of Test Product (T) versus Reference Product (R) for Clopidogrel is given below in Table 4.

Table 4: Statistical Results of Test Product (T) versus Reference Product (R) for Clopidogrel

Parameters	Geometric Least Square Mean		T/R Ratio (%)	90% Confidence Interval	Intra subject CV (R Vs R) (%)
	Test Product (T)	Reference Product (R)			
Ln (C_{max})	1379.8795	1299.7110	106.17	90.09%-125.12%	42.9
Ln (AUC_{0-t})	2133.5174	2187.4334	97.54	85.23%-111.62%	37.4
Ln ($AUC_{0-\infty}$)	2245.1622	2414.0012	93.01	82.93%-104.30%	38.6

The Linear and Semilog Plot of Mean Plasmatic Clopidogrel Concentration vs Time points are provided in Figures 1 & 2 respectively.

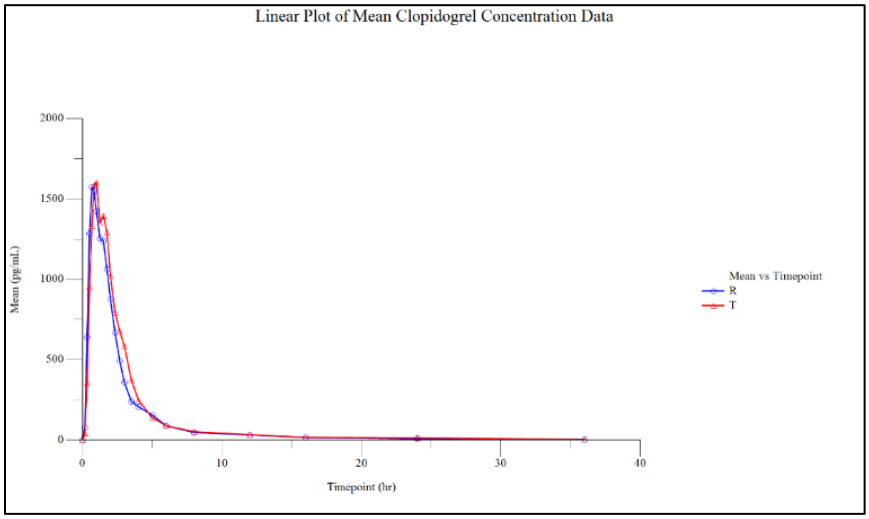


Figure 1: Linear Plot of Mean Plasmatic Clopidogrel Concentration vs Time points (N=40)

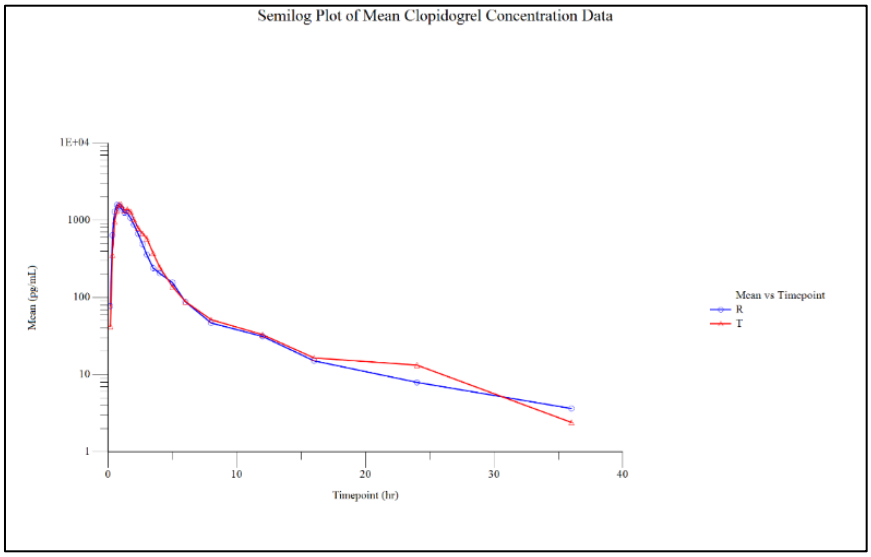


Figure 1: Semilog Plot of Mean Plasmatic Clopidogrel Concentration vs Time points (N=40)

CONCLUSION

The 90% confidence intervals for Ln-transformed pharmacokinetic parameters - C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were 90.09 - 125.12%, 85.23 - 111.62% and 82.93 - 104.30% respectively for Clopidogrel, where C_{max} is within the acceptable limit of widen range 73.18 – 136.65% as per the obtained ISCV of reference formulation and AUC_{0-t} and $AUC_{0-\infty}$ are within the acceptance limit of 80.00 - 125.00%. Based on the results obtained with the present study, it is concluded that the products Test (Clopidogrel Tablets USP 75 mg of Caplin Point Laboratories Ltd., India) in the form of a single tablet and reference PLAVIX (Clopidogrel Tablets 75 mg) of Sanofi-Aventis de Chile S.A. in the form of a single tablet, are bioequivalent under fasting conditions.

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