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Research Article

PLATELET INHIBITORY ACTIVITY AND PHARAMCOKINETCS OF PRASUGREL A NOVEL THIENOPYRIDINE P_2Y_{12} INHIBITOR: A SINGLE DOSE CROSS OVER BIOEQUIVALENCE STUDY IN HEALTHY HUMAN VOLUNTEERS

Sahu Nimain Charan*, P. Sri Divya, S. Ramachandran, MD. Dhana Raju

Department of Pharmacology, GIET School of Pharmacy, Rajahmundry, AP, India

*Corresponding Author Email: snimain@yahoo.com

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ABSTRACT

To compare the bioavailability and bioequivalence of two prasugrel formulations one as a test and the other was the standard. The study was performed according to a randomized, open label, balanced, two-treatment, two-period, two-sequence, single-dose, crossover under fasting period with minimum of seven days wash-out period and was evaluated in 20(+ 2 stand by) subjects. To analyse pharmacokinetic properties, the blood samples were drawn taken up to 36 h after dosing. Plasma concentration of prasugrel was determined using liquid chromatography – tandem mass spectrometry method. Pharmacokinetic parameters tmax, Cmax, AUC0-t, AUC0-∞, t1/2 and λz (Kel) were tested for bioequivalence after log-transformation of data and non-parametric evaluation was done for ratios of tmax. The point estimates and 90 % confidence intervals (CI) for AUC0-t, AUC0-∞, and Cmax for active metabolite (R-138727) were 95.82-105.18, 96.00-104.69 and 90.80-103.20 respectively. These results indicated that the two formulations of Prasugrel were bioequivalent in case of active metabolite (R-138727), thus may be prescribed interchangeably.

Keywords: Prasugrel; Antiplatelet; Bioequivalence and Bioavailability; LC-MS / MS

INTRODUCTION

Bioavailability, Systemic bioavailability, physiological bioavailability, biological availability all these terms are interchangeable and denote the measurement of the rate and extent (total amount) of the drug that reaches the systemic circulation following the administration of a dosage form. Bioavailability means the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action. The pharmacokinetic (Pk) parameters by which we can assess bioavailability are rate, $t_{max,}$ C_{max}, extent, AUC. Bioequivalence studies are the preliminary requirement for generic products to enter in the market. Bioavailability (BA) and Bioequivalence (BE) studies provide important information in the overall set of data that ensure the availability of safe and effective medicines to patients and practitioners. Prasugrel is a thienopyridine prodrug that is metabolized *in vivo* to an adenosine diphosphate (ADP) receptor antagonist, which is a potent inhibitor of ADP-induced platelet aggregation[1-4]. Prasugrel co administered with aspirin is under investigation for the prevention of atherothrombotic events in patients with acute coronary syndrome[5] (myocardial infarction or unstable angina) who undergoes percutaneous coronary intervention (PCI). Thienopyridines block the ADP P_2Y_{12} receptor on platelets and offer additive effects when given with aspirin because of their differing but complementary mechanism of action. Like clopidogrel, prasugrel is a thienopyridyl prodrug. The active metabolite of prasugrel (R-138727), a sulfhydryl compound, binds covalently and irreversibly to the platelet P_2Y_{12} receptor via a disulfide bond.[6] Because of this irreversible binding of the active metabolite, there is permanent blockade of ADP-mediated P_2Y_{12} signalling and inhibition of both glycoprotein IIb / IIIa receptor activation and platelet aggregation.[7] Prasugrel produces inhibition of platelet aggregation to 20 μM or 5 μM ADP, as measured by light transmission aggregometry.[5] Although the activation / metabolism of both prasugrel and clopidogrel involves some of the same Cytochrome peroxidase (CYP) enzyme systems the potential for pharmacokinetic interactions differs between prasugrel and clopidogrel. For example, competition for CYP 3A4 may explain why the antiplatelet effect of clopidogrel is reduced when given concomitantly with atorvastatin or dihydropyridine calcium channel blockers (CCBs). Concomitant administration of dihydropyridine CCBs and clopidogrel also increased the risk of an adverse cardiovascular outcome (adjusted hazard ratio [HR] = 3.5, 95 % CI = 1.4) Prasugrel is rapidly and extensively metabolized to the active metabolite (R-138727) and several inactive metabolites. Metabolism to R-138727 is primarily via CYP 3A4 and CYP2B6. Exposure to the active metabolite and clinical outcomes are not affected by CYP 2C19 and 2C9 polymorphism. Rapid conversion of the parent drug to active metabolite (median time to peak plasma concentration is approximately 30 minutes. Dosage: The regimen for prasugrel typically used in clinical trials has been a 60-mg Loading dose (LD) followed by a 10-mg / d maintenance dose (MD) [8-9]. Median elimination half-life of the active metabolite is approximately 7.4 h. Excretion is primarily urinary (approximately 70 %) and faecal excretion is < 30 %. Food effect: Fasting administration preferred; C_{max} is reduced by 49 % and t_{max} delayed 0.5-1.5 h when administered with high-fat high calorie meal, although area under curve (AUC) is unaffected. Bioavailability is unaffected by food**.**

Safety Profile of Prasugrel

It is not surprising that prasugrel, which has more potent anti platelet effects than clopidogrel, may cause increased bleeding in clinical use. The phase II PRINCIPLE-TIMI 44 study (thombolysis in myocardial infarction), in which the primary end point was anti platelet activity, included 201 patients undergoing PCI who were randomized to prasugrel at a loading dose of 60 mg then 14 days of maintenance therapy with 10 mg per day or clopidogrel 600 mg then 150 mg per day. After 14 days, patient crossed over to the alternate anti platelet agent. During the loading dose and pre-

crossover phase, 19 of 102 (18.6 %) patients receiving prasugrel and 14 of 99 (14.1 %) randomized to clopidogrel had any type of bleeding event (not significant). Prior to crossover, 2 patients (2.0 %) in the prasugrel group experienced a thombolysis in myocardial infarction TIMI minor bleeding episode compared with no patients in the clopidogrel group. After crossover, 4 patients who received clopidogrel followed by prasugrel experienced a hemorrhagic adverse event (TIMI major or minor bleeding) compared with no patients who received prasugrel followed by clopidogrel. No TIMI major bleeding events were observed through day 29 of this study.

Objectives of the Study

To assess the bioavailability and safety of drug Prasugrel (10 mg) tablets compare with reference product (10 mg tablets) in 20 (+ 2 stand by) healthy human adult male subjects under fasting condition.

Study Design

The present study was a randomized, open label, balanced, two-treatment, two-period, two-sequence, single-dose, cross over under fasting period with minimum of seven days wash-out period and was evaluated in 20 (+ 2 stand by) subjects.

Inclusion Criteria and Exclusion Criteria

Healthy human male subjects between 18-45 years weighing at least 50 kg and with a body mass index (BMI) of 18.0 kg / m^2 and ≤ 25.0 kg / m^2 were included in the study. Only those individuals willing to sign the informed consent form were included. Subjects were selected after passing a clinical screening procedure including a physical examination, ECG and clinical laboratory tests (hemoglobin, hematocrit, WBC, platelets, WBC differential, blood urea nitrogen, sGPT, sGOT, alkaline phosphatase, total bilirubin, total protein, fasting glucose, albumin, creatinine, urine analysis, pregnancy test (for female subjects) and negative results of HBsAg, anti HBC and anti HIV. Subjects were excluded if they had a history of any illness of the hepatic, renal and cardiovascular system, took alcohol or other medications for a long period of time, had hypersensitivity to Prasugrel, had received any investigation drug within four weeks (or suitable longer period for slowly eliminated drugs) of enrolment and donation or loss more than 450 ml of blood within 3 months prior to the screening of the study. Subjects with systolic blood pressure less than 90 mm of Hg and with diastolic blood pressure greater than 140 mm of Hg were excluded in the study.

METHODOLOGY

The protocol study was approved by Drugs Controller General of India and reviewed by Institutional Review Board and Independent Ethical Committee. This study was conducted in compliance with the ethical principles of the Declaration of Helsinki for biomedical research involving human volunteers and Good Clinical Practice (GCP). All participants signed a written informed consent after they had been informed of the nature and details of the study. The study was based on a randomized, open label, balanced, two-treatment, two-period, two-sequence, single-dose, crossover under fasting period with minimum of seven days wash-out period was evaluated in 20 (+ 2 stand by) subjects. Subjects were randomized to one of the two sequences to receive the formulations according to randomization scheme. The test preparation was 10 mg of Prasugrel tablets, manufactured by Panacea biotec limited and the reference formulation was 10 mg Effient® tablets, produced by Eli Lily and Company. The sample size n = 20 (+ 2 stand by) subjects was sufficient to ensure power of 80 % for correctly concluding bioequivalence under the following assumption: a = 0.05, $0.95 < \mu T / \mu R < 1.05$ and an intra-subject variability of 20 %. A total of 20 subjects (males) were selected and participated in this study. The demographic data of twenty volunteers are shown in Table 1. All subjects were avoided using other drugs for at least two weeks prior to the study and after its completion. They were also refrained from ingesting alcohol, caffeine, chocolate, tea or coke containing beverages at least 48 h before each dosing and until the collection of the last blood sample. Subjects were confined to clinical unit of Sipra Labs one night before study to assure the fasting condition (10 h before drug administration). On the study day, subjects were given one tablet of either product with 240 ml of water. No food was allowed until 4 h after dose administration. Water intake was allowed 1 h after the dose. Standard meals were served at 4 and 11 h after drug administration. Snack was served at 8 h after drug administration. Subjects were remained upright (sitting or standing) for the first 4 h. Subjects were confined at clinical unit of Sipra Labs for 24 h after dosing and were not permitted to take strenuous exercise during the sampling days. Blood pressure, heart rate, body temperature and adverse events were monitored during blood sampling. 5 ml of the venous blood were collected at pre dose, 0.17, 0.33, 0.50, 0.67, 0.83, 1.00, 1.25, 1.50, 2.00, 3.00, 4.00, 6.00, 8.00, 10.00, 12.00, 16.00, 24.00 and 36.00 h after drug administration in the heparinised tubes. After blood separation, plasma was frozen at -20°C until analysis. After two weeks wash out period, subjects returned to Sipra Labs and the blood sample analysis was repeated in the second period in the same manner to complete the crossover design.

Safety Evaluation

There were no serious adverse events observed in the study.

LC-MS / MS Assay of Prasugrel Active Metabolite (R-138727) and in Plasma

The concentration of Prasugrel active metabolite (R-138727) in plasma was determined using LC-MS / MS method. Rilpivirine was used as the internal standard. The method has already been validated in terms of selectivity, sensitivity, linearity, accuracy and precision, recovery, stability, and also has been verified just before being used in study. The standard calibration curves for Prasugrel were ranged from 1-500.19 ng / mL. The best linear fit and least-squares residual for the calibration curve were achieved with 1 / x 2 weighing factor. The analytical separation (97:3), pumped at 0.6 mL / min for 3.0 minutes run time. The column temperature was maintained at 40°C. Briefly, a 250 µL of human plasma in microtube was added with 20 µL of internal standard (10 ppm). After mixing, 250 µL of acetonitrile was added and vortex mixed for 30 seconds. The mixture was centrifuged at 3000 rpm for 10 minutes. A volume of 5 µL supernatant was injected into LC-MS / MS system.

Pharmacokinetic and Statistical Analysis

The bioequivalence was determined using the primary parameters, AUC_{0-t}, $AUC_{0-\infty}$, C_{max}. C_{max} and t_{max} were obtained directly by inspection of the individual drug plasma concentration time data, and were used as measures of rate of absorption. AUC_{0-t} was calculated using the trapezoidal rule.

The elimination rate constant (Kel) was calculated by the technique of least-squares regression from the data of the last 3-5 points of each plasma concentration data curve. The $AUC_{0-\infty}$ values were determined by adding the quotient of Ct and the appropriate Kel to the corresponding AUC_{0-t}, that is:

$$AUC_{0-\infty} = AUC_{0-t} + Ct / Kel$$

The apparent elimination half-life ($t_{½}$) of Prasugrel in plasma was calculated by using the following equation:

$$t_{½} = (\ln 2) / Kel$$

For the parameters of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} a multiplicative model was assumed, and analysis of variance (ANOVA) was applied using the respective in-transformed data. For estimation of bioequivalence the 90 % CI of the geometric mean ratio test / reference (T/R) for AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} were calculated assuming a multiplicative model. The accepted bioequivalence range for these parameters was 80-125 %. The statistical analyses of least square means (LSM) of test and reference product were performed using WinNonlin Pro® Software version 5.3(Phargist, USA) or above.

RESULTS

Both Prasugrel formulations were well-tolerated at the administered dose and no significant adverse clinical events were observed. There were no serious adverse events. A total of 20 subjects participated in this study and all the subjects were available for pharmacokinetic evaluation. The Prasugrel concentration $v_{s.}$ time profiles of 20 subjects for both formulations is shown in Supplementary Figure 1. The pharmacokinetic parameters that are used to assess the bioequivalence of the test formulation versus the reference were AUC_{0-t}, $AUC_{0-\infty}$ for the extent of the absorption and C_{max} and t_{max} for the rate of absorption. Descriptive statistics of the pharmacokinetic parameter for Prasugrel test and reference are summarized in Table 3 where the geometric mean values and the range for the AUC_{0-t}, $AUC_{0-\infty}$, C_{max} and $t_{½}$ values obtained for each formulation are shown. The pharmacokinetic characteristic t_{max} was presented as mean values. The mean obtained values for test and reference formulations for test and reference formulations in case of active metabolite are shown in the Table 4. The ratio analysis of untransformed data and difference of log transformed primary pharmacokinetic parameters [Cmax, AUC (0-t) and AUC (0-inf)] for Prasugrel (active metabolite) for test and reference formulation were calculated. Arithmetic least square mean (LSM) ratio was found to be 99.56, 100.5, and 100.03 for C_{max}, AUC $_{(0-t)}$ and AUC $_{(0-inf)}$ respectively. The geometric least square mean (LSM) ratio was found to be 96.80, 100.39 and 100.25 for C_{max}, AUC $_{(0-t)}$ and AUC $_{(0-inf)}$ respectively. 90 % confidence intervals C_{max}, AUC $_{(0-t)}$ and AUC $_{(0-inf)}$ were 90.80-103.20, 95.82-105.18, and 96.00-104.6 for log-transformed data respectively.

Table 1: **Summary Data for the Subjects Enrolled in the Study (20 + 2 subjects)**

	Age (year)	Weight(kg)	Height (cm)	BMI (kg / m^2)
Mean	30.50	165.93	59.03	21.37
SD	6.62	7.01	7.69	2.06
CV %	21.70	4.23	13.02	9.62
Median	32.00	165.75	56.65	21.45
Minimum	20.00	153.50	50.00	18.20
Maximum	41.00	178.50	73.80	24.80

Table 2: Parameters of Active Metabolite (R-138727)

Parameter	Test Product	Reference Product
t_{max} (h)	0.59	0.67
C_{max} (µg / ml)	7.2857	7.3182
AUC_{0-t} (µg.h / ml)	7.8361	7.322
$AUC_{0-\infty}$ (µg.h / ml)	7.8798	7.8773

Table 3: Summary Statistics of Untransformed Pharmacokinetic Parameters for Prasugrel in 20 Healthy, Human, Adult Subjects

PK Parametrs	Test Product (T)		Reference Product (R)	
C_{max}(µg / ml)	150.02	347.16	1526.16	241.88
AUC_{0t}(µg.hr / ml)	2621.27	674.32	2603.72	643.40
$AUC_{0-\infty}$(µg.hr / ml)	2731.95	679.44	2720.67	661.96
$t_{1/2}$(h)	11.68	4.51	12.01	4.95
$K_{el}(h^{-1})$	0.07	0.03	0.07	0.03
	Median			
T_{max}(h)	0.59		0.67	

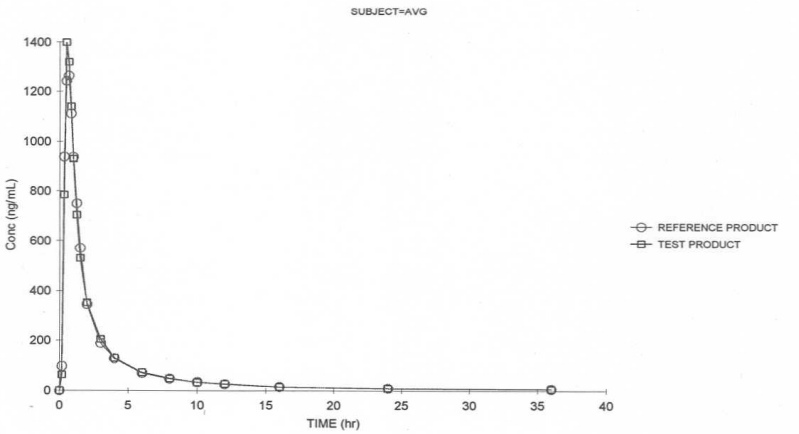
Table 4: Summary Statistics of Log-Transfromed Pharmacokinetic Parameters for Prasugrel in 18 Healthy Human Adult Subjects

Product / Statistics	C_{max}(µg / ml)	AUC_{0-t} (µg.h / ml)	$AUC_{0-\infty}$ (µg.h / ml)
Product T Geometric mean	1429.2722	2530.2079	2643.2997
N	20	20	20
Product R Geometric mean	1507.4504	2520.4386	2636.6607
N	20	20	20
Anti-log of least square mean			
T	7.2857	7.8361	7.8798
R	7.3812	7.8322	7.8773
Geometric mean ratio of t / r (%)	96.80	100.39	100.25
90 % confidence interval	90.80-103.20	95.82-105.18	96.00-104.69
Power (%)	0.998	1.0000	1.0000
Intra-subject CV (%)	11.70	8.50	7.91

ACTIVE METABOLITE-R138727

FIGURE-1

Linear plot mean Plasma Prasugrel concentrations vs Time profile for 20 healthy, human, adult subjects



The results of the bioequivalence analysis for prasugrel are given in the Table 2 and 3. The intra-subject variability of Prasugrel in the AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, and $t_{½}$ estimates from the coefficient of variables as determined by ANOVA. As shown in Table IV, 90 % confidence intervals (CI) of AUC_t, $AUC_{0-\infty}$, C_{max}, and $t_{½}$ log-transformed individual ratios of Prasugrel were included into the range of bioequivalence, i.e. 80-125 % when analyzed by parametric statistics.[8] In the same way, individual t_{max} difference was not statistically different between the two formulations. The mean ratio of $AUC_{0-t}/AUC_{0-\infty}$ for all individuals and for both products indicate an adequate sampling time since the extrapolated portion of the total AUC is less than 20 %. The results for $t_{½}$ in the present study (11.68 ± 4.51 h for test product and 12.01 ± 4.95 h for reference product) were consistent with the results reported in the literatures.

DISCUSSION

During the past quarter of a century, huge advances have been made in our understanding of the Pathophysiology of ACS, and these advances have been accompanied by important breakthroughs in the management of this condition. Platelets play a crucial role in ACS, and newer anti platelet drugs continue to be developed with the goal of maximizing the reduction in atherothrombotic events while minimizing bleeding complications. Prasugrel is one of such drugs, which is an anti platelet agent whose mechanism of action is to irreversibly antagonizing the P_2Y_{12} class of platelet ADP receptor. The present study indicates clinical safety of prasugrel and its bioequivalence with effient® tablet. The study also indicates that prasugrel tablet is well-tolerated in the healthy human volunteers.

CONCLUSION

With the present results available, it may be regarded as prasugrel tablet is safe and well-tolerated in subjects with acute coronary syndrome. The application of either parametric or nonparametric statistics reveals the presence of bioequivalence between Effient® tablet produced by Eli Lilly and Company and Prasugrel tablet produced by Panacea biotech for the rate and extent of absorption. Thus, it can be assumed that the two formulations are therapeutically equivalent and therefore interchangeable.

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