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Research Article

Randomized, Open Labelled, Two-Treatment, Two-Period, Single Dose Crossover Bioavailability & Bioequivalence Study to Compare Two Formulations of Rabeprazole in Healthy Male Subjects

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ABSTRACT

A bioequivalence study of two enteric coated formulations (Rabeloc 20 mg, Cadila Pharmaceuticals Ltd., India & Pariet 20 mg, Janssen Pharmaceuticals, Belgium) of Rabeprazole was carried out on 24 healthy, adult male human subjects under fasting condition. It was an open labeled, balanced, randomized, two - treatment, two - period, two - sequence, single - dose crossover designed study. Both the treatments (either T or R) were administered according to randomization schedule to each subject in each period. Both the period were separated with wash out period of 7 days. After the dosing, the blood samples were collected at pre-decided time intervals for 24 hours. The plasma was separated and analyzed for the concentration of Rabeprazole sodium using a validated HPLC/LC-MS-MS method. Various pharmacokinetic parameters like C_{max}, T_{max}, $T_{1/2}$, AUC_{0-t} and AUC_{0-inf} were calculated from the plasma concentration-time profile of both formulations. Statistical analysis using two one sided *t* test and analysis of variance showed no significant difference in log transformed C_{max}, AUC_{0-t} and AUC_{0-inf}. The 90% confidence interval for AUC, C_{max} ratios were falling within the 80 - 125% for Rabeloc tablet with Pariet 20 mg tablet. Thus, result of the study concluded that the both the preparation of Rabeprazole were bioequivalent.

Keywords: Rabeprazole; Bioavailability; Bioequivalence; Pharmacokinetics

INTRODUCTION

Rabeprazole is a Proton Pump Inhibitor (PPI). Rabeprazole sodium is known chemically as 2-[[[4-(3-methoxypropoxy)-3-methyl-2-pyridinyl]-methyl] sulfinyle]-1 H-Benzimidazole sodium salt. It acts by binding covalently with the H^+K^+ ATPase pump on the gastric parietal cells and inactivates them thereby inhibiting the gastric acid production and thus raises the gastric pH [1]. In the conditions like Gastro Esophageal Reflux Disease (GERD), erosive GERD, dyspepsia, H Pylori infection, gastric as well as duodenal ulcer, the use of anti-secretory agents to obtain a sound control of gastric acidity becomes a compelling need. As compared to H2 receptor antagonists, PPI are found to be more dependably effective in controlling acid secretion [2].

The overall bioavailability of Rabeprazole is 52% which is not influenced either by food or antacids. The plasma protein binding of Rabeprazole is 94.8%-97.5%. It follows non enzymatic metabolic pathway which is the reason for the absence of drug-drug interactions between this PPI and other drugs, which are metabolized by the isoenzymes of the cytochrome P450. Amongst all the PPIs, rabeprazole has the highest pKa of ~5.0. As compared to all the PPIs, rabeprazole has the fastest onset of action. Within 5 minutes, it blocks almost all the active proton pumps completely [3]. The maximal plasma concentration are reached after approximately 3-4 hours of drug administration [4]. Rabeprazole produces significant, profound dose-related inhibition of gastric acid secretion [5]. It was shown in a study done on patients with renal dysfunction that pharmacokinetic profile of Rabeprazole is not affected in the patients with severe renal impairment and no adjustment in dose is required in such patients [6]. The rabeprazole C_{max} and AUC are linear over an oral dose of 10 or 20 mg are administered every 24 hours, the pharmacokinetics of rabeprazole is not altered by multiple dosing. The plasma half-life ranges from 1-2 hours [7]. The time to reach the C_{max} (t_{max}) is dose independent [8].

Rabeprazole is susceptible for degradation by gastric acid, so it is to be administered as enteric coated formulation [1]. Pariet [9] 20 mg tablets containing Rabeprazole 20 mg is commercially available preparation developed by the innovator Janssen Pharmaceutical, Belgium. Cadila pharmaceuticals Ltd., being the innovator of the intravenous formulation of rabeprazole, developed the oral tablets of the same in the MHRA, WHO, USFDA, Zimbabwe, Russia and Malaysia approved plant following strict GMP protocols.

The aim of the study was to compare the single dose oral bioavailability of Rabeloc 20 (containing Rabeprazole sodium 20 mg) tablet, with the innovator in 24 healthy, adult male human subjects under fasting condition and to monitor the safety of the subjects.

MATERIALS AND METHODS

Subjects

Twenty-six (24 + 2 stand by) healthy male adult subjects participated in the study. Weight, height and age of the each subjects were taken before enrollment. The volunteers were declared normal on the basis of medical history, examination, physical examination and laboratory investigations (hematological and urine analysis) done before enrollment in the study. None of the volunteers were having any acute or chronic gastrointestinal, respiratory, cardiovascular or CNS diseases. All the volunteers were instructed to abstain from any xanthine containing food or beverages or alcoholic products for 48 hours prior to dose administration and throughout sampling schedule during each period. They were free of all medication at least 15 days prior to study period and allowed no concomitant medication during the study session. Oral as well as written informed consent was taken from all the volunteers prior to commencement of the study.

Inclusion criteria: Subjects had to fulfill all of the following criteria to be considered for inclusion in to the study.

- The healthy male of 18-45 years of age who has voluntarily given written informed consent to participate in the study.
- Within ± 10% of ideal body weight in relation to height according to Life Insurance Corporation of India height-weight chart for non-medical cases.
- The results should be within normal range of laboratory values for hematology, clinical chemistry and urine analysis.
- The test results should be negative for Hepatitis B surface antigen, Hepatitis C antibody and HIV antibody.
- Subjects must be of normal health as determined by medical history and physical examination performed within 21 days prior to the commencement of the study.

Exclusion criteria: The subjects were excluded based on the following criteria:

- Subjects incapable of understanding informed consent.
- Subjects with blood pressure ≤ 90/ 60 or ≥ 140/ 90, history of hypersensitivity or idiosyncratic reaction to Rabeprazole or any of the excipients or with any evidence of medical or psychiatric illness.

- Regular smokers who smoke more than ten cigarettes daily, drug dependents and/or chronic alcoholics, who have difficulty in abstaining from smoking/drug/alcohol for the duration of the study period.
- Subjects who have taken any systemic medication within the past 15 days prior to the start of clinical period.
- Investigations with blood samples, biochemical analysis and urine samples not falling with in the normal reference ranges or showing the presence of disease markers in laboratory analysis or chest X-ray.
- Subjects who participated significance in any other clinical investigation using experimental drug or had bleeding of more than 350 ml in the past 3 months.
- Coffee or tea consumption > 6 cups/day or any amount within 48 hours prior to the study.

Study design

The study was open labeled, balanced, randomized, two - treatment, two - period, two - sequence, single - dose crossover bioavailability and bioequivalence study. A single oral dose of drug product was administered along with 200 ml of drinking water at room temperature (25.0 ± 4.0°C) and relative humidity (60.0 ± 20.0%) after an overnight fast of 10 hours. After 7 days of washout time post period-1 study, volunteers were given the other drug with the same protocol being followed.

The protocol and Informed Consent Form (ICF) used to obtain informed consent of the study subjects were reviewed by Independent Ethics Committee (IEC). The study was commenced only after obtaining the written approval of the study protocol by the IEC, with or without modification. The designated clinical research personnel informed the subjects (in English or Local language - Gujarati) before initiation of the study through an oral presentation regarding the purpose, procedures to be carried out, potential hazards and rights of the subjects during the course of study. The confidentiality of the name of the study subjects was maintained and is accessible only to the study personnel, quality assurance auditor during audits and if necessary, to the IEC and various regulatory authorities.

Blood sampling

Blood samples were collected by an indwelling cannula placed in a forearm vein in a pre-labelled test tubes containing sodium citrate buffer as the anticoagulant. The pre dose samples were collected within a period of one hour before dosing and post dose samples were collected at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 16.0 and 24.0 hours. As soon as possible after collection, blood samples were centrifuged at 2500 – 3000 rpm for 10 minutes to separate plasma. All the plasma samples were stored in properly labelled containers at -20°C or colder till analysis.

Analytical method

Samples from 24 subjects were analyzed for total active Rabeprazole in plasma using a validated HPLC/LC-MS-MS method. The sensitivity, linearity and validation studies of the method were conducted. During analysis, standard and quality control samples were distributed throughout each batch of study samples analyzed. Rabeprazole sodium was extracted from human plasma by a Liquid-liquid extraction by using 5 ml Tertiary Butyl methyl ether as an extracting solvent. The blinding was maintained with regard to the analyst during the course of analysis. This method is accurate and precise in the range of 45.157 ng/ ml to 1204.184 ng/ ml. Lansoprazole served as an internal standard in the analysis.

Pharmacokinetic analysis

Various pharmacokinetic parameters were calculated for Rabeprazole using WinNonlin-Pro Software (Pharsight. USA). Time of the maximum plasma concentration (T_{max}) and maximum measured plasma concentration over the specified span (C_{max}) were obtained directly from the experimental data. The area under the plasma concentration versus time curve from time zero to the last measurable concentration (AUC_{0-t}) was calculated by linear trapezoidal method. The area under the plasma concentration versus time curve from time zero to infinity (AUC_{0-inf}) was calculated as the sum of AUC_{0-t} plus the ratio of the last measurable concentration to the elimination rate constant. Apparent first order elimination or terminal rate constant (K_{el}) was calculated from a semi-log plot of the plasma concentration versus time curve. The elimination rate constant was calculated by linear least-square regression analysis using the last three (or more) non-zero plasma concentration. The elimination or terminal half-life ($T_{1/2}$) was calculated as 0.693/ K_{el}. No values of K_{el}, $T_{1/2}$ and AUC_{0-inf} were reported for the cases that did not exhibit a terminal log-linear phase in the concentration versus time profile.

Analysis of variance

The untransformed and log-transformed pharmacokinetic parameters (C_{max}, AUC_{0-t}, AUC_{0-inf}) were analyzed by an Analysis of Variance (ANOVA) including the effects for treatments, sequence of dosing, subjects nested within sequences and period of treatment and drug formulations as factors in the statistical model. An F-test was applied to determine whether the differences between treatments and period of treatment were statistically significant ($p < 0.05$). The ANOVA was performed for blood drug concentrations at each sampling time.

Confidence intervals

Consistent with the two one-sided "t" tests for bioequivalence, 90% confidence intervals for the difference between treatments, least-square means were calculated for log-transformed C_{max}, AUC_{0-t} and AUC_{0-inf}. The confidence intervals are expressed as a percentage relative to the LSM of the reference treatments. To be considered as bioequivalent, the 90% confidence intervals of these parameters should be in the interval 80-125% for the log-transformed data.

Ratio analysis

Ratios of means were calculated using the LSM for log-transformed C_{max}, AUC_{0-t} and AUC_{0-inf}. The geometric mean values were reported for the log-transformed parameters. Ratios of means are expressed as a percentage of the LSM for the reference treatment.

Power test

The power (i.e. probability of detecting a 20% differences relative to the references treatment LSM at the 5% significance levels using a t-test under null hypothesis of zero differences) was calculated for log transformed C_{max}, AUC_{0-t} and AUC_{0-inf}.

Bioequivalence criteria

Based on the statistical results of 90% confidence intervals of the means for log-transformed pharmacokinetic parameters C_{max}, AUC_{0-t} and AUC_{0-inf}, conclusions were drawn to whether the test product is

bioequivalent to the reference product, if the 90% confidence interval for C_{max}, AUC_{0-t} and AUC_{0-inf} of the test product falls within the acceptance range of 0.8–1.24 (80%-125%).

RESULTS

Twenty six healthy adult male subjects participated in the study. Weight of the subject was with in limit of 10% of the weight considering normal, considering the height and physical structure. Age of the subject was within range of 18 to 45 years. The 26 subjects ranged in age from 19 to 41 years (mean = 27.81 years), in weight from 43 to 80 kg (mean = 59.38 kg) and in height from 153.0 to 177.0 cm (mean = 165.73 cm). All 26 subjects participated in the period 1 of the study, but one subject (no. 11) didn't report for the period 2 of the study, therefore he was considered as lost to follow-up. Twenty five subjects had taken up in the second period of the study. The mean age of the subjects was 27.81 year whereas the mean weight and height of the subjects were 59.38 kg and 165.73 cm respectively.

Pharmacokinetic parameters (C_{max}, T_{max}, K_{el}, $T_{1/2}$, AUC_{0-t}, and AUC_{0-inf}) were calculated by non-compartmental analysis using a WINNONLIN_PRO pharmacokinetic software (Pharsight Corporation, USA). Pharmacokinetic parameters (C_{max}, T_{max}, K_{el}, $T_{1/2}$, AUC_{0-t}, and AUC_{0-inf}) for both the component of the drug product (i.e. Rabeprazole sodium) are summarized in table 1. Confidence Interval for pharmacokinetic parameters (C_{max}, AUC_{0-t}, and AUC_{0-inf}) after logarithmic transformation are shown in table 2. In this study, the two formulations under the study (i.e. Test and Reference) follow the same pattern in absorption, metabolism and elimination phases for Rabeprazole sodium 20 mg Tablet.

The descriptive statistical analysis of pharmacokinetic parameters (C_{max}, AUC_{0-t}, and AUC_{0-inf}) of Rabeprazole sodium Tablet is shown in table 3. The mean plasma concentration-time curves of Rabeprazole sodium tablet after administration of Rabeloc 20 mg and Pariet 20 mg Tablet, formulation are shown in figure respectively.

DISCUSSION AND CONCLUSIONS

Rabeprazole is the fastest acting PPI which provides a profound gastric acid inhibition. For a generic drug, it is of immense importance to be of bioequivalence to the reference molecule so that it could be assured that the various pharmacokinetic parameters of the drug are at par.

Table 1: Pharmacokinetic parameters.

Rabeprazole sodium 20 mg Tablet		
	Rabeloc 20 mg Tablet	**Pariet 20 mg Tablet**
C_{max} (ng/ ml)	340.279 ± 27.319	322.689 ± 29.877
T_{max} (hr)	2.00 ± 0.213	2.304 ± 0.208
K_{el} (hr^{-1})	0.812 ± 0.087	0.761 ± 0.085
$T_{1/2}$ (hrs)	1.114 ± 0.133	1.329 ± 0.211
AUC_{0-t} (ng/ ml/ hr)	561.767 ± 67.273	552.823 ± 67.350
AUC_{0-inf} (ng/ ml/ hr)	621.907 ± 69.078	622.299 ± 66.486

All values represent Mean ± SEM of twenty three subjects.

Table 2: Summary of confidence interval.

	Rabeprazole sodium 20 mg Tablet
C_{max}	97.27 ± 118.80
AUC_{0-t}	93.94 ± 110.79
AUC_{0-inf}	91.54 ± 106.14

Table 3: Summary statistics of Log-transformed pharmacokinetic parameters in formulation of Rabeloc 20 (Rabeprazole sodium 20 mg) tablet compared with that of Pariet 20 (Rabeprazole sodium 20 mg) after single dose administration in healthy human subjects.

Product/Statistics	**C_{max} (ng/ ml)**	**AUC_{0-t} (ng/ ml)**	**AUC_{0-inf} (ng/ ml)**
Rabeloc			
Geometric Mean	314.210	486.904	550.524
CV% of Geometric Mean	44.686	58.272	53.020
N	23	23	23
Pariet			
Geometric Mean	292.437	478.394	558.983
CV% of Geometric Mean	48.958	58.219	48.680
N	23	23	23
Ratio of Least squares mean (Rabeprazole sodium)			
Rabeloc/Pariet (%)	101.27	100.33	99.77
90% Confidence Interval (T/R) of Rabeprazole sodium from Rabeloc/ Pariet			
Lower Limit (%)	97.27	93.94	91.54
Upper Limit (%)	118.80	110.79	106.14
***p*-value (ANOVA)**			
Treatment	0.2295	0.7165	0.7260
Period	0.8422	0.2720	0.6624
Sequence	0.1456	0.1434	0.2325
Power: Rabeprazole sodium from Rabeloc *vs* Pariet (%)	97.70	99.60	99.89
CV% of Intra Subject Variability			
Rabeloc *vs* Pariet	8.54	7.05	6.32

The relative bioavailability was assessed by the ratio of C_{max} and AUC_{0-inf} values. The ratio of Least Square Means of C_{max}, AUC_{0-t} and AUC_{0-inf} were found to be 101.27%, 100.33% and 99.77% respectively for Rabeloc 20 mg (Rabeprazole sodium 20 mg) compared with that of Pariet 20 mg (Rabeprazole sodium 20 mg).

In a study, rabeprazole was shown to have C_{max} of 437 ng/ ml in fasted individuals, which is slightly higher than which was found in the present study. In the present study, the t_{max} was found to be 2 hours which is lesser than the study done by Yasuda et al. $t_{1/2}$ was comparable in both of the studies [10].

Standard Analysis of Variance (ANOVA) model of a 2 way crossover design with a general linear approach was applied to log-transformed pharmacokinetic parameters, (C_{max}, AUC_{0-t}, AUC_{0-inf}) to determine difference among the Test and Reference products. The model included the effects for treatments, sequence of dosing, subjects nested within sequences and period of treatment and drug products as factors. For each parameter, mean values of the test product (Rabeloc) were compared with the reference (Pariet) using with the type I error rate of 0.05. P value for C_{max}, AUC_{0-t} and AUC_{0-inf} were found to be 0.2295, 0.7165 and 0.7260 respectively. All of these values were not less than 0.05 indicating there was no significant difference between Rabeloc and Pariet.

The C_{max}, AUC_{0-t} and AUC_{0-inf} were further analyzed on a log scale to assess bioequivalence of test product and the reference product. The two one sided *t* test hypotheses for average bioequivalence were tested at the 0.05 level by constructing the 90% Confidence Interval (CI) of the ratio of the test: reference means. Ratio for C_{max}, AUC_{0-t} and AUC_{0-inf} are 101.27, 100.33 and 99.77 respectively which is again conclusive of Rabeloc 20 mg to be bioequivalent with Pariet 20 mg tablet. No toxic or adverse drug reactions were observed in human subjects during the course of study.

Since the 90% CI for AUC, C_{max} ratios were falling within the 80-125% for Rabeloc tablet with Pariet 20 mg tablet, it was concluded that the Rabeloc 20 mg tablet of Cadila Pharmaceuticals Ltd. India is bioequivalent in comparison with Pariet 20 mg tablet of Janssen Pharmaceutical, Belgium in terms of both rate and extent of absorption after single dose administration in healthy human subjects.

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