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Bioequivalence and Pharmacokinetic Study of Ranazoline in Healthy Male Volunteers: An Open label, Randomized, Single-Dose, Two-Way Crossover Study

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ABSTRACT

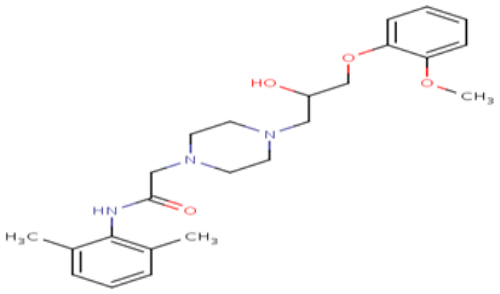
The present study was to assess the relative bioavailability and pharmacokinetic properties of extended release formulations of Ranolazine 1000 mg in healthy male volunteers using a randomized, open-label, balanced, two-treatment, two-period, two sequence, single dose, crossover, bioequivalence study under fasting conditions. Bioavailability of the test product of Ranolazine extended release tablets 1000 mg was compared with that of the reference product of Ranexa® (Ranolazine extended release tablets 1000mg) of CV Therapeutics Inc., California. The plasma samples were collected at 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 6.50, 7.00, 8.00, 10.00, 12.00, 16.00, 24.00 and 48.00 hours post dose after single administration of Ranolazine 1000mg. The plasma Ranolazine concentrations were estimated by using a validated bioanalytical method by LC-MS/MS. A ten day washout period is followed between two treatments. The formulations were considered to be bioequivalent if the 90% CIs for the log-transformed values were within the predetermined equivalence range 80%–125% for AUC and C_{max}. For Ranolazine, at 90% confidence intervals C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were 83.43-113.29, 82.10-102.87 and 80.94-101.85 for log-transformed data respectively. The present results show that the formulation of Ranolazine was bioequivalent to the reference in fasting, healthy, male volunteers.

Keywords: Bioavailability, Bioequivalence, Pharmacokinetics, Ranolazine

INTRODUCTION

Ranolazine, a piperazine derivative, used for the treatment of angina and also for its anti-ischemic effects, is an inhibitor of late sodium channel current and thus decreases sodium entry into ischemic myocardial cells Fig 1. As a consequence, ranolazine is proposed to reduce calcium uptake indirectly via the sodium/calcium exchanger. Ranolazine reduces the frequency of anginal attacks and increases exercise capacity in patients with chronic angina [1] [2] [3]. The drug can be used in combination with other antianginal drugs, which are ineffective individually [4] [5]. Recently extended-release ranolazine was also approved in the United States for the treatment of chronic angina.

Figure 1: Structure of Ranolazine



Ranolazine was patented in 1986 and is available in an oral form having both immediate release and extended release. But the immediate-release ranolazine is not in current use. Immediate release dosage form had an average terminal elimination

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half-life ranging from 1.4 to 1.9 hours and a 10-fold peak/trough difference with dosing of 240 to 400 mg 3 times per day [6]. For the extended release dosage forms the average terminal elimination half-life is 7 hours after multiple dosing to steady state, and the peak/trough difference is 1.6-fold with dosing of 500 to 1000 mg twice daily. The aim of this study was to evaluate the pharmacokinetic characteristics and the bioequivalence of the test formulation (Extended release tablet 1000mg) and the reference formulation Ranexa® (Ranolazine extended release tablets 1000mg) of CV Therapeutics Inc., California in healthy human male volunteers, in fasted state in order to determine whether any observed differences and exceeded regulatory guidelines for bioequivalence.

SUBJECTS AND METHODS

Subjects:

Twelve healthy, adult, male volunteers were selected for the study. Each volunteer was required to provide written informed consent for participation before the study. Medical history, physical examination, electrocardiography and various laboratory tests (hematology, blood biochemistry, hepatic function, and urinalysis) were carried out prior to the beginning of the study. Inclusion criteria included being within age 18 – 40 years and ideal body mass index. Exclusion criteria included smoking; the presence of heart, kidney, neurologic, or metabolic disease, a history of drug hypersensitivity and current pharmacologic treatment. Volunteers were instructed to adhere to a standard protocol and to refrain from administering any medication 1 week before and during the course of the study.

Study Design:

The study was carried out in accordance with the provisions of the current version of the ICH 'Guidance for Good Clinical Practices', ICMR 'Guidelines for Biomedical Research on Human Participants' and the principles enunciated in the Declaration of Helsinki (WMA General Assembly, Seoul, October 2008) [7] [8]. Study was conducted in a randomized, open-label, balanced, two-treatment, two-period, two sequence, single dose, crossover, bioequivalence study with 10 days washout period between the dose administrations. Subjects arrived at the study center the day before the study and fasted overnight before drug administration. According to the randomization, subjects were divided into 2 groups. During the first period, volunteers from group A received a single 1000 mg tablet of the reference product Ranexa® (Ranolazine extended release tablets 1000mg) of CV Therapeutics Inc., California while volunteers from group B received a single 1000 mg tablet of the test product (Ranolazine). The drug was administered with 250ml of water. After the 10 days washout, the alternate treatment was administered.

Blood Sampling Schedule:

In each period, 21, (1 x 5-mL) blood samples were collected. The predose blood sample (1 x 5-mL) was collected within 1 hour prior to dosing. The post-dose blood samples (1 x 5-ml each) were collected at 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 6.50, 7.00, 8.00, 10.00, 12.00, 16.00, 24.00 and 48.00 hours post dose. 48.00 hour sample time point was collected on ambulatory basis. For each subject the total number of blood samples drawn during the study was 42. The total volume of blood drawn including 15-ml for screening and 18-ml of blood discarded (i.e., 0.5 ml of blood is discarded via an indwelling cannula up to 12 hours) was not exceed 243-ml for the entire study. Plasma was immediately separated by centrifugation at 3000 RPM for 10 minutes at 4°C ± 2°C using Heareus Centrifuge.

Fasting/Meals:

All subjects were on fast overnight for a period of 10 hours before commencement of dosing. Drinking water was not allowed from one hour. Uniform and low fat meals were provided to all the subjects. A standard meal (lunch), snacks, and dinner, at four, eight and twelve hours, respectively was given after the drug administration.

Tolerability:

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Tolerability was assessed using vital signs (blood pressure, temperature, and heart rate) taken before dosing and approximately every 4 hours after administration in each study period. Subjects were interviewed during the study concerning the occurrence of adverse events.

Ranolazine quantification in Human Plasma:

The plasma Ranolazine concentrations were estimated by using a validated bioanalytical method LC-MS/MS using gliclazide as an internal standard as described by [9] [10]. The analyte and internal standard gliclazide were extracted from plasma by liquid-liquid extraction, using ethyl acetate and separated on hypersil BDS column (C18 5µm; 50L x 2.1mm I.D) using acetonitrile : ammonium formate (80:20 v/v), at a flow rate of 1.0ml/min. Detection is carried out by reaction monitoring on a Qtrap TM LC-MS-MS system. (MDS Sciex API - 4000). The injector volume was 30 µl and coloumn oven temperature maintained was 40°C.

Pharmacokinetic and statistical analysis:

The pharmacokinetic parameters like AUC0–t (area under the plasma concentration-time curve measured at t hours), $AUC_{0-\infty}$ (area under the plasma concentration-time curve measured at ∞ hours), AUC_{0-t}/ $AUC_{0-\infty}$, C_{max} (maximum observed drug concentration during the study), T_{max} (time to observe maximum drug concentration), K_{el} (apparent first-order terminal rate constant) and $T_{½}$ (terminal half-life) for Ranolazine were calculated using WinNonlin Pro® software version 5.2.1 (Pharsight, USA). C_{max} and T_{max} were determined directly from the respectively observed plasma concentration-time data. Analysis of variance (ANOVA) was performed (∞=O.05) on the Untransformed Pharmacokinetic parameters AUC_{0-t}, $AUC_{0-\infty}$, C_{max} and T_{max}. Additionally, Log-transformed data was used for analysis of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max}. The analysis of variance model included sequences; subjects tested within sequence, period and drug formulation as factors. Ratio analysis was reported for untransformed and log-transformed AUC_{1ast}, AUC_{inf} and $C_{max.}$ The geometric mean value was reported for log transformed data. For bioequivalence evaluation, in accordance with current FDA guidelines, the products were considered bioequivalent if the 90% Confidence Interval for Cmax, AUC_{0-t} and $AUC_{0-\infty}$ fell within the range of 80% to 125%.

RESULTS AND DISCUSSIONS

Quantification of Ranolazine:

The linear range of Ranolazine was 10-5000 ng/ml with correlation coefficient of 0.9937. The lower limit of quantification was found to be 10 ng/ml. Intra-assay precision was found to be between 4.6% and 10.7% and inter assay precision was between 5.5% and 11.1%. Intra-assay accuracy ranged from 91.06% to 98.43% and interassay accuracy from 98.81% to 103.52%.

Pharmacokinetic parameters:

The results of pharmacokinetic parameters of ranolazine such as C_{max} (ng/ml), T_{max} (hr), AUC_{0-t} (ng.h/ml), $AUC_{0-\infty}$ (ng.h/ml), $t_{1/2}$ (hr), K_{el} (h^{-1}), AUC_{0-t}/ $AUC_{0-\infty}$ for test and reference formulations were reported in Table 1. The profile of log mean plasma concentrations of Ranolazine versus time in subjects (n=12) for test product and reference product were given in Fig 2.

Table 1: Pharmacokinetic parameters after administration of 1000mg of ranolazine in test and reference formulations in 12 healthy male volunteers

	TEST		REFERENCE	
Variable	**Mean ± SD**	**CV (%)**	**Mean ± SD**	**CV (%)**
C_{max} (ng/ml)	8.131± 1584.698	46.63	8.159 ± 1873.800	48.4
T_{max} (hr	4.583 ± 1.294	28.23	4.625 ± 1.680	36.33
AUC_{0-t} (ng.h/ml)	10.521± 13486.313	36.38	10.605 ± 20931.630	51.89
$AUC_{0-\infty}$ (ng.h/ml)	10.597± 14366.320	35.90	10.694 ± 20872.458	47.36

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$t_{1/2}$ (hr)	11.353 ± 10.205	89.89	12.024 ± 4.890	40.67
K_{el} (h^{-1})	0.0886 ± 0.0450	50.86	0.0646 ± 0.0196	30.28
AUC_{0-t}/ $AUC_{0-\infty}$	93.85± 13.55	14.44	92.09 ± 10.18	11.05

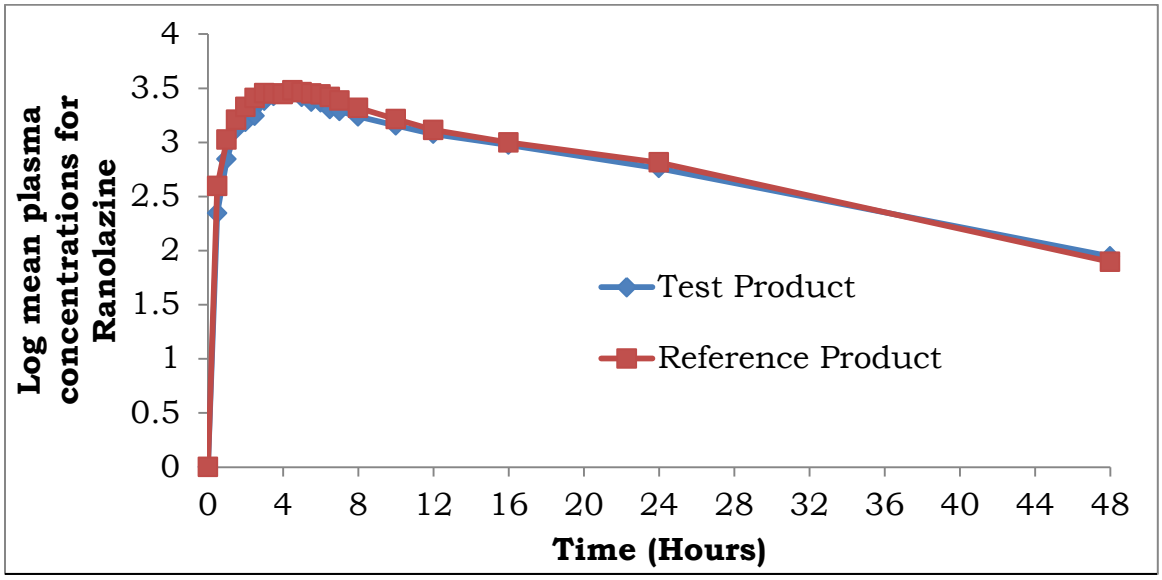

Figure 2: Log mean plasma concentrations of Ranolazine vs time profile for 12 subjects

Ranolazine least square mean ratios (C_{max}, AUC_{0-t} and $AUC_{0-\infty}$) were 97.22, 91.90 and 90.79 for log-transformed data respectively indicating a comparable Bioequivalence of test formulation to the reference formulation. Ranolazine 90% confidence intervals (C_{max}, AUC_{0-t} and $AUC_{0-\infty}$) were 83.43-113.29, 82.10-102.87 and 80.94-101.85 for log-transformed data respectively (Table 2). The 90% confidence intervals for the ratio of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ values for the test and reference were within the Bioequivalance acceptable range 80-125% for the log-transformed data as per the established regulatory guidelines.

Table 2: Summary statistics of LOG-Transformed Pharmacokinetic Parameters for Ranolazine in 12 Healthy Male volunteers

	Parameter	C_{max}	AUC_{0-t}	$AUC_{0-\infty}$
Test Product (T)	Geometric Mean	3398.508	37070.125	40016.795
	SD	1584.698	13486.313	14366.320
	CV%	46.63	36.38	35.90
Reference product (R)	Geometric Mean	3495.714	40336.321	44074.394
	SD	1873.864	20931.630	20872.458
	CV%	53.60	51.89	47.36
Least mean squareS	T	8.131	10.521	10.597
	R	8.159	10.605	10.694
Geometric Mean Ratio	T/R (%)	97.22	91.90	90.79

CONCLUSION

In this small study of 12 healthy male volunteers, no statistically significant differences in Cmax, AUC_{0-t}, and $AUC_{0-\infty}$ were found between the test and reference formulations of ranolazine 1000mg extended release tablets. The 90% CIs for the mean

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ratio values for the test and reference formulations of Cmax, AUC_{0-t}, and $AUC_{0-\infty}$ indicated that the reported data were entirely within the bioequivalence acceptance range proposed by the FDA of 80% to 125% (using log-transformed data).

↓ REFERENCES

1. Zerumsky K and McBride BF; Ranolazine in the management of chronic stable angina; Am. J. Health Syst. Pharm; 2006; 63(23); 2331–2338.
2. Jose J, Jimmy B, Saravu K and Shastry BA; Ranolazine: A novel therapeutic option in chronic stable angina; Kath. Univ. Med. J; 2007; 5(4); 596-599.
3. Markus J; Clinical pharmacokinetics of ranolazine; Clin. P kinetics; 2006; 45(5); 469-491.
4. Tavazzi L; Ranolazine a new antianginal drug; Fut. Cardiol; 2005; 1(4); 447-455.
5. Stanley WC; Ranolazine a new approach for treatment of stable angina pectoris; Fut. Drugs; 2005; 3(5); 821-829.
6. Rousseau MF, Pouleur H, Cocco G and Wolff AA; Comparative efficacy of ranolazine versus atenolol for chronic angina pectoris; Am. J. Cardiol; 2005; 95(3); 311–316.
7. ICH - International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). Guideline For Good Clinical Practice E6(R1), Current Step 4 version (1996). Available from: <http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products /Guidelines /Efficacy /E6_R1/ Step4/E6_R1__Guideline.pdf> [9 January 2014]
8. ICMR - Ethical Guidelines for Biomedical Research on Human Participants. Indian Council of Medical Research (ICMR), New Delhi, (2006). Available from:<http://icmr.nic.in/ethical_guidelines.pdf> [9 January 2014]
9. Jerling M and Abdallah H; Effect of renal impairment on multiple-dose pharmacokinetics of extended-release ranolazine; Clin. Pharmacol. Ther; 2005; 78(3); 288–297.
10. Jerling M, Huan BL, Leung K, Chu N, Abdallah H and Hussein Z; Studies to investigate the pharmacokinetic interactions between ranolazine and ketoconazole, diltiazem, or simvastatin during combined administration in healthy subjects; J. Clin. Pharmacol; 2005; 45(4); 422–433.