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Research Article

BIOEQUIVALENCE STUDY OF CHIRALLY PURE S-METOPROLOL IR 50 mg TABLET: A RANDOMIZED, OPEN LABEL, SINGLE DOSE, CROSSOVER STUDY IN HEALTHY ADULT INDIAN SUBJECTS

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ABSTARCT

Aim: To estimate bioavailability of single oral dose of S-metoprolol immediate release tablets containing 50mg S-Metoprolol (test formulation) manufactured by Emcure pharmaceuticals Ltd., India and compare with single oral dose of BETALOC tablets containing 100mg metoprolol (reference formulation) manufactured by Astrazeneca pharma India Ltd., India, using a randomized two-way crossover design under fasting condition.

Material and Methods: Using a one center, open labeled, randomized, two way, two period, two treatment, single dose cross over design, test and reference formulations were administered to 24 healthy adult male volunteers under non-fed condition, with 7 days washout period between dosing. Pharmacokinetic parameters, $C_{max,}$ AUC_{0-t}, $AUC_{0-\infty}$ and $C_{max/}$ $AUC_{0-\infty}$ were calculated from the plasma concentration-time data of each individual and during each period by applying non-compartmental analysis. Data for test and reference formulations were analyzed statistically to test for bioequivalence of the two formulations.

Results: All 24 subjects who received the two formulations on two occasions with a washout period of 7 days completed the study and provided an adequate amount of blood at each sampling point. After oral administration the value of C_{max} (ng/ml), T_{max} (hr), AUC_{0-t} (ng/ml*hr)and $AUC_{0-\infty}$ (ng/ml*hr)for reference and test formulation were 50.030 and 53.765, 1.865 and 2.177, 352.247 and 387.927 and 363.753 and 402.552, respectively. The ratio of least square means for $C_{max,}$ AUC_{0-t}, and $AUC_{0-\infty}$ were 98.96%, 103.38% & 103.88% respectively. The 90% confidence interval was noted with all values within the acceptance limit. No adverse event was observed in any of the subjects during the study. Both clinical and laboratory parameters of all subjects showed no clinically significant changes.

Conclusion: The test formulation containing S-Metoprolol IR 50mg (manufacture by Emcure Pharmaceutical Ltd., Pune, India) was bioequivalent to reference formulation BETALOC tablets containing 100mg metoprolol (Astrazeneca pharma India Ltd., India). Both formulations were well tolerated. The test formulation can be considered a pharmaceutically and therapeutically equivalent alternative to BETALOC.

Keywords: S-Metoprolol- Bioequivalence- Pharmacokinetics

INTRODUCTION

All the beta-blockers are racemic mixtures of two enantiomers, R and S. Both these isomers may exhibit differing pharmacological properties and so currently used racemic beta-blockers are actually fixed-dose combinations of two pharmacokinetically and pharmacodynamically different isomers.[1] Metoprolol is one of the most widely used cardioselective beta blocker in the treatment of hypertension, angina and congestive heart failure . The cardiac ß-blocking activity of metoprolol resides with S-enantiomer, with S: R activity ratio being 33:1. The β1 receptor affinity of the S-form is about 500 times greater than that of R-form. R-enantiomer has rather strong activity in blocking β2 receptors with the S: R ratio being 1:10.[5, 7] This implies that higher doses of racemic metoprolol will lead to loss of cardioselectivity and side-effects due to β2 blocking property of R-isomer. Higher cardioselectivity is desirable especially when beta blockers are to be used in patients with COPD, diabetes, CHF and when higher doses of beta blockers are to be used. S-Metoprolol is the S-isomer of racemic metoprolol and exhibits greater affinity and higher activity in blocking β1 receptors than the R-isomer. S-metoprolol at half the dose of racemate has been proved to be effective in the treatment of hypertension & angina in patients with or without co-morbidities such as COPD, diabetes and hyperlipidemia. There is adequate pharmacokinetic data available on S-metoprolol extended release formulation. However for S-Metoprolol immediate release formulation it is important to study pharmacokinetic parameters of therapeutically active isomer in the harmony.

MATERIAL AND METHODS

Study design & approvals:

A one center, open labeled, randomized, two-way, two-period, two-treatment, single dose, crossover bioequivalence study of 50mg S-Metoprolol tablets in twenty four healthy adult male subjects under fasting condition.

The study was conducted in accordance with the ethical principles that have their origins in the WMA Declaration of Helsinki and in compliance with ICH GCP and the local guidelines of ICMR. Formal approval for the protocol & informed consent was obtained from IRB before the start of the study. The standard primary pharmacokinetic parameters were followed as per guidelines in the "Guidance for Industry-Bioavailability and Bioequivalence Studies for Orally Administered Drug Products – General Considerations USFDA, CDER March 2003".

Subject selection:

The study was conducted at Therapeutic Drug Monitoring Laboratory. Human subjects were contacted through the subject bank available with the study centre. Only Asian males of Indian origin were selected as study subjects. Subjects were instructed during screening to refrain from using tobacco or betel nut or products containing the same and from consuming any alcoholic products, grapefruit juice, xanthine-containing foods or beverages (like chocolate, tea, coffee or cola drinks) from 48 hours prior to dosing till the completion of study.

The study was carried out in twenty four subjects. An equal number of subjects were randomly assigned to each dosing sequence of treatments (formulations), separated by 7 days of washout period.

All subjects under went a screening procedure comprising of clinical examination, recording of electrocardiogram and laboratory investigations of blood and urine, which was within 14 days prior to first dosing. Urine screen for drugs of abuse and alcohol breath test were performed at admission of each period for all subjects. Clinical examination (vital signs including sitting blood pressure, radial pulse, oral temperature and respiratory rate), physical examination and systemic examination were repeated at the time of admission, before discharge in each period and at the end of study.

Study procedures:

Subjects were housed in the clinical facility minimum 13 hours predose from a time adequate to ensure 10 hours fasting before dosing and were allowed to leave the facility after 24.00 hour post-dose sample in each period. As per the randomization schedule, one tablet of test (T) product i.e., S- metoprolol 50 mg tablets or reference (R) product i.e., Betaloc tablets containing 100mg metoprolol was administered to each subject with 240 mL of water at ambient temperature in each period by trained study personnel. Subjects were instructed not to chew or crush the tablet but to consume it as a whole. Compliance for dosing was assessed by a thorough check of the oral cavity immediately after dosing. Administration of investigational products was carried out while the subjects were in sitting posture and they were instructed to remain seated for two hours after dosing in each period except when clinically indicated to change the posture or in case of any natural necessity. Thereafter, the subjects were allowed to engage in normal activities while avoiding strenuous physical activity. Drinking water was prohibited for one hour before and one hour after dosing. At other times, drinking water was provided *ad libitum.* A standardized lunch, snacks and dinner served to subjects 4 hours, 6-8 hours and 12-14 hours post-dose respectively.

Blood sample collection:

On day 1 of each study phase, the cannula was placed into the subject's forearm about an hour before the scheduled time of dosing and it was retained for approximately 12 hours in most of the subjects who could tolerate the cannula. Twenty one samples were collected from each subject during each period. The venous blood samples (5mL each) were withdrawn at pre-dose (before dosing, in the morning of the day of dosing) and at 0.00 and 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 3.00, 5.00, 7.00, 10.00, 12.00, 14.00, 16.00, 18.00, 20.00, 24.00 hrs post-dose. Blood samples were obtained from an antecubital vein by an indwelling venous cannula using coded, 05-ml sterile K_2EDTA Vaccutainers. They were centrifuged at 4000 rpm for ten minutes to separate plasma. The centrifugation was done within 10 minutes of the blood collection and temperature maintained at 0-4°C. The plasma was separated and stored in duplicate polypropylene vials. The collected samples were stored frozen at -70°C ± 10°C until assayed.

Subjects safety:

Vital signs were measured and recorded in sitting position of volunteers before drug administration and up to two hours post dose, thereafter vital signs were measured and recorded in supine condition. Vital signs monitored at 0.00hrs, 2.00hrs, 4.00hrs, 8.00hrs, 12.00hrs and, 24.00hrs. The patients were under close medical supervision through out study duration for evidence of any adverse event.

Chromatographic conditions:

S-Metoprolol concentrations were measured using the LC/MS/MS method developed and validated at the analytical facility. The analytical method was validated prior to the start of the study. The validation parameters include specificity, ruggedness, LOD, LOQ, calibration curve, precision, accuracy, recovery, quality control samples & stability. LC/MS/MS (AB Sciex Instruments) equipment consisted of Perkin Elmer Series 200 pump fitted with Perkin Elmer Series 200 auto sampler and Chiral column Aspec Chirobiotic T (150mm x 4.6 mm, i.d. 5µ). Plasma samples were extracted by using liquid liquid extraction procedure. Linearity was achieved over a concentration range from 0.50 ng/mL to100 ng/mL.

Pharmacokinetic analyses:

To demonstrate bioavailability characteristics of the S-metoprolol and bioequivalence of the test with reference product, the following primary measures obtained from the plasma concentration time profile were compared: maximum measured plasma concentration (C_{max}), area under the plasma concentration versus time curve from time 0 to the last time point with measurable concentration ($AUC_{0\text{-}t}$) calculated by the linear trapezoidal rule and the area under the plasma concentration versus time curve from time 0 to time infinity ($AUC_{0\text{-}INF}$) calculated as the sum of $AUC_{0\text{-}t}$ plus the ratio of the last measurable plasma concentration to the elimination rate constant. Secondary pharmacokinetic parameters include Kel i.e. the elimination rate constant estimated by a non-linear least-squares regression analysis of the individual concentrations observed as a function of time during the elimination phase and the elimination $t_{½}$ is obtained by dividing 0.693 by elimination rate constant (Kel).

Statistical analyses:

Mean, standard deviation, minimum, maximum and coefficient of variation were calculated for the plasma concentration verses time profile for test and reference formulations. Mean, standard deviation, median, minimum, maximum, coefficient of variation and geometric mean were calculated for all pharmacokinetic parameters. The pharmacokinetic parameters for S-Metoprolol were calculated using WinNonlin software. Descriptive statistics of S-Metoprolol (Test and Reference) were obtained for all pharmacokinetic parameters i.e. C_{max}, $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$, T_{max}, K_{el}, $t_{1/2}$, $AUC_{0\text{-}t}/AUC_{0\text{-}\infty}$. The statistical comparison of the pharmacokinetic parameters was carried out using SAS® obtained from 24 subjects who completed both treatment periods. Bioequivalence between the products was determined by calculating the 90% CI for the ratio of the Cmax, AUC0–t, and AUC0–∞ values for the test and reference products, using logarithmic transformed data. An analysis of variance (ANOVA) on natural log transformed data was conducted to test for period, product, and group effects. In accordance with current FDA guidelines,[5] the products were considered bioequivalent if the 90% CI for Cmax, AUC0–t, and AUC0–∞ fall within the range of 80% to 125%.

RESULTS

Linear mean plasma concentration verses time curves for S-metoprolol test (T) and reference (R) formulations shown in the Figure 1. Pharmacokinetic parameters of S-metoprolol 50mg tablet as test formulation and BETALOC tablets containing 100mg metoprolol as reference formulation for 24 healthy adult male subjects shown in table 1. The sequence, period and formulation effects for ln-transformed pharmacokinetic parameter for C_{max}, $AUC_{0\text{–}t}$, and $AUC_{0\text{–}\infty}$ were statistically insignificant. Descriptive statistics of the ratio of least square means for C_{max}, $AUC_{0\text{–}t}$, and $AUC_{0\text{–}\infty}$ were 98.96%, 103.38% & 103.88% respectively. The 90% confidence interval for the ln-transformed for C_{max}, $AUC_{0\text{–}t}$ and $AUC_{0\text{–}\infty}$ was 87.29-112.19%, 92.46-115.59% & 92.89-116.18% respectively within the range of 80% to 125% (table 2). The intra-subject variability for ln-transformed data for C_{max}, $AUC_{0\text{–}t}$ and $AUC_{0\text{–}\infty}$ was 25.72%, 22.81% & 22.85% respectively (table 3).

Table 1: Pharmacokinetic parameters of S-Metoprolol tablets containing 50 Mg S-Metoprolol (Test formulations) and Betaloc tablets containing 100 Mg Metoprolol (Reference formulations) administered to 24 healthy adult male subjects

Pharmacokinetic Parameters	Reference				Test			
	Mean	S.D.	S.E.	%CV	Mean	S.D.	S.E.	%CV
C_{max} (ng/ml)	50.030	15.8094	3.2271	31.60	53.765	28.4920	5.8159	52.99
$AUC_{(0\text{-}t)}$ (ng/ml*hr.)	352.247	220.1659	44.9412	62.50	387.927	270.8314	55.2832	69.82
$AUC_{(0\text{-}\infty)}$ (ng/ml*hr.)	363.753	233.7610	47.7163	64.26	402.552	288.1428	58.8169	71.58
C_{max} / $AUC_{(0\text{-}\infty)}$ (hr^{-1})	0.1666	0.0545	0.0111	32.70	0.1565	0.0436	0.0089	27.86
T_{max} (hr)	1.865	0.5613	0.1146	30.11	2.177	1.1829	0.2415	54.34
K_{el} (hr^{-1})	0.216	0.0780	0.0159	36.15	0.223	0.0997	0.0204	44.65
$T_{½}$ (hr)	3.563	1.1232	0.2293	31.52	3.616	1.2533	0.2558	34.66

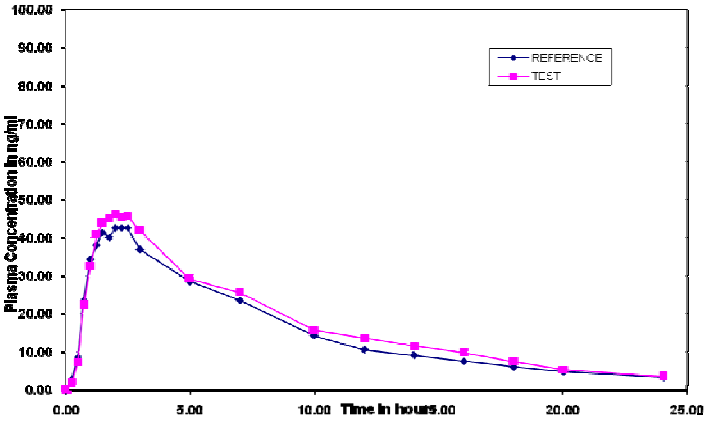

Fig. 1: Mean Plasma Concentration V/S Time Curve In Ng/Ml

Table 2: Bioequivalence Evaluation

Parameters	Ratio of least square mean (test /reference %)	90% confidence Interval		
ln C_{max}	98.96	87.29	-	112.19
ln $AUC_{(0-t)}$	103.38	92.46	-	115.59
ln $AUC_{(0-\infty)}$	103.88	92.89	-	116.18

Table 3: Intrasubject Variability for S-Metoprolol

Data	% Intrasubject variability
ln C_{max}	25.72
ln AUC $_{(0-t)}$	22.81
ln AUC $_{(0-\infty)}$	22.85

DISCUSSION

The different pharmacokinetic properties of stereoisomers of metoprolol are due to the differences in metabolism of its isomers. Metoprolol is predominantly eliminated by hepatic metabolism. Most oxidation of the drugs is performed by one of several oxidative enzyme systems associated with cytochrome P450. CYP2D6 belongs to cytochrome P450 super-family. In humans, metoprolol is eliminated by several pathways, including benzylic hydroxylation (alpha-hydroxylation) which results in an active metabolite and accounts for ~10% of the dose.[4] This pathway is stereoselective for S (–)-metoprolol. The major metabolic pathway is however O-demethylation and further oxidation to a carboxylic acid metabolite that accounts for 65% of the dose. O-demethylation favors R (+)-Metoprolol and is responsible for the stereoselectivity observed in the plasma concentrations of Metoprolol.[3,4] In this study the S-metoprolol was detected in plasma from bioavailability of S-Metoprolol immediate release formulation, where $AUC_{(0-t)}$ observed as 387.927 ng/mL and the C_{max} 53.765 ng/mL was achieved with in 2.177hr (T_{max}).

Immediate beta-blocker therapy appears to reduce the magnitude of infarction , the rate of reinfarction and the frequency of life-threatening ventricular tachyarrhythmias in patients with STEMI. According to 2004, STEMI guidelines, oral beta-blocker therapy should be administered promptly to those patients without a contraindication, irrespective of concomitant fibrinolytic therapy or performance of primary PCI. A prompt IV beta-blocker therapy is indicated in STEMI patients without contraindications, especially if a tachyarrhythmia or hypertension is present. After IV therapy, oral therapy with up to 50 mg of metoprolol every 6 hrly is indicated for first 48 hours after which the patients can be controlled on extended release metoprolol (up to 100 mg twice daily).[8]

CONCLUSION

From our results, it is evident that pharmacokinetics of S-metoprolol immediate release (manufacture by Emcure Pharmaceutical Ltd., Pune, India) are comparable to the pharmacokinetics of BETALOC tablets containing 100mg metoprolol (Astrazeneca pharma India Ltd., India). Thus it can be used in the immediate treatment of myocardial infarction before starting the maintenance doses of S-metoprolol extended release. The use of S-metoprolol would provide a more cardioselectivity, greater potency, uniform pharmacokinetics and lesser chance of drug interactions as compared to single dose 100mg metoprolol.

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REFERENCES

1. *Cruickshank JM.* Are we misunderstanding beta-blockers. Int J Cardiology. 2007; *120*: 10–27.
2. *Helfand M and Peterson K.* Drug Class Review on Beta Adrenergic Blockers. Available form: http://www.ohsu.edu/drugeffectiveness/reports. Accessed on 2008. January 22.
3. *Lennard MS, Silas JH, Freestone S, Ramsay LE, Tucker GT and Woods HF*. Oxidation phenotype--a major determinant of Metoprolol metabolism and response. New England J of Med.1982; *307*:1558-1560.
4. *Murthy SS, Shetty U, Nelson WL, Jackson PR, and Sennard MS.* Enantioselective and diastereoselective aspects of the oxidative metabolism of Metoprolol. Biochemical Pharmacology. 1990; *40*:1637-1644.
5. *Nathanson JA.* Stereospecificity of β adrenergic antagonists: R-enantiomers show increased selectivity for β-2 receptors in ciliary process. J Pharm and Exp Ther. 1988; *245*: 94-101.
6. US Dept of Health and Human Services, Food and Drug Administration (FDA), Center for Drug Evaluation and Research (CDER). Guidance for Industry Bioavailability and Bioequivalence Studies for Orally Administered Drug Products—General Considerations, 2002 [FDA Web site]. http://www.fda.gov/cder/ guidance/4964dft.htm. Accessed January 28, 2008.
7. *Wahlund G, Nerme V, Abrahamsson T and Sjoquist P.O.* The β 1- and β 2-adrenoceptor affinity and β 1-blocking potency of S- and R-Metoprolol. British J Pharm. 1990; *99(3)*: 592-6.
8. ACC/AHA Guidelines for the Management of Patients with ST-Elevation Myocardial Infarction—Executive Summary. 2004 American Heart Association, Inc. *Circulation.* 2004; *110*:588-636.