Bioequivalence Study of Two Formulations of Bisoprolol Fumarate Tablets in Healthy Subjects

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ABSTRACT

The aim of this study was to evaluate the bioequivalence of Diopolol (containing Bisoprolol fumarate 10 mg) tablet of SAVA Healthcare Ltd, India with Concore (Containing Bisoprolol hemifumarate 10 mg) tablet of Merck Serono, Germany in healthy adult volunteers. This open label, balanced, single-dose, randomized, two period, two sequences ,crossover oral bioequivalence study was conducted in 24 healthy human adult male subjects under fasting condition. Subjects received bisoprolol 10 mg of either test or reference formulation with a washout period of 7 days. After study drug administration, serial blood samples were collected over a period of 48 hours. The plasma concentrations of bisoprolol were determined by a validated method using LC/MS/MS. Pharmacokinetic parameters C_{max}, T_{max}, $T_{1/2}$, AUC_{0-t}, $AUC_{0-\infty}$, and k_{el}, were determined for both the formulations. The formulations were to be considered bioequivalent if the geometric least square mean ratio of test and reference of Cmax, AUC_{0-t}, and $AUC_{0-\infty}$, were within the predetermined bioequivalence range of 80% to 125%. A total of 24 subjects were enrolled. No significant differences were found based on analysis of variance. The 90% confidence intervals (CI) of Cmax, AUC_{0-t} and $AUC_{0-\infty}$, of bisoprolol were 103.29 - 115.15, 103.73 - 116.62, and 94.78 - 116.64 respectively This study shows that the test formulation is bioequivalent to the reference formulation for bisoprolol.

Keywords: Bioequivalence, Bisoprolol, Pharmacokinetics

INTRODUCTION

Bisoprolol [(RS)-1-{4-[(2-isopropoxyethoxy)methyl]phenoxy}-3-(isopropylamino)propan-2-ol] is a synthetic beta 1-selective adrenoceptor antagonist; it has higher selectivity as compared to other β1-selective β-blockers such as atenolol, metoprolol and betaxolol[1,2]. Bisoprolol undergoes minimal first pass metabolism, thus has a biological availability of about 90% after oral administration. Bisoprolol is rapidly absorbed after oral administration and the absorption is not affected by food and because of low protein binding ability (~30%) the pharmacokinetics are not influenced by pathophysiological changes in the plasma proteins. Peak plasma concentrations occur within 2-4 hours of dosing with 5 to 20 mg, and mean peak values range from16 ng/mL at 5 mg to 70 ng/mL at 20 mg. The kinetics of bisoprolol is linear and independent of age.

Bisoprolol has both hydrophilic and lipophilic nature to some extent thus shows favorable pharmacokinetic properties such as high absorption rate and long plasma elimination half-life compared to other beta- blockers[7]. Bisoprolol shows a balanced elimination as half of the dose is metabolized by the liver to an inactive metabolite and the rest is eliminated by the kidney.

The aim of this study was to compare bioequivalence of single dose of DIOPOLOL (containing Bisoprolol Fumarate 10 mg) Tablet of SAVA Healthcare Ltd, India as Test with CONCORE (Containing Bisoprolol hemifumarate 10 mg) Tablet of Merck Serono, Germany as Reference; in healthy, adult, human male subjects under fasting condition.

MATERIAL AND METHODS

The study was designed as an open label, balanced, single-dose, randomized, two period, two sequences, crossover bioequivalence study. All the subjects provided written informed consent to participate in the study prior to enrolment and were free to withdraw at any time during the study. The study was conducted in compliance with the ICH GCP, ICMR guidelines, and declaration of Helsinki at the research facility of Auriga Research Ltd., New Delhi.

Study Population

The study was approved by an Independent ethics committee, prior to enrollments. The population consisted of 24, adult, healthy human male between 20-42 years of age, height and weight ranging between 153-182 cm and 50-80 kg and BMI ranging from 21.36-24.15.

Prior to drug administration all subjects underwent clinical and physical examination which included recording of ECG and laboratory investigations of blood (blood glucose, urea, creatinine, AST, ALT, alkaline phosphatase, total bilirubin, triglyceride, total cholesterol, hemoglobin, total & differential white cell counts) and urine based on which they were

considered medically fit by the investigator. All subjects provided written informed consent to participate in the study, prior to enrolment and were free to withdraw at any time during study.

Study Design

The study was an open label, balanced, single-dose, randomized, two periods, two sequences, cross over bioequivalence study in 24 healthy male volunteers under fasting condition. A wash out period of 07 days was maintained between each consecutive dosing to minimize the carry over effects and to eliminate the drug from the body.

The volunteers administered one of the two study drugs after an overnight fast. The dose administration was performed as per the randomization schedule. Subjects received single oral dose of DIOPOLOL (Containing 10mg Bisoprolol fumarate) tablet of SAVA Healthcare Ltd., India as Test or CONCORE (containing 10mg of Bisoprolol hemifumarate) tablet of Merck Serono, Germany as Reference.

Drug Administration

After an overnight fast of at least 10 hrs, either the test or the reference product was orally administered with 240 ml of water. Subjects received an alternative treatment in the successive period following randomized cross-over design. At the end of the study each subject received each treatment once.

The clinical staff ensured that the study participant has swallowed medication by performing oral cavity check using torchlight and spatula. The clock time for each dose administered was recorded on the Case Report Forms. All volunteers were instructed to remain seated or semi-inclined and avoid severe physical exertion for the first 02 hours after dosing.

Blood Sampling

In each Period, total 15 blood samples were collected per subject as per the following schedule; Pre-dose blood sample was collected just after phlebotomy within 02.00 hours prior to drug administration (at 00.00 hr) and the post dose samples were collected at 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00, 6.00, 8.00, 10.00, 12.00, 24.00, 48.00 hours.

The number of blood collections for drug analysis was 30 samples per subject in the study. Blood samples were collected into pre labeled K_2EDTA vacationers and separated plasma samples were transferred into pre labeled polypropylene tubes as single aliquot. After centrifugation, plasma separated from blood samples was stored in a freezer at -80^0C or colder until Bio-analysis.

Method of Analysis

Drug analysis

The plasma levels of Bisoprolol were determined by a validated method using LC/MS/MS in the Bio Analytical department at Arbro Pharmaceuticals Ltd.The concentration range for Bisoprolol employed in the standard curve ranged from 2.65ng/mL to 210.80ng/mL respectively. Sample preparation process was accomplished by protein precipitation technique. The processed sample was chromatographed and analysed on Chromolith 50X4.6, 5µm column using mobile phase (0.1% FA in water & 0.1% FA in methanol). Briefly, samples were vortexed to ensure complete mixing of contents, then pipette out 0.200 mL of sample into RIA vials and addition of 0.040mL (from 10µg/ml) of internal standard and again vortex. After that 0.800 ml of 0.3% formic acid in ACN was added and vortexed for 10 min. Sample were centrifuged at 14000 rpm for 10 minutes. The upper layer was transferred into injector vials and injected on LC/MS/MS System.

Chromatographic Conditions

The LC-MS/MS method for determination of Bisoprolol in human plasma samples was performed using a mobile phase consisting of 0.1% formic acid in water and 0.1% formic acid in methanol. Metoprolol, a structural analogue of bisoprolol was used as an internal standard[10]. The compounds were eluted at a flow rate of 0.5 ml/ min. The typical standard retention times were 1.810 min for Bisoprolol and 1.30 min for Metoprolol.

Pharmacokinetic and Statistical Analysis

Based on the plasma concentrations of Bisoprolol, the pharmacokinetic parameters were calculated by using "Non-compartmental model" and ANOVA statistics. All pharmacokinetic analysis carried out by using WinNonlin Version 5.3. The elimination rate constant (kel) was obtained as the slope of the linear regression of the log-transformed concentration values versus time data in the terminal phase. The elimination half-life (t½) was calculated as 0.693/ kel. The area under the curve to the last measurable concentration (AUC_0-t) was calculated by the linear trapezoidal rule. The area under the curve extrapolated to infinity (AUC_0-∞) was calculated as the sum of the AUC_0-t plus the ratio of the last measurable concentration to the elimination rate constant. The variance model included sequence, subjects nested in sequence, period, and product as factors. A 5% level of significance used for all comparisons (period, product and sequence). Inconsistent with the two one-sided tests for bioequivalence, 90% confidence intervals for the ratios of means was calculated for both untransformed and log-transformed AUC0-t, AUC0-∞ and Cmax for losartan and its active metabolite losartan carboxylic acid. The formulations were to be considered bioequivalent if the log transformed ratios (test/reference) of Cmax, AUC0–t, and AUC0–∞ were within the predetermined bioequivalence range of 80% to 125%.

Safety and tolerability

For monitoring the safety of subjects, their vital signs, well-being assessments, and observations of medical examination were recorded at regular interval as mentioned in the protocol.

There was one adverse event reported during the wash out period, the subject no. 01, experienced mild cough & cold. Subject No 01 also had increased eosinophil count in post-study safety lab analysis. Subject No 04 and 06 had increased total S. bilirubin in post-study safety lab analysis. All the deranged parameters of subjects 01, 04 and 06 were found to be within normal range after repeat safety analysis. Adverse events were followed up until resolution.

RESULT AND DISCUSSION

The GeoLSM (%) and CI of Cmax were found to be 109.06% and 103.29% to 115.15%. Similarly, for AUC_{0-t} and $AUC_{0-\infty}$ was found to be 109.99% and 103.73% to 116.62%, 105.14% and 94.78% to 116.64% respectively, which showed all the values within the acceptable range of 80% to 125%.
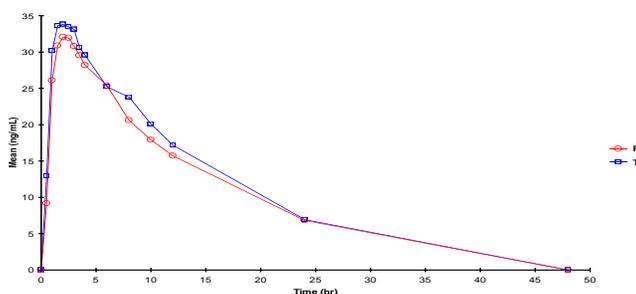

Figure 1: Linear plot of mean plasma concentration of bisoprolol (ng/ml) *versus* time for all 24 subjects
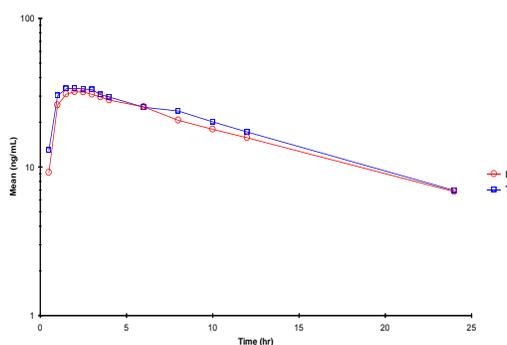

Figure 2: Log plot of mean plasma concentration of bisoprolol (ng/ml) *versus* time for all 24 subjects

The plasma drug concentration-time curves show that the mean concentration of Bisoprololis similar for the two formulations over 48 hours sampling period.

Table 1. Primary and secondary pharmacokinetic parameters of bisoprolol after administration of test and reference product

Products	Test			Reference		
Parameters	Mean	±SD	CV%	Mean	±SD	CV%
AUC_{0-t} ng.hr/ml	439.22	103.84	23.64	405.56	113.68	28.03
$AUC_{0-\infty}$ ng.hr/ml	541.90	131.72	24.31	556.85	346.54	62.23
C_{max} ng/ml	37.83	7.68	20.31	34.78	7.46	21.45
T_{max} (hr)	1.83	0.65	35.67	2.33	1.07	45.86
$T_{1/2}$ (hr)	23.50	2.45	10.42	23.00	3.39	14.73
λ_z (1/hr)	0.08	0.02	22.55	0.08	0.03	33.14

Table 3. Summary of pharmacokinetic bioequivalence parameter for bisoprolol

Pharmacokinetic Parameter	Least square Means		Geometric Mean		Point Estimator (%)	90 % C.I	
	Test	Reference	Test	Reference	Test/Reference	Lower	Upper
C_{max} (ng/ml)	3.61	3.53	37.12	34.03	109.06	103.29	115.15
AUC_{0-t} (ng.hr/ml)	6.05	5.96	426.14	387.44	109.99	103.73	116.62
$AUC_{0-\infty}$ (ng.hr/ml)	6.27	6.22	526.35	500.60	105.14	94.78	116.64

The log-transformed pharmacokinetic parameters, C_{max}, $AUC_{(0-48)}$, and $AUC_{(0-\infty)}$ of Bisoprolol were subjected to analysis of variance (ANOVA) with the main effects of sequence, treatment, and period at 5% level of significance. The coefficient of variance corresponding to intra- subject variability for C_{max}, $AUC_{(0-48)}$, and $AUC_{(0-\infty)}$ was 10.9%, 11.85%, and 21.16%.

As the confidence interval of the primary pharmacokinetics parameters lay within the acceptable bioequivalence range of 80-125% the test and the reference drug are considered bioequivalent. The adverse event observed were not serious and had no relationship with the study drug. Thus the drug was considered safe and well tolerated among the study population. The intra subject variability was found not to be more than 30% thus Bisoprolol is considered not to be highly variable in the systemic circulation of the subject.

CONCLUSIONS

Based on the results it was concluded that the test (t) DIOPOLOL (containing Bisoprolol fumarate 10 mg) tablet of SAVA Healthcare Ltd, India is bioequivalent to reference (r) CONCORE (Containing Bisoprolol hemifumarate 10 mg) tablet of Merck Serono, Germany, in healthy, adult, human male subjects under fasting condition.

REFERENCE

1. Pharmacodynamic profile of the selective beta 1-adrenoceptor antagonist bisoprolol. Harting J, Becker KH, Bergmann R, Bourgois R, Enenkel HJ, Fuchs A, Jonas R, Lettenbaur H, Minck KO, Schelling P, et al. Arzneimittelforschung. 1986 ;36(2):200-8

2. Direct labelling of myocardial beta 1-adrenoceptors. Comparison of binding affinity of 3H-(-)-bisoprolol with its blocking potency. Kaumann AJ, Lemoine H. NaunynSchmiedebergs Arch Pharmacol. 1985;331(1):27-39.

3. Lancaster SG, Sorkin EM. Bisoprolol. A preliminary review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in hypertension and angina pectoris. Drugs. 1988;36(3):256–285.

4. Concor® 5 and Concor® 10 film-coated tablets [package insert]. Available from http://home.intekom.com/pharm/merckp/concor.html July 27, 2012.

5. Bisoprolol in the treatment of chronic heart failure: from pathophysiology to clinical pharmacology and trial results Marco Metra, Savina Nodari, Tania Bordonali, Patrizia Milani, Carlo Lombardi, Silvia Bugatti, Benedetta Fontanella, Giulia Verzura, Rossella Danesi, and Livio Dei CasTherClin Risk Manag. 2007; 3(4): 569–578.

6. Heart failure: epidemiology and prevention in India. Huffman MD, Prabhakaran D. Natl Med J India. 2010;23(5):283-8.

7. Wotschokowsky M. Pharmakologisch relevante physico chemische Eigensch aften von Bisoprolol-hemifumarat (EMD 33 512). Merck KGaA, Darmstadt, 1985.

8. ZHU LM et al. The efficacy of once-daily bisoprolol, lacidipine and lisinopril on the 24-hour blood pressure in patients with essential hypertension. J Hypertension 1997;15 (Suppl 4): 212

9. Merk cardiovascular, Bisoprolol: Cardioselective Beta-Blocker, Merckhttp://www.bisoprolol-slides.info/homesite/teaser.pdf February 2001

10. Gabriela Peste, Nela Bibire, Mihai Apostu, Aurel Vlase,1 and Corneliu Oniscu.A New Liquid Chromatography-Tandem Mass Spectrometry Method for Determination of Bisoprololin Human Plasma Samples. J Biomedicine and Biotechnology 2009, Article ID 736327.