Available online on 15.12.2022 at http://ajprd.com

Asian Journal of Pharmaceutical Research and Development

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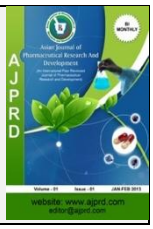

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Research Article

Bioequivalence study of BETALOC-XR™ (Drug International Ltd, Bangladesh) and METOPROLOL 100 STADA™ (STADA Pharm Gmbh, Germany) as open-label, two-way crossover and randomized fashion among healthy male volunteers

Uttam Kumar Sarker[1*], Fatiha Farhana[1], Atikul Islam[1], Md.Shamsuzzoha[1], Mir Misbahuddin[2], Md. Elias Mollah[3], Balaram Roy[1], Md. Rabiul Islam[3`]

[1]Department of Chemistry, Hajee Mohammad Danesh Science and Technology University, Dinajpur;
[2]Department of Pharmacology, Bangabandhu Sheikh Mujib Medical University, Dhaka;
[3]Department of Chemistry, Jahangirnagar University, Savar, Dhaka, Bangladesh.

ABSTRACT

Bioequivalence study of two oral formulations of metoprolol 100 mg tablet was brought about in 16 healthy male Bangladeshi normal individuals as crossover-randomized fashion. The test and reference formulated products were BETALOC-XR™ (Drug International Ltd, Bangladesh) and METOPROLOL 100 STADA™ (STADA Pharm Gmbh, Germany). Each tablet was delivered with 150 mL of water to every normal individual after whole night fasting on two treatment days separated by more than one week washout period. After administering drug, blood samples were collected serially for a period of 24 hours. A validated HPLC method was used to estimate the plasma concentrations of metoprolol. The pharmacokinetic parameters C_{max}, T_{max}, $AUC_{0\rightarrow 24h}$, $t_{1/2}$, and K_{el} were determined in this study. The mean (± SD) $AUC_{0\rightarrow 24h}$ for test drug BETALOC-XR™ was 490.3 ± 187.5 ng/hr/mL whereas for reference drug METOPROLOL 100 STADA™ it was 559.9 ± 267.2 ng/hr/mL. The relative bioavailability (BETALOC-XR™/METOPROLOL 100 STADA™ ratio) was 87.5%. The C_{max}, t_{max}, half-life of elimination ($t_{1/2}$) and the rate of elimination (K_{el}) for test drug was 170.9 ± 78.3 ng/mL, 2.3 ± 0.8 hours, 1.2 ± 2.0 hour and 0.4362 whereas for reference drug was 209.1 ± 91.3 ng/mL, 1.7 ± 0.7 hours, 1.3 ± 2.2 hour and 0.5875 respectively. Based on these statistical hypothesis it was conjecture that a BETALOC-XR™ tablet is bioequivalent to a METOPROLOL 100 STADA™ tablet.

Keywords: Bioequivalence, Metoprolol, HPLC, Pharmacokinetics, Drug International Ltd.

ARTICLE INFO: Received 10 Sept. 2022; Review Complete 15 Oct. 2022; Accepted 20 Nov. 2022; Available online 15 Dec. 2022

Cite this article as:

Sarker UK, Farhana F, Islam A, Shamsuzzoha M, Misbahuddin M, Mollah ME, Roy B, Islam MR, Bioequivalence study of BETALOC-XR™ (Drug International Ltd, Bangladesh) and METOPROLOL 100 STADA™ (STADA Pharm Gmbh, Germany) as open-label, two-way crossover and randomized fashion among healthy male volunteers, Asian Journal of Pharmaceutical Research and Development. 2022; 10(6):09-13.

DOI: http://dx.doi.org/10.22270/ajprd.v10i6.1184

*Address for Correspondence:
Uttam Kumar Sarker, Department of Chemistry, Hajee Mohammad Danesh Science and Technology University, Dinajpur

INTRODUCTION

The term bioequivalence (BE) study between test and reference products is the lack of a notable difference in the rate and extent of absorption to which the active ingredient in pharmaceutical equivalents becomes obtainable at the area of drug action when the drug administered in an appropriately designed study at the same molar dose of the therapeutic ingredient under uniform experimental conditions[1] (FDA, 2003). BE documentation play an important role in new drug development. Bioequivalence refers to the quality of a locally manufactured drug by comparison with standard drug of internationally reputed company. Bioequivalence studies are designed to compare the *in vivo* performance of a generic pharmaceutical product compared to a reference pharmaceutical product. A common design for a bioequivalence PK study involves administration of the test and reference products to healthy subjects occasionally in patients by two way cross-over and randomized fashion,

with each administration separated by a washout period. The washout period is chosen to ensure that drug given in one treatment is entirely eliminated prior to administration of the next treatment i.e.10 times of elimination half- life. The rise and fall of these drug concentrations over time in each subject in the study provide an estimate of how the drug substance is absorbed into the test and reference products and released from the body. To allow comparisons between the two products, these blood (to include plasma or serum) and urine concentration time curves are used to calculate certain bioequivalence metrics of interest. These metrics are calculated for each subject in the study and the resulting values are compared statistically. If the pharmacokinetic parameters such as AUC_{0-t}, C_{max}, $t_{1/2}$, K_{el} etc. for the new drug coexist with the reference properties lie in the range between confidence interval (CI) 80-125%, then the test drug will said to be bioequivalent with the reference drug. BE also requires the similar bio availabilities, efficacy and safety of the two preparation in the above mentioned range (80-125%). In BE study the comparison of C_{max} and AUC_{0-t} are the main key features which are most frequently used in-vivo studies to construct BE. The statistical analysis of the observed data generates an estimated value, called confidence interval (CI) and it propounds a range of likely values of an unacquainted metrics.

Metoprolol is almost beta-1 selective blockers used for the treatment of angina pectoris, hypertension, post myocardial infarction and congestive heart failure, abnormal fast heart rate, supraventricular tachycardia, ventricular tachycardia[2] and a lot of evidence showed that it decline the mortality and morbidity in populations.[3-5] Metoprolol is also used as an adjunct treatment of hyperthyroidism.[6] It is restricted for asthma patients. It is readily distributed throughout the body having a high volume of distribution about 3-6 litre/Kg[7-9]. Metoprolol is not bound to plasma protein to significant extent[10]. About less than 5% unchanged metoprolol eliminated by excretion in the urine after oral administration is enormously biotransformed by cytochrome P450 2D6 (CYP2D6) in the liver[11]. Metoprolol can be metabolized by α-hydroxylation and o-demethylation as a substrate by cytochrome P450 2D6 and convert into α-hydroxymetoprolol and o-desmethylmetoprolol[12-13]. These metabolized are also β-1 blocking agent but these are not considered due to less potent compare to the parent drug. Metoprolol has some common side effect including abdominal pain, feeling tired, nausea, feeling faint[2]. Serious toxicity may arise due to excess dosage[14-15]. Pregnancy risk in metoprolol intake has not been prohibited[16]. It is reported that metoprolol is safe in breastfeeding[17]. The various type salt of metoprolol, metoprolol succinate and metoprolol tartarate are accepted in various conditions and are not interchangeable[18-20].

The availability of test metoprolol offers a more economical surrogate for patients requiring β-blocker therapy. That's why BE study is require to ensure the quality of a locally manufactured drug by comparison with standard drug of internationally reputed company.

The purpose of the study: The purpose of the study was to assess the bioequivalence of a test product BETALOC-XR™ (metoprolol 100 mg per tablet) with a reference product METOPROLOL 100 STADA™ by estimation of plasma concentration using HPLC and evaluation of bioequivalence parameters.

Protocol: Bioequivalence study was carried out as randomized, open-label, two-way crossover fashion with washout period of more than 7 days. There was no utmost deflection made from the accepted protocol.

MATERIALS AND METHODS:

Study subjects:

Volunteers were collected from the volunteer bank which was made by counseling with every individual. Total number of subjects was sixteen in this study and the average age, height and weight of the volunteers with standard deviation were 27.3 ± 5.1 years; 165.1 ± 5.8 cm; 56.7 ± 5.1 kg during screening examination. The blood samples were cumulated by two phases after drug dosing. This was in Phase I: 8/12/2012 to 9/12/2012 and 15/12/2012 to 16/12/2012 and in Phase II**:** 12/12/2012 to 13/12/2012 and 22/12/2012 to 23/12/2012. The blood samples were evaluated from: 25/1/2013 to 1/3/2013

Study Design

Every healthy individual secured as a single dose treatment in accordance with crossover randomized fashion (according to the protocol) having more than seven days washout period. Test and reference product denoted by T and R. A crossover design can be represented as (TR, RT), where TR is the first succession of treatments and RT represents the second succession of treatments. Under the (TR, RT) design, healthy qualified subjects who are randomly allocated to succession 1 (TR) will receive the test product (T) first and then cross-over to receive the reference product (R) after more than seven days of wash-out period.

The Institutional review board: The protocol and the ethical feature of this study were approved by the Institutional review board of Khwaja Yunus Ali Medical College and Hospital. This encompasses of seven members committee including a local religious leader (Imam), a lawyer and a woman representatives. The protocol was ratified with insignificant mitigation.

This study was regulated in accordance with the International Conference of Harmonization (ICH) Good Clinical Practice (CGP) guidelines arrogated by the European Agency for the assessment of Medicinal products (EMEA).

Hospital admission: The person who has result in normal range of some clinical screening for example CBC, Urine R/E, RBS, kidney function test, liver function test etc. selected as a volunteer. Volunteers were admitted into twelve bedded bioequivalence ward in the hospital one day before starting the study. At a time eight normal individuals were admitted in the ward during study.

Informed consent: Before starting the treatment the motive of the study was explicated to every volunteer in regional language (Bengali) by medical officer. When volunteer was acknowledged to engage in the study after careful reading the consent form written informed consent

was only taken from him. If any question elevated by the volunteer was explained details with the medical officer.

Drug dosing and Sample collection

In this treatment every volunteer was given a single dose of test or reference product of metoprolol with 250 millilitre water after whole night fasting. Breakfast was supply to the volunteers after 4 hour of the drug dosing. Volunteers were permit to consume water after 2 hour of the dosing and then breakfast, lunch and dinner were given according to the time schedule. Volunteers were under direct medical observation at the study place. The blood samples were taken immediately before (2 mL in each time) and at 0.33, 0.66, 1, 1.5, 2, 3, 4, 8, 12 and 24 hours after dosing metoprolol. The blood samples were accumulated in EDTA tube and were centrifuged at 4000 rpm for 10 min. The plasma was separated and kept frozen at –80°C in eppendrof tube. The same method was repeated to finish the crossover study after seven days.

Chromatographic condition for drug analysis

HPLC with UV-Visible detector was utilized to exploration metoprolol drug. HPLC grade solvents and analytical grade reagent and chemicals were used in this study. The HPLC method was flourish and approved before the study by following international guidelines.[21]

Agilent Germany 1200 series reverse-phase high performance liquid chromatography (HPLC) was used for the determination of metoprolol which comprised solvent reservoir, degasser, solvent delivery binary pump, auto sampler, column and diode array detector. ChemStation software was utilized to integrate the signal. C18 column (4.6 mm×150 mm) with particle size 5 µm (Sigma Aldrich) was employed for chromatographic separation. The mobile phase was used for analysis consisted of 18% acetonitrile (HPLC grade; E. Merck, Germany) and 82% sodium di-hydrogen phosphate buffer (pH 5.0, 20mmol, adjusted with o-phosphoric acid) was delivered at a rate of 0.8 mL/min. The freshly prepared phosphate buffer was filtered using 0.45 µm nylon filters. The wavelength was (221±2 nm) at 30°C. Injection of sample (20 µL) was done using an auto sampler. The peak with retention time and area were defined using software. The half-life of elimination ($t_{1/2}$) and the rate of elimination (K_{el}) of metoprolol were used to further characterize the pharmacokinetic outcome of this study.

Sample preparation for the HPLC injection

A volume of 200 µL sodium di-hydrogen phosphate buffer (pH 5.0, 20 mmol, adjusted with o-phosphoric acid) was added to 100 µL plasma and it was vortexed for 3 min after then 1 mL acetonitril was added and vortexed for 3 min. The mixture was centrifuged at 13500 rpm for 5 minutes. The supernatant was evaporated by nitrogen gas and water bath at 45°C.The residue was reconstituted with 400 µL mobile phase and was vortexed for 1 min and was injected 20 µL in the HPLC systems.

Statistical analysis

The pharmacokinetic parameters maximal plasma concentration (C_{max}), time for the maximal plasma concentration (T_{max}), half-life ($t_{½}$), area under the curve ($AUC_{0\rightarrow t}$, $AUC_{0\rightarrow \alpha}$,) and the elimination rate constant (K_{el}) for the test and reference products were calculated by two-way analysis of variance (ANOVA) procedures using Thermo Kinetica 2000 software[22]. The 90% confidence interval was evaluated by applying online software.

Pharmacokinetic analysis

The pharmacokinetic parameter C_{max}, T_{max}, $AUC_{0\rightarrow 24h}$, $t_{1/2}$, and K_{el} was determined. The mean (± SD) $AUC_{0\rightarrow 24h}$ for metoprolol of test drug for 16 volunteers was 490.3 ± 187.5 ng/hr/mL whereas it was 559.9 ± 267.2 ng/hr/mL for METOPROLOL 100 STADA™. The relative bioavailability (BETALOC-XR™/METOPROLOL 100 STADA™ ratio) was 87.5%. The C_{max}, t_{max}, half-life of elimination ($t_{1/2}$) and the rate of elimination (K_{el}) of test drug was 170.9 ± 78.3 ng/mL, 2.3 ± 0.8 hours, 1.2 ± 2.0 hour and 0.4362 whereas reference drug was 209.1 ± 91.3 ng/mL, 1.7 ± 0.7 hours, 1.3 ± 2.2 hour and 0.5875 respectively.

Tolerance: The Single dose of metoprolol (100 mg) of both products was well tolerated by volunteer.

RESULT AND DISCUSSION

An open-label, randomized, crossover design in-vivo bioequivalence studies were accomplished contrasting between a test product with a reference product by estimation of plasma concentration using HPLC and evaluation of bioequivalence parameters. There is no clinically remarkable difference in the rate and extent to bioequivalence of any local test product with reference innovator product at which the agile body of the drug becomes obtainable at the area of operation. Healthy male volunteers were introduced in this study and a single dose under the fasting condition was administered to each volunteer. The current study had some limitations that should be considered. There was no unpredicted occurrence that could have altered the study result. There was no discontinue and all volunteers who started the study continued to the end and were released from the study center in sound health. The reported analytical method was manifested sensitive and accurate for the determination of metoprolol in plasma. The plasma assay procedures were validated. The limit of detection (LOD) was 2 ng (3SD) whereas limit of quantification (LOQ) was 6 ng (10SD). The retention time of metoprolol was 6 min. Our study examined the pharmacokinetic properties and bioequivalence of two formulations of metoprolol in healthy Bangladeshi male volunteers. The pharmacokinetic parameters calculated for both the test and reference formulations were not significantly different, which reflects the comparable pharmacokinetic characteristics of two formulations[23]. Metoprolol was readily absorbed for both formulations from the gastrointestinal tract and metoprolol was quantifiable at the first sampling time (0.33 h) for most of the volunteers. By hepatic metabolism metoprolol is substantially eliminated. Different oxidative enzyme system consociate with cytochrome P450 involve for the oxidation of this drug. Among them CYP2D6 reside on cytochrome P450 super family. Figure 1 shows mean concentration-time profiles of the study and indicating that the mean plasma drug concentration profiles of the two formulations were almost uniform.

Plasma concentrations maximum were reached at 2.0-3.0 h after drug dosing and then vanished reasonably but the metoprolol was found until the last blood sample Table 1 shows the pharmacokinetic parameters for two studies. All measured pharmacokinetic parameters were in good coexistence with reported values. The pharmacokinetic parameters AUC, Cmax and Tmax are important for bioequivalence study and could affect the therapeutic use of a drug and the extent of absorption is a key characteristic of a drug formulation[24]. The relative bioavailability (BETALOC-XR™/METOPROLOL 100 STADA™ ratio) was 87.5%. The C_{max}, t_{max}, half-life of elimination ($t_{1/2}$) and the rate of elimination (K_{el}) of mtoprolol of test drug were 170.9 ± 78.3 ng/mL, 2.3 ± 0.8 hours, 1.2 ± 2.0 hour and 0.4362 respectively. The C_{max}, t_{max}, half-life of elimination ($t_{1/2}$) and the rate of elimination (K_{el}) of metoprolol for reference drug was 209.1 ± 91.3 ng/mL, 1.7 ± 0.7 hours**,** 1.3 ± 2.2 hour and 0.5875 respectively. The mean and standard deviation of $AUC_{0\rightarrow t}$, $AUC_{0\rightarrow \alpha}$, Cmax, Tmax, $t_{1/2}$ and Kel of the two products did not differ significantly; recommend that the blood profiles produced by Betaloc are comparable to those produced by metoprolol. Analysis of variance (ANOVA) for these parameters, showed no statistically significant difference between the two formulations. 90% confidence intervals also demonstrated that the ratios of $AUC_{0\rightarrow t}$, $AUC_{0\rightarrow \alpha}$, Cmax, Tmax, $t_{1/2}$ and Kel of the two formulations lie within the FDA acceptable range of 80%–125%.

CONCLUSION

BETALOC-XR™ is bioequivalent to METOPROLOL 100 STADA™ (test) (reference) based on the rate and extent of absorption and can be used interchangeably in clinical setting. The 90% confidence intervals for the BETALOC-XR™ (test) and METOPROLOL 100 STADA™ (test) (reference) were found within the acceptance range of 80–125%.

ACKNOWLEDGEMENT

We acknowledge the help of Mr. Mohammed Yusuf, Technical Director of Khwaja Yunus Ali Medical College Hospital. We are also grateful to all the volunteers who participated in this study.

Table I: Pharmacokinetic parameters following oral administration of BETALOC-XR™ (test) and METOPROLOL 100 STADA™ (reference)

	BETALOC-XR™				METOPROLOL 100 STADA™			
Parameters	mean	SD	90%CI		mean	SD	90%CI	
AUC (ng/hr/mL)	490.3	187.5	95.8	104.1	559.9	267.2	95.0	104.9
Cmax (ng/mL)	170.9	78.3	95.2	104.7	209.1	91.3	95.5	104.4
Tmax (hour)	2.3	0.8	96.1	103.8	1.7	0.7	95.8	104.1
half-life (hour)	1.2	2.0	86.5	113.4	1.3	2.2	83.4	116.5
Kel	0.4362		93.7	106.2	0.5875		94.5	105.4

Table-2: Demographic data for bioequivalence study of metoprolol among 16 volunteers

		Range
Age (years)	27.3 ± 5.1	18-35
Height (cm)	165.1 ± 5.81	160-180
Weight (Kg)	56.7 ± 5.1	50-75
BMI (kg/m^2)	20.83± 1.34	18-24
Gender	Male	
Race	Asian	

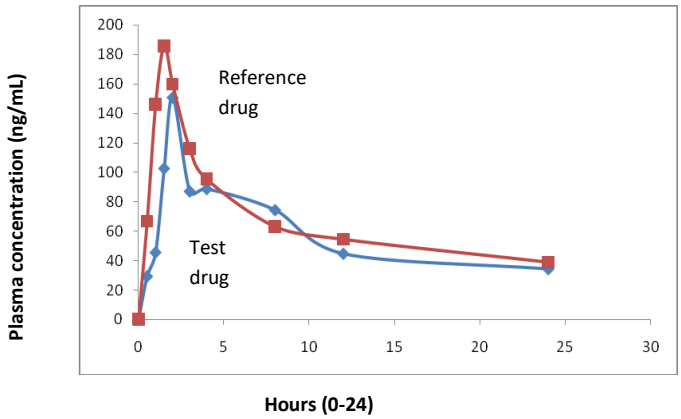

Figure 1: Mean plasma concentrations of metoprolol in 16 human volunteers

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