Single Dose Bioavailability Study of Bilayer Matrix Tablets Containing Antihypertensive Agents

K. Veeran Gowda, A. Ghosh, U. Bhaumik, S. Agarwal,
P. Senthamil Selvan, B.K. Sahoo, U. Nandi and T.K. Pal*
Department of Pharmaceutical Technology, Jadavpur University
Kolkata-700 032, India
Email: tkpal12@gmail.com

The objective of this study was to determine the relative bioavailability of a fixed dose combination (FDC) product containing metoprolol tartrate 100 mg SR and ramipril 10 mg tablet. Two individual products containing metoprolol tartrate SR and the other containing ramipril were selected as the reference preparations as there was no single preparation containing these two drugs. The pharmacokinetics of metoprolol tartrate and ramipril individually after oral administration have been evaluated. However, there is no report available on the combined pharmacokinetics and bioavailability of this particular FDC. The study was designed as a single dose fasting, two periods, two way cross over study with two week wash out period. A validated liquid chromatographic mass spectrometry method (LCMS/MS) was used for the simultaneous determination of both the drugs in human plasma. No statistical differences were obtained between two preparations with respect to the mean pharmacokinetic parameters. The relative extent of absorption as assessed by the AUC ratio (test/reference) and C_{max}, the average values were found to be within the acceptable range of 0.80-1.25. The results clearly indicated that both the preparations are bioequivalent in terms of rate and extent of drug absorption. Both the preparations were well tolerated and no adverse reactions were observed during the entire course of the study.

Key Words: Pharmacokinetics, Metoprolol tartrate, Ramipril, Bilayer matrix tablets.

INTRODUCTION

Metoprolol[1,2] is a β1 selective adrenoceptor antagonist and has a plasma half life of 3-4 h. Metoprolol is completely and rapidly absorbed through out most of the gastrointestinal tract. It undergoes extensive first pass metabolism[3,4]. The relationship between plasma concentrations and β1 blocking effect is well defined for metoprolol[5,6]. Therefore, metoprolol is considered as an ideal candidate to be formulated into controlled release dosage form

so that it can provide a uniform effect for 24 h in hypertension and coronary heart disease. Like other β-blockers, metoprolol is used as a racemic mixture and its pharmacological activity is confined to the S-enantiomer[7].

Ramipril is an angiotensin converting enzyme (ACE) inhibitor and is chemically, 2-[n-[(S)-1-(ryhoxycarbonyl)-3-phenylpropyl)]-L-alanyl]-(1S, 3S,5S)-2-azabicyclo[3-3-0]octane-3-carboxylic acid[8]. Hepatic cleavage of ester group converts ramipril to its active diacid metabolite, ramiprilat[9,10] and reaches peak concentrations within 2-4 h after dosing[11]. The elimination half life of ramipril is 2 h while that of ramiprilat is 13 to 17 h due to enzyme binding. Other metabolites of ramipril are inactive. It is used to treat hypertension and congestive heart failure[12-14]. It is a prodrug that acts on the rennin angiotensin aldosterone system by inhibiting the conversion of the inactive angiotensin I to the highly potent vasoconstrictor, angiotensin II.

A combination formulation of metoprolol tartrate and ramipril is useful in the treatment of hypertension and other heart diseases. It is also beneficial in terms of its convenience and patient compliance. A bilayer matrix tablet containing metoprolol tartrate 100 mg as SR and ramipril 10 mg as IR was prepared in the laboratory. The bioavailability of the prepared product was evaluated in this study by comparing its pharmacokinetics with the two reference products containing these drugs as individual preparations.

EXPERIMENTAL

Products studied: *Test preparation:* Bilayer matrix tablet containing metoprolol tartrate 100 mg SR and ramipril 10 mg IR prepared in the laboratory was selected as the test product for this study.

Reference preparation: Two separate preparations *viz.*, a capsule containing metoprolol tartrate 100 mg XR (Metolar XR®) and a tablet containing ramipril 10 mg (Cardace® 10) purchased from a local pharmacy were selected as the reference preparations.

Study design, study subjects: The study was designed as a single dose fasting two periods; two way cross over study with a wash out period of 2 week. The protocol was reviewed and approved by Institutional Ethical Committee of Jadavpur University prior to the start of the study.

Six healthy male volunteers were enrolled for the study based on their laboratory tests (serum chemistry, hematology, urine analysis), medical history, physical examination and HIV screening. Their age varied 24 and 31 years (28 ± 3.16), weight ranged between 64 to 95 Kg (75 ± 12.34) and had height an height ranging from 167.50 to 182.00 cms. No alcohol or concomitant medication was allowed 72 h prior to the initial administration of dose and for the entire course of the study. None of the subjects had a previous history of allergy to antihypertensive agents or controlled substance abuse.

Drug administration and sample collection: All the subjects assembled in the clinical pharmacological unit (CPU) at 6.00 a.m. on the study day of each period after overnight fasting of 10 h. Their total pulse rate and blood pressure was recorded. Each subject was randomized at the beginning of the study to receive either a single dose of the test FDC or reference preparations during each period along with 240 mL of water. Following a seven day wash out period, all subjects received the alternate formulation during period II.

The subjects were fasted for 10 h before dosing and until 4 h post dose collection. A series of blood samples were collected prior to and following administration of the drug during periods I and II. According to FDA and EMEA regulations, the sampling schedule should be planned to provide a reliable estimate of the extent of absorption[15,16]. Usually the sampling time should extend to at least three terminal elimination half lives of the active ingredient. Time periods between sampling should not exceed one terminal half life[17]. Blood samples were collected immediately prior to dosing and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 18.0 and 24.0 h post dosing in test tubes with EDTA at each time point and were stored frozen at -20 °C with appropriate labeling of volunteer code no., study date and collection time till the date of analysis.

Sample preparation: Liquid-liquid extraction procedure was used for the extraction of the drug from the plasma. Calibration standards, quality control samples were treated with 3 mL mixture containing diethyl ether and dichloromethane (70:30 v/v). 50 μL of internal standard (100 ng/mL) were added to each plasma sample (0.25 mL) and vortex mixed for 10 min followed by centrifugation for another 10 min. The organic layer containing the analytes was separated, transferred to a separate test tube and evaporated to dryness under a stream of N_2 at 40 ºC. The residue obtained on drying was reconstituted with the 250 μL of mobile phase. The reconstituted sample was transferred to an auto sampler vial and injected into the liquid chromatography mass spectrometry (LC-MS/MS) system.

Analytical determination by LCMS/MS: Analysis of the drugs (metoprolol and ramipril) content in the plasma samples was carried out by a validated liquid chromatography tandem mass spectrometry (LCMS/MS) method previously reported by us[18]. Mobile phase used for separation of the analytes was methanol:10 mm ammonium formate buffer (97:3 v/v). The flow rate was set at 1 mL/min. The injection volume was 20 μL and the total run time was 5 min. The column was maintained at ambient temperature (23 ºC) whilst the autosampler temperature was set at 10 ºC.

Pharmacokinetic analysis: The pharmacokinetic parameters for both metoprolol and ramipril were determined using non-compartmental method. The maximum plasma concentration (C_{max}) and time to peak plasma

concentration (t_{max}) were directly obtained from the data of both the drugs. The elimination half life ($t_{1/2}$) was calculated as 0.693/ke, where ke is the elimination rate constant which was in turn calculated as the slope of the regression line of natural log transformed plasma concentration-time profile curve. The area under the plasma concentration-time curve (AUC_{0-t}) was calculated from the measured levels, from time zero to time of last quantifiable level, by the linear trapezoidal rule. $AUC_{0-\infty}$ was calculated using the formula: $AUC_{0-\infty} = AUC_{0-t} + C_{last}/ke$, where C_{last} is the last quantifiable plasma level.

Statistical analysis: An analysis of variance (ANOVA) was performed on pharmacokinetics parameters like C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ for both test and reference preparations using general linear model (GLM) procedures in which sources of variation were subject, treatment and period. The 90 % confidence interval of test/reference ratios for C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ and their log transformed values were determined. Bioequivalence between the formulations can be concluded when the 90 % confidence interval for the pharmacokinetic parameters of the two products are found within the acceptable range of 80-125 %.

RESULTS AND DISCUSSION

The demographic data of the subjects indicated that they formed a homogenous population in terms of age, weight and height. No adverse events were reported during the entire study and the drugs were well tolerated by the volunteers. No dropouts were reported as well.

The analytical method described for the simultaneous determination of both metoprolol and ramipril in human plasma was shown to be accurate and sensitive. Atenolol was used as an internal standard in the analysis. Metoprolol, ramipril and atenolol were eluted at retention times of 0.80, 0.92 and 0.32 min, respectively. No plasma interference was seen at the retention time of the analytes. The peaks of metoprolol, ramipril and atenolol were well resolved.

Tables 1 and 2 summarizes the mean values of pharmacokinetic parameters obtained after administration of test and reference preparations. The mean plasma concentration-time profile graphs obtained after administration of the test and reference preparations of metoprolol and ramipril are shown in Figs. 1 and 2, respectively. The parameters tmax and $AUC_{0-\infty}$ are related to the rate and extent of absorption, respectively, while C_{max} is related to both the processes. The extent of absorption is a key characteristic of a drug formulation and therefore the AUC is an important parameter for analysis in a comparative bioavailability study. However, the other two parameters, t_{max} and C_{max} are also important features of plasma level profile that are related to the therapeutic use of many drugs and hence are also considered in the analysis.

TABLE-1
PHARMACOKINETIC PARAMETERS OF METOPROLOL TARTRATE
100 mg SR (MEAN ± SD) WITH TEST AND REFERENCE PREPARATIONS

Parameter	Reference	Test
C_{max} (ng/mL)	108.38 ± 3.80	105.85 ± 3.17
AUC_{0-t} (ng h/mL)	947.43 ± 23.62	923.83 ± 19.28
$AUC_{0-\infty}$ (ng h/mL)	985.56 ± 24.51	958.10 ± 18.87
T_{max} (h)	4.00 ± 0.00	4.00 ± 0.00
$t_{1/2}$ (h)	4.63 ± 0.13	4.63 ± 0.04
Kel (h^{-1})	0.15 ± 0.004	0.15 ± 0.001

TABLE-2
PHARMACOKINETIC PARAMETERS OF RAMIPRIL 10 mg IR
(MEAN ± SD) WITH TEST AND REFERENCE PREPARATIONS

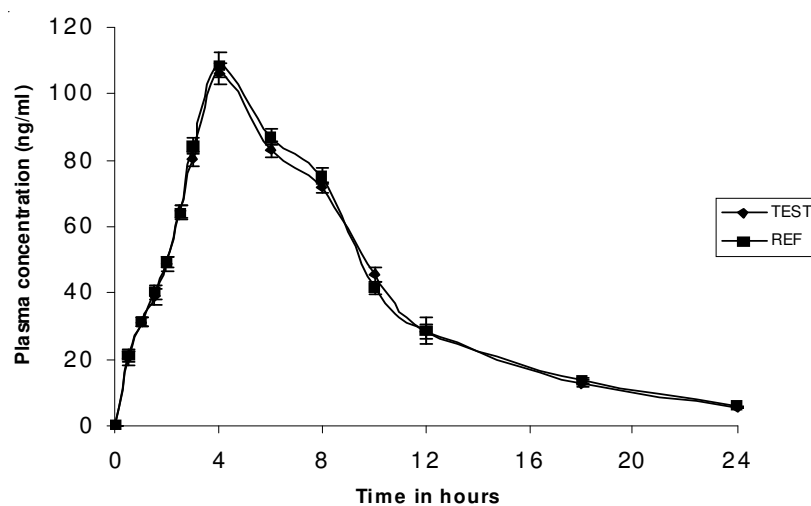| Parameter	Reference	Test
C_{max} (ng/mL)	26.940 ± 1.510	25.790 ± 2.360
AUC_{0-t} (ng h/mL)	67.202 ± 1.920	64.791 ± 3.010
$AUC_{0-\infty}$ (ng h/mL)	79.446 ± 2.260	77.670 ± 2.901
T_{max} (h)	0.583 ± 0.204	0.583 ± 0.204
$t_{1/2}$ (h)	1.504 ± 0.125	1.563 ± 0.114
Kel (h^{-1})	0.464 ± 0.042	0.445 ± 0.035

Fig. 1.   Mean plasma concentration profile of test and reference preparations of
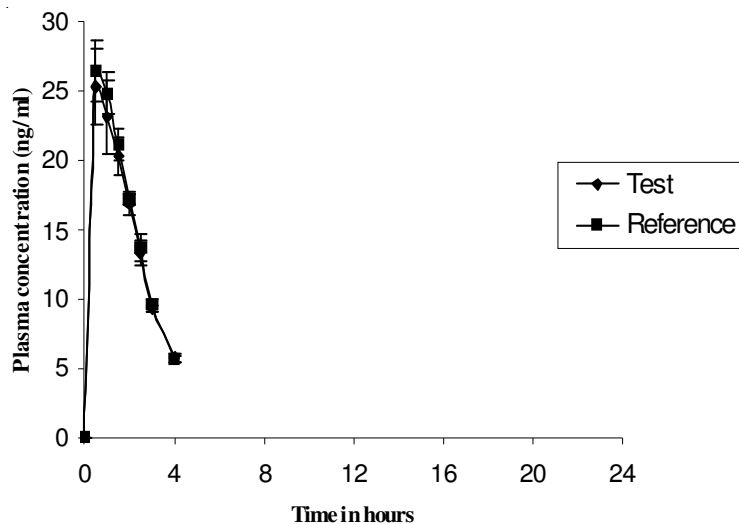metoprolol tartrate 100 mg SR in 6 healthy volunteers

Fig. 2.   Mean plasma concentration profile of test and reference preparations of
ramipril 10 mg in 6 healthy volunteers

The mean t_{max} values were 4.0 and 0.583 h for metoprolol and ramipril, respectively. The mean elimination half life of metoprolol was 4.63 for both test and reference and 0.445 and 0.465 h^{-1} for test and reference preparations of ramipril. Thus, a wash-out period of two weeks was sufficient due to the fact that no sample prior to the administration in phase II showed any level of the two drugs.

The 90 % confidence interval for the ratio of logarithmically transformed $AUC_{0-\infty}$ values were in the range of 0.99-1.00 for metoprolol and 0.987-1.001 for ramipril (Table-3). The values of 90 % confidence interval for untransformed and log tramsformed values of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were well within the acceptable bioequivalence limit of 0.80-1.25[19,20].

TABLE-3
90 % CONFIDENCE INTERVAL FOR TEST AND REFERENCE
PREPARATIONS OF METOPROLOL TARTRATE AND RAMIPRIL

Pharmacokinetic parameter	CI Range	
	Metoprolol tartrate 100 mg SR	Ramipril 10 mg IR
C_{max}	0.9336-1.0169	0.8999-1.0564
ln C_{max}	0.9863-1.0036	0.9666-1.0055
AUC_{0-t}	0.9502-1.0042	0.9327-0.9970
ln AUC_{0-t}	0.9927-0.9999	0.9834-0.9992
$AUC_{0-\infty}$	0.9481-0.9961	0.9476-1.0077
Ln $AUC_{0-\infty}$	0.9924-1.0000	0.9879-1.0016

Variation in the $AUC_{0-\infty}$ values among the subjects can be attributed to the differences in the body weight and drug disposition among the volunteers. The values of pharmacokinetic parameters obtained for metoprolol were relatively comparable with those reported by other workers[21,22]. The results of ramipril pharmacokinetics were compared with results of ramipril 5 mg dose reported earlier[23,24]. The findings indicated that ramipril follows a dose dependent pharmacokinetics. This may be due to the different patient population groups employed in the studies. The relative bioavailability based on C_{max} was found to be 97.66 %, 95.73 % for metoprolol and ramipril, respectively. The overall bioavailability judged from AUC_{0-t} was found to be 97.50 % for metoprolol and 96.41 % for ramipril.

Conclusion

Statistical analysis of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ clearly indicated no significant difference between two individual reference formulations and test preparation containing fixed dose combinations of metoprolol and ramipril. The confidence intervals for the ratios of the mean of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ indicated that these values are well within the bioequivalence acceptable range of 0.80 to 1.25. Hence, bioequivalence between two formulations can be concluded.

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