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Bioequivalence Study of Fixed Dose Combination of Atorvastatin and Ezetimibe Tablet in Healthy Volunteers by LC-MS/MS Method

K. VEERAN GOWDA, D. SENTHIL RAJAN, U. MANDAL, W.D. SAM SOLOMON, P. SENTHAMIL SELVAN, A. BOSE, A.K. SARKAR, T.K. CHATTARAJ† and T.K. PAL*
Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700 032, India
E-mail: tkpal_12@yahoo.com, mailveeran@yahoo.com

This study presents the results of two period, two treatment, cross-over investigations on 12 healthy male volunteers to assess the bioequivalence of two fixed dose combination (FDC) products of two manufacturers containing atorvastatin 10 mg and ezetimibe 10 mg. The two products tested were capsule Zetitor-10 (Caplin Point Laboratories Ltd., Pondichery, India) as a reference product and test product containing atorvastatin (10 mg) and ezetimibe (10 mg) caplsule. Both products were administered orally as a single dose separated by a one-week washout period. Atorvastatin and ezetimibe were identified and quantified using LCMS/MS for the pharmacokinetic study. The results of this investigation indicated that there were no statistically significant diffrences between the two products in either the mean concentration-time profiles or in the obtained pharmacokinetic parameters, including area under the serum concentration-time curve concerning the relative extent of absorption, assessed by the AUC ratio (Test/Reference). The average value was found to be 1.00 ± .09 with a 90% confidence limits (CL) of 0.82-1.18. Thus, these findings clearly indicate that the two products are bioequivalent in terms of rate and extent of drug absorption.

Key Words: Atorvastatin, Ezetimibe, Pharmacokinetics, Bioequivalence, Fixed dose combination, LC-MS/MS.

INTRODUCTION

A combination tablet formulation of atorvastatin and ezetimibe is beneficial in terms of its convenience and patient compliance. It is also necessary to confirm that the pharmacokinetics of the component drugs is compatible and that the bioavailability is not affected relative to concomitant administration of the two agents. The combination of statins with other agents has also in some cases increased efficacy but has likewise been limited by toxicity. Monotherapy with either ezetimibe or statins has

†N.R.S. Medical College & Hospital, Kolkata, India.

demonstrated the ability to significantly lower the low density lipoprotein cholesterol. Simultaneous administration of the two agents benefits from their two distinct mechanisms of action: inhibition of biliary and dietary cholesterol absorption by ezetimibe and inhibition of hepatic cholesterol synthesis by statins[1]. Some of the currently available fixed dose combination lipid lowering drugs[2] are extended release niacin/lovastatin, whilst atorvastatin/amlodipine, ezitimibe/simvastatin, *etc*. This paper describes the bioequivalence study of fixed dose combination product containing atorvastatin 10 mg + ezetimibe 10 mg.

Atorvastatin is hydroxyl methyl glutaryl reductase (HMG-CoA) inhibitor with lipid lowering effects. Atorvastatin 10 mg daily for 4 years was effective at reducing the risk of a first major cardiovascular event, including stroke in a large placebo controlled multicentre trial in patients with type 2 diabetes[3]. Lovastatin, pravastatin and simvastatin are derived from fungal metabolites and have half lives of 1-3 h. Atorvastatin, fluvastatin, pitavastatin and rosuvastatin are fully synthetic compounds with elimination half lives ranging from 1 to 19 h. Atorvastatin is a lipophilic compound and lipophilic statins are more susceptible to metabolism by cytochrome P450 system except for pitavastatin[4]. Statins are well tolerated and adverse events like rhabdomyolysis are rare. Various HPLC and LC-MS/MS methods for the identification of atorvastatin in tablets and human plasma have been reported[5-9].

Ezetimibe is the first member of a new class of selective cholesterol absorption inhibitors that effectively blocks intestinal absorption of dietary and biliary cholesterol without affecting absorption of fat soluble vitamins or triglycerides[10-12]. It is the first member of a new class of selective cholesterol absorption inhibitors. Absorption of ezetimibe is rapid and not altered by food[13]. The estimated terminal half life of ezetimibe and ezetimibe glucuronide is *ca.* 22 h. There are no clinically significant effects of age, sex or race on ezetimibe pharmacokinetics and no dosage adjustment is necessary in patients with mild hepatic impairment or mild to severe renal insufficiency. The major metabolic pathway for ezetimibe consists of glucuronidation of the 4-hydroxyphenyl group by uridine 5´-diphosphate glucuronosyltransferase isoenzymes to form ezetimibe-glucuronide in the intestine and liver[14]. The drug is not metabolized by cytochrome P450 system but extensive glucuronidation takes place in the intestine. So far no drug interaction has been associated with major changes in either the pharmacokinetics of ezetimibe or coadministered drugs[15].

Sistla *et al.*[16] have reported a reverse phase high performance liquid chromatography (HPLC) method for the determination of ezetimibe in dosage forms. Quantification of ezetimibe in human plasma, urine and faeces using liquid chromatography mass spectrometry (LC-MS/MS) method has been reported by Oswald *et al*[17].

EXPERIMENTAL

Study population and study design

Twelve normal subjects were enrolled in the study. The participants were aged between 20 and 40 years (mean 29.8 years), their weight varied from 53 to 89 kg (mean 72.5 kg) and height from 166 to 185 cm (mean 172.6 cm). All of them underwent a prestudy examination, ECG recording and hematological and urine analysis 7 d prior to the study. Approval from Drugs Control General of India (DCGI) and Institutional Ethical Committee of Jadavpur University was obtained prior to the start of the study. All subjects gave written informed consent before participation.

The study was a single dose, fasting two period, two way crossover study with a wash out period of one week. All the volunteers assembled in Clinical pharmacology unit (CPU) ward at 6.00 am on the study day of each session, after overnight fasting of 10 h. Their total pulse rate, blood pressure was recorded. The subjects received either of the study preparations. According to FDA and EMEA regulations, the sampling schedule should be planned to provide a reliable estimate of the extent of absorption[18,19]. This is generally achieved if AUC_{0-t} is at least 80% of $AUC_{0-\infty}$. Usually the sampling time should extend to atleast three terminal elimination half lives of the active ingredient. Time periods between sampling should not exceed one terminal half life[20]. A total of 15 blood samples were collected at 0 h (before drug administration) and at 0.5, 1.0, 1.5, 2.0, 3.0, 3.5, 4.0, 6.0, 8.0, 12.0, 24.0, 48.0, 72.0, 96.0 h (after drug administration) in the test tubes with EDTA at each time point. Breakfast, lunch and dinner was provided after 3, 6 and 13 h, respectively after drug ingestion. Collected blood samples were centrifuged immediately, plasma was separated and stored frozen at -20ºC with appropriate labeling of volunteer code No., study date and collection time, till the date of analysis.

Analytical determinations

All the chemicals used in the analysis of the fixed dose combination were of HPLC grade. HPLC Agilent 1100 series (Agilent technologies, Waldbronn, Germany) equipped with G1312A binary pump, G1379A degasser, G1367A thermostat, G1316A thermostatted column compartment and G1323B control module was employed for the analysis. The chromatography was on Zorbax C18 column (5 μm, 50 × 4.6 mm i.d.) at 30ºC temperature. The mobile phase composition was 0.01 M ammonium formate buffer (pH 5.0) and methanol (8:92 v/v) which was pumped at flow rate of 0.2 mL/min.

Mass spectrometric detection was performed on API 3000 triple quadrupole instrument using MRM. The main working parameters of the mass spectrometer are summarized in Table-1. Data processing was performed on Analyst 1.3 software package.

TABLE-1
TANDEM MASS SPECTROMETER MAIN WORKING PARAMETERS

Parameter	Value	
Source temperature (ºC)	400	
Dwell time per transition (m s)	200	
Ion source gas (Gas 1) (psi)	25	
Ion source gas (Gas 2) (psi)	25	
Curtain gas (psi)	7	
Collision gas (psi)	7	
Ion spray voltage (V)	5500	
Entrance potential (V)	10	
	Atorvastatin / ezetimibe	Tizanidine
Collision energy (V)	45	48
Collision cell exit potential (V)	17	7
Declustering potential (DP) (V)	63	85
Ion transition for atorvastatin/ezetimibe (m/z)	559.20/440.30	253.8/44.15

Pharmacokinetic and statistical analysis

Each atorvastatin and ezetimibe plasma concentration time profile was analyzed by non compartmental methods. The plasma concentration - time profile graph obtained after administration of test and reference preparation for ezetimibe and atorvastatin are shown in Figs. 1 and 2. C_{max} and t_{max} were determined as the highest observed concentration and the time to reach the maximum concentrations, respectively. The elimination half life ($t_{1/2}$) was calculated from the slope of the terminal log linear phase. AUC_{0-t} (where t is the time at which last quantifiable concentration is observed) was calculated using trapezoidal rule. $AUC_{0-\infty}$ was calculated as the sum of AUC_{0-t} and the extrapolated area determined by dividing the observed concentration at the time of last quantifiable concentration by the slope of the terminal log linear phase[21]. An Analysis of Variance (ANOVA) was performed on the pharmacokinetic paramters C_{max} and $AUC_{0-\infty}$ using general linear model (GLM) procedures, in which sources of variation were subject, treatment and period. The 90% confidence intervals of the test/reference ratios for C_{max} and $AUC_{0-\infty}$ (log transformed) were determined. Bioequivalence between two formulations can be concluded when the 90% confidence intervals for these pharmacokinetic parameters of two products are found to be within the acceptable range of 80-125 %.

is reference formulation graph & is test formulation graph obtained by plotting time on X-axis and plasma concentration in nanogram per mL on Y-axis

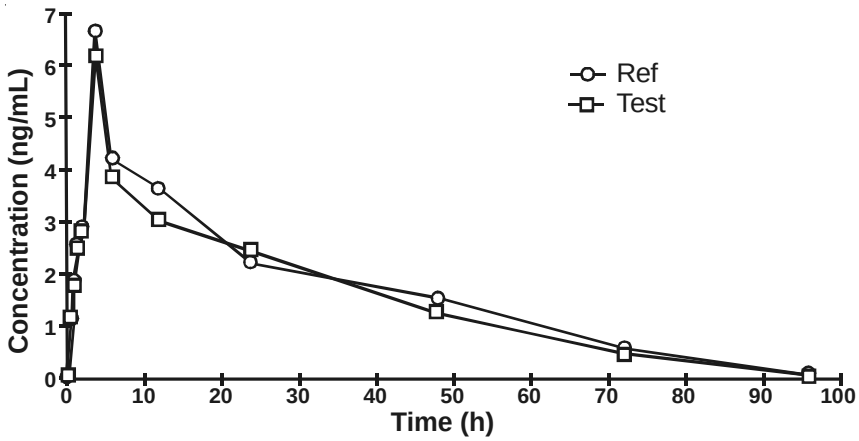

Fig. 1. Average plasma concentration-time curve of ezetimibe 10 mg after single dose administration of reference and test formulations in 12 healthy Indian male volunteers

is reference formulation graph & is test formulation graph obtained by plotting time on X-axis and plasma concentration in nanogram per mL on Y-axis

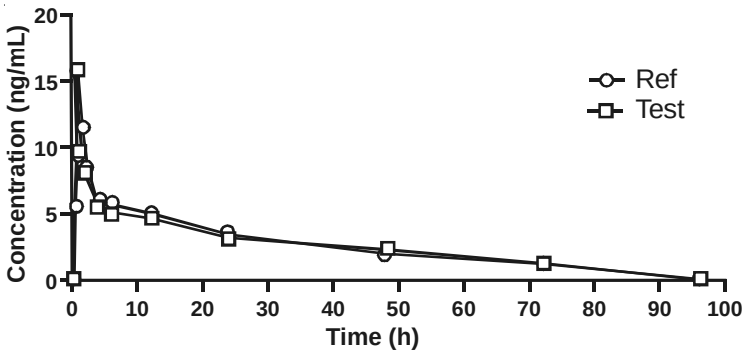

Fig. 2. Average plasma concentration-time curve of atorvastatin 10 mg after single dose administration of reference and test formulations in 12 healthy Indian male volunteers

RESULTS AND DISCUSSION

Administration of the reference preparation, capsule Zetitor-10 as a single dose in the fasting state produced the maximum plasma concentration of 14.803 ± 1.716 ng/mL (C_{max}) at the time 0.708 ± 0.257 h (t_{max}) for atorvastatin and produced the maximum plasma concentration of 5.395 ± 0.639 ng/mL (C_{max}) at the time 3.833 ± 1.801 h (t_{max}) for ezetimibe whereas the test preparation produced the maximum plasma concentration 14.331 ± 4.942 ng/mL (C_{max}) and 5.323 ± 0.675 ng/mL (C_{max}) at the time 0.667 ± 0.389 h and 3.792 ± 3.041 (t_{max}) for atorvastatin and ezetimibe, respectively (Tables 2 and 3).

TABLE-2
PHARMACOKINETIC PARAMETERS OF ATORVASTATIN (MEAN ± SD) IN 12 VOLUNTEERS WITH TEST AND REFERENCE PREPARATION

Parameters	Test	Reference
C_{max} (ng/mL)	14.331 ± 4.942	14.803 ± 1.716
t_{max} (h)	0.667 ± 0.389	0.708 ± 0.257
K_e (h^{-1})	0.019 ± 0.007	0.019 ± 0.003
AUC_{0-96} (ng h/mL)	216.076 ± 69.149	231.125 ± 11.250
$AUC_{0-\infty}$ (ng h/mL)	275.358 ± 88.796	303.344 ± 17.821
$t_{1/2}$ (h)	31.600 ± 11.833	37.486 ± 6.785

TABLE-3
PHARMACOKINETIC PARAMETERS OF EZETIMIBE (MEAN ± SD) IN 12 VOLUNTEERS WITH TEST AND REFERENCE PREPARATION

Parameters	Test	Reference
C_{max} (ng/mL)	5.323 ± 0.675	5.395 ± 0.639
t_{max} (h)	3.792 ± 3.041	3.833 ± 1.801
K_e (h^{-1})	0.020 ± 0.006	0.020 ± 0.005
AUC_{0-96} (ng h/mL)	115.945 ± 28.808	117.919 ± 20.779
$AUC_{0-\infty}$ (ng h/mL)	152.869 ± 37.512	157.949 ± 33.281
$t_{1/2}$ (h)	37.900 ± 11.139	37.493 ± 9.653

Administration of the reference preparation, capsule Zetitor-10, produced the area under plasma concentration time curve (AUC_{0-t}) 231.125 ± 11.125 ng h/mL and 117.919 ± 20.779 ng h/mL for atorvastatin and ezetimibe, respectively, whereas administration of the test preparation of FDC of atorvastatin 10 mg + ezetimibe 10 mg produced the area under plasma concentration curve (AUC_{0-t}) 216.076 ± 69.149 ng h/mL and 117.445 ± 28.08 ng h/mL for atorvastatin and ezetimibe, respectively (Tables 2 and 3).

When administered as a single dose, in the fasting state, the reference preparation, capsule Zetitor-10, produced the area under plasma concentration time curve upto infinity ($AUC_{0-\alpha}$) 303.344 ± 17.821 ng h/mL and 157.949 ± 33.281 ng h/mL for atorvastatin and ezetimibe, respectively; whereas administration of the test preparation of FDC of atorvastatin 10 mg + ezetimibe 10 mg produced area under plasma concentration time curve upto infinity ($AUC_{0-\alpha}$) 275.358 ± 88.796 ng h/mL, 152.869 ± 37.512 ng h/mL for atorvastatin and ezetimibe, respectively.

Administration of reference preparation capsule Zetitor-10, produced the plasma elimination half life, ($t_{1/2}$) 37.486 ± 6.785 h and 37.493 ± 9.653

h for atorvastatin and ezetimibe, respectively whereas administration of the test preparation of capsule atorvastatin 10 mg + ezetimibe 10 mg produced the plasma elimination half life ($t_{1/2}$) 31.600 ± 11.833 h and 37.900 ± 11.139 h for atorvastatin and ezetimibe, respectively.

Administration of the reference preparation capsule Zetitor-10, produced the plasma elimination constant (K_{el}) 0.0195 ± 0.03 and 0.020 ± 0.005 h^{-1} for atorvastatin and ezetimibe, respectively, whereas administrtion of the test preparation produced the plasma elimination constant (k_e) 0.0191 ± 0.007 h^{-1} and 0.0201 ± 0.006 h^{-1} for atorvastatin and ezetimibe, respectively.

On the basis of comparison of the AUC_{0-t} for atorvastatin 10 mg + ezetimibe 10 mg, after single dose administration, the relative bioavailability of the test preparation was 99.90% and 100.40% for atorvastatin and ezetimibe, respectively of that of the reference preparation, capsule Zetitor-10.

90% Confidence interval for C_{max}, AUC_{0-96} and $AUC_{0-\infty}$ values are summarized in Table-4. The values were well within the acceptable range of 0.80-1.25 ANOVA (subject, period, treatment) was applied to the C_{max}, ln C_{max}, Auc_{0-t} and ln AUC_{0-t} values. There was no statistically significant difference for the treatment values. The pharmacokinetic parameters of the test were comparable with that of the reference. None of the volunteers complained of any adverse reaction during the study periods.

TABLE-4
90 % CONFIDENCE INTERVAL C_{max}, AUC_{0-96} and $AUC_{0-\infty}$

Parameter	Atorvastatin (10 mg)	Ezetimibe (10 mg)
C_{max}	0.935 – 1.003	0.943 – 0.998
AUC_{0-96}	0.954 – 0.994	0.912 – 1.058
$AUC_{0-\infty}$	0.948 – 1.033	0.895 – 1.038

On the basis of the pharmacokinetic parameters studied, it can be concluded that the test preparation containing atorvastatin 10 mg and ezetimibe 10 mg, [a product of Aeon Therapeutics (India) Pvt. Ltd., Chennai, India] is bioequivalent with the reference preparation, capsule Zetitor-10, containing atorvastatin 10 mg and ezetimibe 10 mg (a product of Caplin Point Laboratories Ltd., India).

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REFERENCES

1. P. Cole and X. Rabasseda, *Drugs Today (Barc.)*, **41**, 317 (2005).
2. H. Bays and E.A. Stein, *Exp. Opin. Pharmacother.*, **4**, 1901 (2003).
3. K.F. Croom and G.L. Plosker, *Drugs*, **65**, 137 (2005).
4. M. Schachter, *Fundam. Clin. Pharmacol.*, **19**, 117 (2005).
5. S. Erturk, E. Sevinc Aktas, L. Ersoy and S. Ficicioglu, *J. Pharm. Biomed. Anal.*, **33**, 1017 (2003).
6. A. Zarghi, A. Shafaati, S.M. Foroulan and A. Khaddam, *Arzneimittelforschung*, **55**, 451 (2005).
7. M. Hermann, H. Christensen and J.L. Reubaset, *Anal. Bioanal. Chem.*, **382**, 1242 (2005).
8. M. Jemal, Z. Ouyang, B.C. Chen and D. Tentz, *Rapid Commun. Mass Spectrom.*, **13**, 1003 (1999).
9. W.W. Bullen, R.A. Miller and R.N. Hayes, *J. Am. Soc. Mass Spectrom.*, **10**, 66 (1999).
10. L. Reyderman, T. Kosoglou, P. Statkevich, L. Pember, T. Boutros, S.E. Maxwell, M. Affrime and V. Batra, *Int. J. Clin. Pharmacol. Ther.*, **42**, 512 (2004).
11. M. Farnier, *Int. J. Clin. Pract.*, **56**, 611 (2002).
12. J. Ducobu and J. Sternon, *Rev. Med. Brux.*, **25**, 451 (2004).
13. F. Ezzet, G. Krishna, D.B. Wexler, P. Statkevich, *Clin Therm.*, **23**, 871 (2001).
14. T. Kosoglou, P. Statkevich, A.O. Johnson, J.F. Paolini, A.J. Bergman, *Clin. Pharmacokinet.*, **44**, 467 (2005).
15. C. Simard and J. Turgeon, *Can. J. Clin. Pharmacol.*, **10A**, 20 (2003).
16. R. Sistla, V.S. Tata, Y.V. Kashyap, D. Chandrasekar and P.V. Diwan, *J. Pharm. Biomed. Anal.*, **39**, 517 (2005).
17. S. Oswald, E. Scheuch, I. Cascorbi and W. Siegmund, *J. Chromatogr. B. Analyt. Biomed. Life Sci.*, **830**, 143 (2005).
18. US Food and Drug Administration, Centre for Drug Evaluation and Research Guidance: Bioavailability and Bioequivalence Studies for Orally Administered Drug Products - General Considerations, October (2000).
19. Committee for Proprietary Medicinal Products: Working party on the Efficacy of the Medicinal Products, Note for guidance: Investigation of Bioavailability and Bioequivalence (1991).
20. R.L. Nation and L.N. Sansom, *Pharmacol. Ther.*, **62**, 41 (1994).
21. W.A. Ritschel, in: Handbook of Basic Pharmacokinetics, Drug Intelligence, Hamilton, edn. 4, p. 588 (1992).

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