Single dose pharmacokinetics of atorvastatin oral formulations using a simple HPLC-UV method

Muhammad Sohail[1], Mahmood Ahmad*[2] and Muhammad Usman Minhas[2]
[1]Department of Pharmacy, COMSATS Institute of Information Technology, Abbottabad, KPK-Pakistan
[2]Faculty of Pharmacy and Alternative Medicine, the Islamia University of Bahawalpur, Punjab, Pakistan

Abstract: The study was aimed to assess pharmacokinetics of atorvastatin (40 mg) in healthy fasted human subjects by a simple and inexpensive high performance liquid chromatography. Experimental design of the study was a randomized, two way, two periods, crossover study (single dose in fasted conditions). Eighteen (18) healthy male volunteers were enrolled according to FDA guidelines. The plasma samples were assayed using an isocratic High Performance Liquid Chromatography (HPLC) system of Agilent technologies USA consisted of an isocratic pump with column of Thermo Electron Corporation USA (ODS hypersil C_{18} 4.6 mm x 250 mm), a UV-visible detector set at λ_{max} 237 nm. Maximum plasma concentrations (C_{max}) of atorvastatin (Mean ± SEM) for the reference product (A) found to be 13.739±0.210ng/ml & 13.374±0.145ng/ml for test product (B). T_{max} values (Mean±SEM) of atorvastatin were 1.222±0.060 hours and 1.167±0.057hours for reference and test products, respectively. The values of $AUC_{0-\infty}$ (Mean ± SEM) for the reference (A) and test product (B) were 73.955±1.715ng.h/ml and 77.773±1.858ng. h/ml, respectively. Other pharmacokinetic parameters of both products were also determined. A statistical non-significant difference between pharmacokinetic parameters has been found and both brands of atorvastatin showed the same rate and extent of absorption in healthy fasted human volunteers after single dose. A simple and cost effective HPLC method was developed and applied.

Keywords: Atorvastatin, Pharmacokinetics, HPLC, Bioavailability

INTRODUCTION

The statins inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase competitively. HMG-CoA reductase has a key role in biosynthesis of cholesterol. The HMG CoA reductase inhibitors (statins) can cause comparatively large decline in plasma cholesterol levels (Katsiki *et al*., 2010; Mihos *et al*., 2010; Mener *et al*., 2010). Generally statins are well tolerated but common muscular side effects are associated with higher doses (Davidson *et al*., 2007). Atorvastatin has good dose-response relationship for therapeutic effects (Gandelman *et al*., 2011) and only dissolution release data cannot give the clear image for the *in vivo* performance of atorvastatin. New formulations of atorvastatin must be studied for bioavailability as well as its pharmacokinetics.

Atorvastatin is an effective 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase inhibitor, shown in fig. 1. Atorvastatin is a safe and potent member of statins family; it is thought to be 100 times more potent as compared to other members of statins family (Jamshidi *et al*., 2007). Atorvastatin is included in the second generation HMG-CoA reductase inhibitors (Teraoka *et al*., 2011). Recommended dose range of atorvastatin is 10-80 mg once a day in adults and rapidly absorbed after oral administration. Maximum plasma concentration is achieved within 1 to 2 hours (Pfizer, 2009). Atorvastatin is a drug of low solubility and high permeability (BCS Class II drug) (Wu C-Y *et al*., 2005). Atorvastatin showed complex pharmacokinetics and has linear pharmacokinetic profile with respect to AUC and non-linearity is reported with respect to its C_{max}. The orally developed dosage forms of atorvastatin must be studied to confirm its bioavailability and pharmacokinetics (Gandelman *et al*., 2011).

MATERIALS AND METHOD

Chemicals

Atorvastatin was provided by PharmEvo laboratories (pvt.) limited, Karachi, Pakistan. Acetonitrile, Methanol, Phosphoric acid, were obtained from Merck-Germany. HPLC-grade water (double distilled) was used during the experimental work.

Methods

The study was a single dose, randomized; two treatments and cross over study. Volunteers had no any major clinical, medical history and had been confirmed. The age was between twenty to thirty two years, male and healthy non-smoker volunteers with approximately same body weight. Written informed consent was obtained from each volunteer before the start of study. Eighteen healthy human volunteers were scheduled to participate in the study. Each volunteer was given single dose of product (A) and product (B) 40 mg tablet orally. The study was approved by the Pharmacy Research Ethics Committee

**Corresponding author:* e-mail: ma786_786@yahoo.com

(PREC), Faculty of Pharmacy and Alternative Medicine, the Islamia University of Bahawalpur, Pakistan.

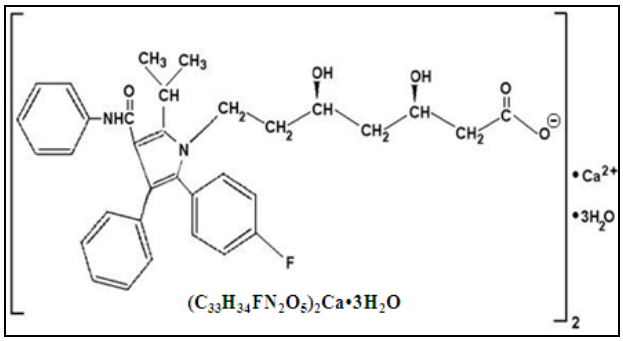

Fig. 1: Structure of atorvastatin

Sample collection and treatment

A 22-gauge cannula was inserted into the vein of forearm for blood samples collection. A blood sample was drawn before drug was given (zero time). Similarly blood samples were drawn at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 hours after the administration of atorvastatin. Each time a 5 ml blood sample was drawn. Labeled disposable plastic centrifuge tubes were used for sample collection. 100 µl of heparin was introduced in to each sample just after the collection in tubes. Blood Samples were then centrifuged at 3000 rpm for 5 minutes. The upper plasma layer was collected from each sample and stored in labeled vials. These plasma samples containing vials were immediately frozen at -20°C in freezer and kept there until assay**.**

Chromatographic Analysis

Instrumentation

An isocratic chromatographic system by Agilent technologies, USA 1200 series equipped with UV-visible variable wavelength detector. Agilent ChemStation operating software was used to control and operate the instrument. The Hypersil ODS C_{18} (5µm, 4.6mm×250mm) column was used.

Mobile phase

The double distilled deionized water and Acetonitrile were mixed at ratio of 45:55 in flask. The pH was adjusted to 2.5 using phosphoric acid, filtered (by Millipore 0.45 µm filter) and degassed in ultrasonic bath for 15 min.

Calibration curve

A calibration curve was constructed to encompass anticipated range of plasma atorvastatin concentration found in healthy volunteers. Blank plasma was spiked with atorvastatin drug solutions to give concentrations of 2.5, 5, 7.5, 10, 12.5,15 and 17.5ng/ml. Each sample solution was injected into HPLC system to produce a calibration curve that has been shown in fig. 2.

Sample preparation and analyses

A 100µl of plasma sample was taken in a glass-stoppered tube and 20µl of drug solution was added to it. Then the mixture was vortexed for 2 minutes on a vortex mixer. Then to this solution 200µl of acetonitrile was added to it and then vortexed for two minutes to achieve maximum extraction of drug to the organic phase. After this step tubes were placed in the centrifuge machine and spun at 3500 rpm for duration of 5 minutes to get the maximal separation of the two phases. Then these tubes were removed slowly and 150µl of upper organic layer was separated using micropipette and transferred to clean glass tubes and 150µl mobile phase was added to the organic layer. The mixture was then vortexed again two minutes. Then solutions were transferred to labeled epindroff tubes and were stored till injection in to HPLC system. The samples were extracted shortly before analysis.

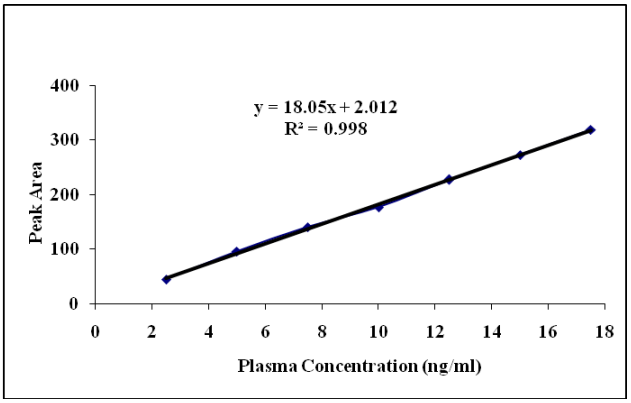

Fig. 2: Standard curve for atorvastatin in spiked plasma

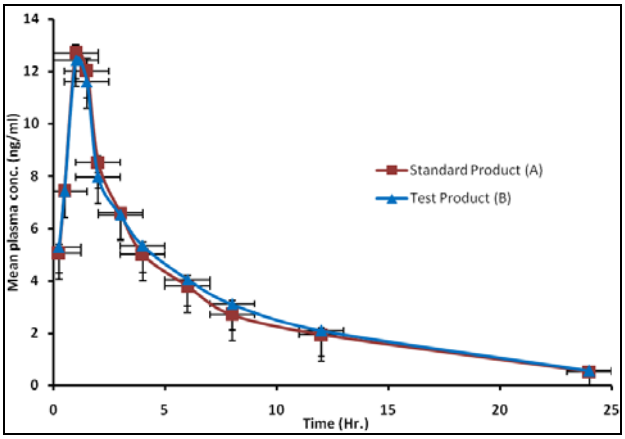

Fig. 3: Mean ± SEM plasma concentrations of atorvastatin (Product-A) (n=18) and atorvastatin (Product-B) (n=18) in human volunteers, administered in an oral dose of 40mg

Analysis was performed by using high performance liquid chromatography, equipped with a pump (Agilent 1200 series) and a variable wavelength detector (VWD, UV detector, Agilent USA). A column used was Hypersil ODS C_{18} (5µm, 4.6mm×250mm) for separation of the

sample. The mobile phase was pumped at a rate of 1 ml/minute. Sample amount injected to the system is 20µl, with a run time of 12 minutes and analyzed at λ_{max} 237 nm.

Pharmacokinetic parameters

Pharmacokinetic parameters were computed by non-compartmental method of analysis using Kinetica PK/PD software version 4.4.1 (Thermo Electron Corporation, USA) and Microsoft Excel 2007. The area under concentration time curve ($AUC_{0-\infty}$), maximum concentration of atorvastatin in plasma (C_{max}) and time to these concentrations (T_{max}) were determined by Kinetica PK/PD software. Other Bioparameters such as AUMC, MRT, $t_{1/2}$, V_d, K_e, and Cl were calculated using Kinetica.

STATISTICAL ANALYSIS

The statistical parameters mean, standard deviation (SD) and standard error of mean (SEM) were calculated using Microsoft Excel 2007 and Kinetica version 4.4.1 (Thermo Electron Corporation, USA). A software MedCal® (version 11.2.1.0.) was used to find out Paired t-test which was used to calculate the difference whether significant or non-significant at 95% confidence interval between the values of both brands of atorvastatin, product (A) a reference and product (B) a test product.

RESULTS

The maximum plasma concentrations (C_{max}) of atorvastatin (13.374±0.145ng/ml) and (13.739±0.210 ng/ml) was found in about 1.167±0.057 hour (T_{max}) and 1.222±0.060 (T_{max}) for both test and reference products respectively. Total area under the curve (AUC), values were mean ± SD (77.773±1.858ng h/ml) and (73.955 ±1.715ng h/ml) for both test and reference products. Comparison of mean values of plasma concentrations of atorvastatin brands are shown in fig. 3. The clearance values mean ± SD (0.519±0.012ml/min) and (0.519± 0.012ml/min) for both test and reference products respectively. The half –life ($t_{1/2}$) expressed in hours are mean ± SD (6.756±0.309) and (6.654±0.164) for test and reference products respectively. The results show that all the pharmacokinetic parameters for both atorvastatin tablets are statistically non-significant. Bioequivalence and Pharmacokinetic parameters of atorvastatin, Test and reference products are presented in table 1.

DISCUSSION

Statistical evaluation of the two atorvastatin products does not show any significant difference as shown in table 1. For the determination of bioavailability and pharmacokinetic parameters, 40mg of atorvastatin was administered to each of 18 healthy human volunteers. The plasma drug concentrations were determined by sensitive and validated HPLC method. The plasma concentrations at different time intervals were used to compute the bioavailability and pharmacokinetic parameters of drug in two different groups.

In the present study, the values for C_{max} are found in the range of 12.271-13.998ng/ml (Mean ± SEM, 13.374± 0.145ng/ml) for test product (B) and 11.038-15.036ng/ml (Mean ± SEM, 13.739±0.210ng/ml) for standard product (A). The values are in agreement with the previous study conducted by Le Ma *et al.,* (2007) where they administered two tablets of atorvastatin 20mg to 18 Chinese volunteers and found a value of C_{max} (Mean± SD) as 9.54±3.68ng/ml in test and 8.54±5.06ng/ml in reference product. Similarly, in another pharmacokinetic study conducted by Kantola *et al.*, (1998) the C_{max} value was reported to be 13.4±9.5µg/l, by administering 40 mg of atorvastatin, which is also comparable with the present study.

In the present study, T_{max} values of test product and standard product in human volunteers were 1.167± 0.243h (Mean ± SD) and 1.222±0.256hr (Mean ± SD), respectively. The range 1-1.5 hr for test product and the same range of 1-1.5 hr for standard product was seen in this study. These values for T_{max} are similar as previously reported values of T_{max} ranging from 1.36±0.68hr (Ma Le *et al*., 2007). Another pharmacokinetic study conducted by Kantola *et al.,* (1998) reported T_{max} as 1hr, administering 40mg of atorvastatin, which is also similar to the present study. Similarly a previous pharmacokinetic study performed by Lins RL *et al.,* (2003) reports same results for T_{max} as 1 hr similar to this study.

In the present study, the value of $AUC_{0-\infty}$ (Mean ± SEM) for test product & standard product in healthy volunteers is 77.773±7.884ng. h/ml and 73.955±7.278ng. h/ml, respectively. There is statistically no significant difference ($P>0.05$, 0.2365) between $AUC_{0-\infty}$ of test and standard products. In the previous bioequivalence study conducted by Le Ma *et al.,* (2007) on healthy Chinese volunteers, $AUC_{0-\infty}$ (Mean ± S.D) was found to be 58.32±23.09ng. h/ml and 59.44±21.88ng. h/ml for standard and test products, respectively. This is similar to the values of $AUC_{0-\infty}$ in the present study.

It can be concluded on the basis of statistical non-significant difference between pharmacokinetic parameters (C_{max}, T_{max}, $AUC_{0-\infty}$, $AUMC_{0-\infty}$, $t_{1/2}$, Ke, MRT, V_d and Cl_T) and the value of Relative Bioavailability (105.0%) that both brands of atorvastatin showed the same rate and extent of absorption in healthy human adult male volunteers. Therefore, Product-A and Product-B have comparable pharmacokinetics when administered in same oral doses under the same experimental conditions (single dose in human healthy adult male and fasted subjects).

Table 1: Pharmacokinetic parameters of atorvastatin (Mean ± SEM) administered in an oral dose of 40 mg in healthy human volunteers (n=18)

Parameters	Product-A (Mean ± SEM)	Product-B (Mean ± SEM)
C_{max}(ng/ml)	13.739±0.210	13.374±0.145^{ns}
T_{max}(h)	1.222±0.060	1.167±0.057^{ns}
$AUC_{0-\infty}$(ng h/ml)	73.955±1.715	77.773±1.858^{ns}
$AUMC_{0-\infty}$(ng h^2/ml)	623.518±20.771	694.572±31.073^{ns}
MRT (h)	8.403±0.127	8.903±0.285^{ns}
K_{el} (h^{-1})	0.105±0.003	0.105±0.003^{ns}
$t_{1/2}$ (h)	6.654±0.164	6.756±0.309^{ns}
V_d(L/Kg)	5.249±0.190	5.062±0.252^{ns}
Cl (ml/min)	0.519±0.012	0.519±0.012^{ns}

ns=non-significant, P value >0.05

The value of Relative Bioavailability (105.0%) that both brands of atorvastatin showed the same rate and extent of absorption in healthy human adult male volunteers. Therefore, Product-A and Product-B are bioequivalent when administered in same oral doses under the same experimental conditions (single dose in human healthy adult male and fasted subjects).

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