DETERMINATION OF PHARMACOKINETIC PROFILE OF HIGH ORAL DOSE OFAN INNOVATOR BRAND OF ATORVASTATIN IN HEALTHY PAKISTANI VOLUNTEERS

Imrana Maqsood, Zarafshan Bader*, Ayesha Janjua*, Shezadi Sabah Imran, Aroosa Ishtiaq Butt, Mehwish Nawaz Qaisrani***

Fazaia Medical College, Islamabad Pakistan, *Foundation University Medical College, Islamabad Pakistan, **Wah Medical College, Wah Cantt/National University of Medical Sciences (NUMS) Pakistan

ABSTRACT

Objective: To observe the pharmacokinetic pattern of an innovator atorvastatin formulation, among Pakistani healthy volunteers.

Study Design: Quasi-experimental design.

Place and Duration of Study: It was conducted at research center of Army Medical College, from Dec 2011 to Aug 2012.

Methodology: Twenty-four healthy male volunteers among Pakistani population participated in present study after approval by ethical committee. Each subject was given single dose of two oral tablet of atorvastatin each containing 40mg of atorvastatin after an overnight fast. Blood samples were collected at different time interval up to 48 hours. Atorvastatin was detected in human plasma using validated methodology involving use of highly sensitive HPLC technique. Acetonitrile and 0.05M sodium phosphate buffer were used for mobile phase and for internal standard progesterone was used. Pharmacokinetic indicators, including C_{max}, T_{max}, t1/2, $AUC_{(0\text{-}t)}$, and $AUC_{(0\text{-infinity})}$, were calculated by using Computer program (APO, MWPHARM, and Ver. 3.60).

Results: Pharmacokinetic parameters of 80 mg of tablet atorvastatin were observed in human plasma after oral administration of single oral dose. Drug was detected in plasma with mean peak concentration C_{max} 23.97 µg/l and time to reach peak concentration T_{max} 3.29 hours. Elimination half-life and elimination rate constant of atorvastatin was 6.11 ± 2.21 hours and 0.13 ± 0.06 l/hr respectively. Applying trapezoidal rule (t=48), for calculating AUC, it was to be found 175.24 ± 59.28 hr/µg/l for $AUC_{0\text{-}48}$ and was 174.28 ± 60.50 hr/µg/l for $AUC_{0-\infty}$ MRT for atorvastatin was calculated as 8.81 ± 3.20 hours.

Conclusion: Pharmacokinetic profile of branded atorvastatin formulation in Pakistani population was in close comparable limits with pharmacokinetic of atorvastatin in different ethnic groups.

Keywords: Atorvastatin, HPLC-UV, Hyperlipidemia, Pharmacokinetic studies, Statins.

INTRODUCTION

Hyperlipidemia is a major health issue all over the world. Occurrence of this metabolic disorder has increased gradually both in developed as well as in developing countries like Pakistan due to increase sedentary life style[1]. In hyperlipidemia there is usually an increase levels of plasma lipids, including cholesterol, cholesterol esters, phospholipids and triglycerides or increase in plasma lipoproteins like very low-density lipoprotein (VLDL), low-density lipo-protein (LDL), and decrease in high-density lipoprotein (HDL) levels[2]. Hyperlipidemia is the major leading cause for occurrence of cardiovascular diseases (CVD)[3]. WHO has highlighted CVD a major cause of death in all over the world, which is contributing to about more than 30% of all deaths. The rate of death due to this disease is 82% in low- and middle-income countries and according to a prediction, about 23.6 million people will die from CVD by the end of year 2030[4]. Statins, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, are lipid lowering drugs, have brought a big revolution in treatment of CVD[3]. They are useful in treatment of hyperlipidemia by decreasing LDL cholesterol, reducing triglycerides, and slightly raising HDL levels. Statins are found to be effective in primary and

Correspondence: Dr Imrana Maqsood, Associated Professor, Department of Pharmacology & Therapeutics, Fazaia Medical College, Islamabad Pakistan

Received: 24 Apr 2019; revised received: 10 Dec 2019; accepted: 16 Dec 2019

secondary prevention of coronary artery disease[5]. Statins act primarily by reversible inhibition of HMG-CoA reductase which catalyzes the conversion of HMG-CoA to mevalonate, a rate-limiting step in cholesterol biosynthesis, in this way decrease the endogenous cholesterol production[6]. Lovastatin, was the first statin approved for use, other approved statins which are now available worldwide are simvastatin (1991), pravastatin (1991), fluvastatin (1994), atorvastatin (1997), rosuvastatin (2003), and pitavastatin (2009)[7]. Atorvastatin, FDA approved anti hyperlipidemic agent, is an important member of statin family, chemically it is [R- (R*, R*)]-2-(4-fluorophenyl)-β, δ-dihydroxy-5- (1-methylethyl)-3-phenyl4 [(phenylamino) carbo-nyl]-1 H-pyrrole-1-heptanoic acid. It is abso-bed rapidly after oral administration with oral bioavailability of about 14%[8]. Peak plasma levels are obtained within 1–2 hours. It is metabolized extensively by cytochrome P450 3A4 (CYP3A4) into two major active metabolites, ortho- and parahydroxy atorvastatin[9].

Different chromatographic methods have been used for quantification of atorvastatin and its metabolites in human plasma but the methodology used in our study was simple, sensitive and accurate for estimation of atorvastatin and it was further modified by using progesterone as internal standard. Lipitor is being widely used for pharmacokinetic and bioequivalence studies of atorvastatin. Although similar studies have been performed by using low doses of atorvastatin among Pakistani population but high dose(80mg) pharmacokinetic data of this important, most widely used antihyperlipidemic innovator product is very limited. So, our objective was to obtain pharmacokinetic profile of 80mg lipitor under indigenous conditions.

METHODOLOGY

A quasi-experimental study was conducted in centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College, Rawalpindi, and was completed in nine months, from December 2011 to August 2012. It followed the guidelines laid on current Good Clinical Practices[10] and the Declaration of Helsinki[11]. Ethics Committee of Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College, Rawalpindi, approved the study protocol (certificate No.02/CREAM-A). Sample size was calculated using epi tools with precision of 1, confidence level of 0.90 and population standard deviation of 2.94[12]. The twenty-four healthy male subjects were selected by using convenient sampling technique. All participants were informed about nature, purpose, related investigational procedures and possible consequences of study. They were enrolled after signing an informed written consent. Medical health fitness of each participant was assessed by taking complete history and by medical examination and laboratory tests. Subjects with significant medical history of any disease, history of smoking, drug abuse and any kind of drug allergy were excluded.

Calcium salt of atorvastatin for calibration, progesterone as an internal standard, HPLC grade water, HPLC grade methanol and acetonitrile, sodium dihydrogen phosphate, disodium hydrogen phosphate, o-Phosphoric Acid, sodium hydroxide and sodium chloride were used. For pharmacokinetic study of atorvastatin, method developed by using high performance liquid chromatography (HPLC) with ultraviolet (UV) detector was followed[13,14]. Methodology was slightly changed by using progesterone as internal standard.

Two tablets of an innovator brand of atorvastatin, by (Pfizer, Pakistan), each containing 40mg of atorvastatin, were given as a single oral dose to every participating individual with an overnight fastof 8-10 hours. No food was allowed until four hours after dosing. Repeated blood sampling was done by putting an indwelling cannula. Each time 5ml of blood was drawn. First blood sample was taken prior to administration of drug (0hrs) then at 0.15, 0.30, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 8, 12, 24, 36, 48 hours after dosing. Immediate centrifugation of blood samples was done at 4500 × g for 5 min, after transferring them into heparinized tubes. Plasma obtained after

centrifugation of blood samples were stored at -80°C until analysis.

Chromatographic Conditions And Preparation of Standard Solutions

Pharmacokinetic parameters of an innovator brand of atorvastatin were calculated by using High Performance Liquid Chromatography (HPLC) method. HPLC by Perkin-Elmer, UV-Vis detector, Column Oven and Vacuum degasser (Series 200) was used. HPLC system had C18-RT analytical column, Merck (150×4.6mm I.D, 5µm) and guard column (1cm ×4.0mm I.D., 5µm particle size) for chromatographic separation. Wavelength of UV– detector was at 247nm and column oven temperature was set at 62°C. Mobile phase was prepared daily by combining 0.05M solution of sodium phosphate buffer and acetonitrile in concentration of 40% and 60% respectively. Calcium salt of atorvastatin was dissolved in methanol to prepare stock solution containing atorvastatin 1mg/ml. Stock solution of progesterone was prepared in 50:50 acetonitrile: water with a target concentration of 1 mg/ml. Sodium phosphate buffer (0.5 M) was prepared in water and adjusted to pH of 5.5. All the stock solutions were kept in a refrigerator at 4°C and were usable for at least 4 weeks.

Now 750µl of acetonitrile, 200µl saturated solution of NaCl and 20µl of progesterone all were added in 500µl of plasma obtained after centrifugation. All chemicals were mixed by vortex mixer, then centrifuged for 15 minutes[13]. Supernatant obtained was transferred into autosampler vials for analysis.

Calibration Curve and Pharmacokinetic Data Analysis

Calibration curves were prepared for atorvastatin and progesterone by spiking their standard solutions with drug free plasma. Calibration curves were made by linear least-squares regression analysis which plotted peak-area ratios of AT/I.S. versus the atorvastatin concentrations in solution and it was found linear as shown in fig-1. Pharmacokinetic parameters of each subject were obtained by applying one compartment model and using computer software programmer, APO, MW PHARM version 3.60 Mediware Holland. The maximum plasma concentration (C_{max}) and the time at which maximum concentration produced (T_{max}) were calculated directly from concentration time data (fig-2). Area under the curve (AUC h/µg/L) was calculated by applying trapezoidal rule. Statistical analysis of pharmacokinetic data was done by using SPSS version 15.0 and Microsoft Excel with Statistical Data Analysis Tool Pack version 2007, SP1 (MSO).

RESULTS

Twenty-four healthy male Pakistani volunteers with mean age 25.75 ± 4.17 years, mean weight 64.41 ± 5.33 kilograms and mean height of

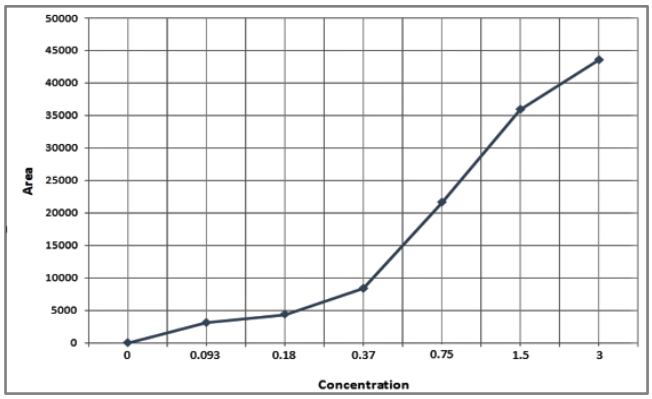

Figure-1: Calibration curve.

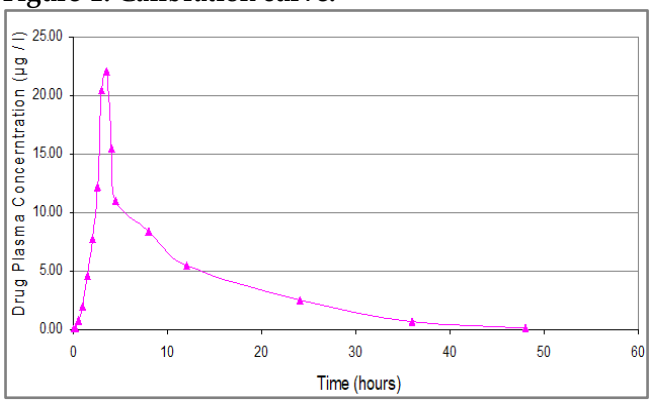

Figure-2: Plasma concentration-time curve.

171.76 ± 4.22 cms were enrolled for this study. All the enrolled subjects participated till the end of study and no drug reaction was observed in any participant. Atorvastatin after oral administration absorbed from the gastrointestinal tract and all

subjects showed detectable amount of atorvastatin in their plasma after a mean lag time of 0.61 ± 0.35 hours. The pharmacokinetic parameters of 80mg of atorvastatin were obtained by applying one compartment model and using computer software programmer, APO, MW PHARM. are summarized in table. By applying trapezoidal rule (t=48) mean value of area under the curve (AUC_{0-48}) and ($AUC_{0-\infty}$) for atorvastatin was calculated.

Table: Pharmacokinetic parameters of atorvastatin.

Pharmacokinetic Profile of atorvastatin (Lipitor)- 80mg	
Lag time (h)	0.61 ± 0.35
Absorption rate constant, ka (l/h)	0.76 ± 0.87
Absorption half-life, t1/2 (h)	1.89 ± 1.37
Volume of distribution, Vd (L)	4867.92 ± 3785.33
$AUC_{(0-48)}$ (h.µg/l)	175.24 ± 59.28
$AUC_{(0-\infty)}$ (h.µg/l)	174.28 ± 62.50
Time to peak, T_{max} (h)	3.29 ± 0.25
Peak concentration, C_{max} (µg/l)	23.97 ± 7
Elimination Half Life, t1/2 (h)	6.11 ± 2.21
Elimination Rate Constant, k (l/h)	0.13 ± 0.06
Mean Residence Time, MRT(h)	8.81 ± 3.20
Clearance, CL (l/h)	571.07 ± 447.72

DISCUSSION

In present study pharmacokinetic profile of an innovator brand of atorvastatin was studied in twenty-four healthy Pakistani male volunteers after giving them orally a single dose of 80mg of atorvastatin. In present study, pharmacokinetics of atorvastatin in local population under indigenous conditions have been studied and the obtained pharmacokinetic data of a standard reference drug was then compared with the data collected from other South-Asian countries. Single oral dose of 80 mg of atorvastatin from Pfizer pharmaceutical company were administrated in fasting subjects and serial blood sampling was done at up to 48 hours at suitable time intervals. Atorvastatin was absorbed rapidly from gastrointestinal tract and was detected in plasma soon after one hour of administration. This absorptive parameter is in line with established pharmacokinetic characteristics of atorvastatin, assessed in china in 2010[15]. Absorption half-life of atorvastatin was 1.89 hours with absorption rate constant of 0.76 hours in Pakistani subjects. Mean lag time was 0.61 hours and the apparent volume of distribution (Vd) was estimated to be 4867.92 liters. Pharmacokinetic comparison of these parameters with other studies could not be done as lack of availability of data for these profiles. The peak plasma concentration C_{max} was 23.97 ± 7.0 µg/l and mean time to reach the peak concentration T_{max} was 3.29 hours after administration of 80mg of atorvastatin orally. A pharmacokinetic study done in healthy Indian population showed C_{max} 74.94 ± 56.01ng/ml and T_{max} 2.13 ± 1.20 hours, with 80mg of atorvastatin[16]. In another pharmacokinetic study performed in healthy Bengali volunteers C_{max} and T_{max} was found to be 26.166 ± 2.948 and 2.396 ± 0.589 respectively[12]. Comparable results of these pharmacokinetic parameters were also shown in a study conducted in healthy Pakistani subjects with 40mg of an innovator brand of atorvastatin in which C_{max} was 50.5 ± 0.9 and T_{max} 3.0 ± 0.217. AUC, is the area under the plasma drug concentration-time curve, it shows the body exposure to drug after administration of a given dose of the drug. It was calculated by using linear trapezoidal method[15,16]. It was 175.24 ± 59.28 hr/µg/l for $AUC_{(0-48)}$, and was 174.28 ± 62.50 hr/µg/l for $AUC_{0-\infty}$ in Pakistani subjects after oral administration of 80mg of atorvastatin. Theses results were in comparable range in a study conducted with same dose of an innovator brand of atorvastatin in Indian population[16]. $AUC_{(0-t)}$, was estimated 271.562 ± 52.318 hr/ng/ml and 309 ± 59.594 hr/ng/ml for $AUC_{0-\infty}$ in a similar study conducted in Bangladesh[12]. Therefore, both the rate and extent of absorption of 80mg of lipitor in Pakistani subjects and in people of other South-Asian countries were in closer similar limits. The elimination rate constant, Ke, can be defined as the fraction of drug that is eliminated per unit of time. It was found to be 0.13 ± 0.06 l/hr for atorvastatin in our study. It was 0.10 ± 0.04l/hr with 40mg of atorvastatin in indians[18] 180.053 ± 0.006 l/hr in Bengali subjects[12]

and was found to be 0.089 ± 0.025 l/hr in healthy Chinese volunteers[19]. Mean elimination half-life of atorvastatin in present study was 6.11 ± 2.21 hours. Comparable data for elimination half-life of atorvastatin were observed in Chinese population in two different studies, which were 7.45 ± 3.34 hours[15] and 8.50 ± 2.74 hours[19]. In Indians it was calculated as 10.96 ± 2.81 hours[16] and in Bengalis it was 13.78 ± 1.55 hours[12]. Mean Residence Time (MRT) for 80mg of atorvastatin formulation was calculated as 8.81 ± 3.20 hours. Lack of data for this parameter in different studies made difficulty in comparison. It was observed as 12.22 ± 3.47 hours only in one study conducted in Chinese population with 20 mg of atorvastatin.

So, it is cleared from above mentioned discussion that pharmacokinetic parameters in present study are consistent with those obtained from Indians, Bengali and Chinese populations.

CONCLUSION

The pharmacokinetic data obtained after oral administration of 80mg of an innovator brand of atorvastatin did not show gross difference between Pakistani population and other ethnic groups mentioned in various studies. Therefore, it can be concluded that genetic makeup of healthy Pakistanis as well as environmental factors, do not play any significant role in development of pharmacokinetic variations among Pakistanis and in different ethnic groups.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

REFERENCES

1. Akram M. Epidemiology and risk factor for hyperlipidemia in Pakistan. J Nutr Disorders Ther 2017; 7(Suppl-3): 96-99.
2. Shattat GF. A Review Article on Hyperlipidemia: Types, treatments and new drug targets. Bio Med Pharmacol J 2014; 7(2): 399-409.
3. Mahmood D, Jahan K, Habibullah K. Primary prevention with statins in cardiovascular diseases: A Saudi Arabian perspective. J Saudi Heart Assoc 2015; 27(3): 179–91.
4. Biglu MH, Ghavami M, Biglu S. Cardiovascular diseases in the mirror of science. J Cardiovasc Thorac Res 2016; 8(4): 158–63.
5. Trentman TL, Avey SG, Ramakrishna H. Current and emerging treatments for hypercholesterolemia: A focus on statins and proprotein convertase subtilisin/kexin Type 9 inhibitors for perioperative clinicians. J Anaesthesiol Clin Pharmacol 2016; 32(4): 440-45.
6. McFarland AJ, Anoopkumar-Dukie S, Arora DS, Grant GD, McDermott CM, Perkins AV et al. Molecular mechanisms underlying the effects of statins in the central nervous system. Int J Mol Sci 2014; 15(11): 20607–37.
7. Newman CB, Preiss D, Tobert JA, Jacobson TA, Chin GC, Tannock LR et al. Statin safety and associated adverse events: A scientific statement from the american heart association. Arterioscler Thromb Vasc Biol 2019; 39: e38–81.
8. Choi Y, Lee S, Jang IJ, Yu KS. Pharmacokinetic interaction between fimasartan and atorvastatin in healthy male volunteers. Drug Des Devel Ther 2018; (12): 2301-09.
9. Patiño-Rodríguez O, Martínez-Medina RM, Martínez-Delgado M, Mares-GarcíaAS, Escobedo-Moratilla A. Absence of significant pharmacokinetic interaction between atorvastatin and fenofibrate: a randomized, crossover, study of a fixed-dose formulation in healthy Mexican subjects. Front Pharmacol 2015; 6(4): 1-6.
10. Vijayananthan A, Nawawi O. The importance of good clinical Practice guidelines and its role in clinical trials. Biomed Imaging Interv J 2008; 4(1): e5-9.
11. World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. J Am Med Assoc 2013; 310(20): 2191-94.
12. Khan SI, Saha SK, Poddar SK, Bachar R, Al-Shoyaib A, Chakrabarty JK, Promite S, et al. Bioequivalence studies and pharmacokinetic properties of atorvastatin 40mg tablet in healthy bengali subjects. MOJ Bioequiva Bioavailability 2017; 4(2): 241-46.
13. Zarghi A, Alireza S, Foroutan SM, Arash K. A simple and rapid HPLC method for the determination of atorvastatin in humanplasma with UV detection and its application to pharmacokinetic studies. Arzneimittel-Forschung 2005; 55(8): 451-54.
14. Bahrami G, Mohammadi B, Mirzaeei S, Kiani A. Determination of atorvastatin in human serum by reversed-phase high performance liquid chromatography with UV detection. J Chromatogr B Analyt Technol Biomed Life Sci 2005; 826(1-2): 41-45.
15. Liu YM, Pu HH, Liu GY, Jia JY, Weng LP, Xu RJ, et al. Pharmacokinetics and bioequivalence evaluation of two different atorvastatin calcium 10-mg tablets: a single-dose, randomized-sequence, open-label, two-period crossover study in healthy fasted chinese adult males. Clin Ther 2010; 32(7): 1396-07.
16. Partani P, Verma SM, Gurule S, Khuroo, Monif T. Simultaneous quantitation of atorvastatin and its two active metabolites in human plasma by liquid chromatography/(-)electrospray tandem mass spectrometry. J Pharm Anal 2014; 4(1): 26–36.
17. Sher M, Hussain G, Hussain MA, Akhtar T. Development and validation of high-performance liquid chromatographic-ultraviolet (HPLC-UV) method for the bioequivalence study of atorvastatin. Asian J Pharm Pharmacol 2012; 6(32): 2424-28.
18. Polagani SR, Pilli NR, Gajula R, Gandu V. Simultaneous determination of atorvastatin, metformin andglimepiride in human plasma by LC–MS/MS and itsapplication to a human pharmacokinetic study. J Pharm Anal 2013; 3(1): 9–19.
19. Ma L, Dong J. Development and Validation of Atorvastatin by LC–ESI–MS and application in bioequivalence research in healthy chinese volunteers. Chromatographia 2007; 65(11-12): 737-41.