PHARMACOKINETIC STUDY OF ATORVASTATIN AFTER SINGLE DOSE ADMINISTRATION AMONG PAKISTANI POPULATION

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ABSTRACT

Objective: To obtain pharmacokinetic data of Orvastin, a newly launched formulation of atorvastatin, in healthy males of Pakistan.

Study Design: It was quasi-experimental design.

Place and Duration of Study: Study was conducted at Centre for Research in Experimental and Applied Medicine (CREAM) Army Medical College, Rawalpindi and duration of study was about ten months.

Material and Methods: Twenty-four healthy male subjects were taken conveniently from Pakistani population. Two tablets of Orvastin, each containing atorvastatin 40mg, were administered orally as a single dose. Multiple blood samples were taken with small gaps in between up to the period of 48hrs. High Performance Liquid Chromatography (HPLC) with UV-detector was used for quantification of atorvastatin in plasma; wavelength of UV-detector was adjusted at 247nm. Mobile phase was made up of 60 percent acetonitrile and 40 percent 0.05M sodium phosphate buffer. Flow rate of mobile phase was maintained at 1.5ml/min with 5.5 pH. Progesterone was used as an internal standard. Stock solutions of atorvastatin were made by dissolving it into methanol and acetonitrile was used for making stock solution of progesterone. Calibration curves were made for atorvastatin and internal standard from concentration time data, values for time to achieve maximum plasma concentration. (Tmax) and maximum plasma concentration (Cmax) were directly calculated. Computer program (APO, MW PHARM, and Ver. 3.60) was used for calculation of pharmacokinetic profile of atorvastatin.

Results: Atorvastatin was detected in plasma samples of all volunteers. The absorption rate constant (Ka) was 0.41 l/hr. Cmax was 26.69 ± 6.67 µg/l and Tmax was 3.33 ± 0.41 hrs. Apparent volume of distribution (Vd), of atorvastatin, was 3244.84 ± 1237.36 liters. The elimination rate constant was 0.15 l/hr. Elimination half-life of atorvastatin was 6.14 hours. Trapezoidal rule was used for calculation of AUC_{0-48} and $AUC_{0-\infty}$ and it was found to be 208.77 h/µg/l and 208.74 h/µg/l respectively. Clearance of atorvastatin was 420.87 ± 170.64 liters/hour and Mean Residence Time (MRT) was 8.86 ± 5.01 hours.

Conclusion: Pharmacokinetic data of new formulation of atorvastatin is in comparable range with other brands of atorvastatin used in different ethnic groups.

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

INTRODUCTION

The main important cause of death all over the world in both in men and women is the coronary heart disease (CHD) which has close association with hyperlipidemia[1]. Hyperlipidemia is a metabolic disorder of lipid metabolism, presented with hypercholesterolemia and hypertriglyceridemia, the most common form of hyperlipidemia is hypercholesterolemia[2]. Hyperlipidemia is a major causative factor related with atherosclerosis of blood vessels and its associated complications, presented with coronary heart disease, cerebral strokes and peripheral vascular disease[3]. It has been estimated that by the end of year 2020, cardiovascular diseases will be the main cause of death and morbidities all over the world[4]. Elevated levels of the plasma lipids, including cholesterol, cholesterol esters, triglycerides and

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phospholipids are found in hyperlipidemia[5]. It has been shown before in different clinical studies that there are reduced chances of atherosclerosis associated complications like coronary heart disease in patients who are taking lipid-lowering drugs regularly for both primary prevention and secondary prevention[1]. Both in adults and children, for treatment of hyperlipidemia statins are mainly used[6]. These are the best selling drugs in the world, having excellent safety profile with mild adverse reactions and have documented beneficial effects in cardiovascular diseases[7]. Statins, also known as HMG-CoA reductase inhibitors are competitive inhibitor of the rate-limiting enzyme, 3-hydroxy 3-methylglutaryl coenzyme-A (HMG-CoA) reductase in cholesterol synthesis. Inhibition of this enzyme reduces hepatic cholesterol synthesis, which in turn leads to upregulation of hepatic low-density lipoprotein (LDL) receptors. Increase number of LDL receptors enhances removal of LDL-cholesterol particles from the plasma[8]. Atorvastatin is an important member of statins, chemically it is calcium (3R,5R)-7-[2-(4-fluorophenyl)-5- (1-methylethyl)-3- phenyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl]-3,5-dihydroxyheptanoate trihydrate and have anti-inflammatory, antioxidative, and antithrombotic effects which helps to decrease morbidity and mortality in patients with atherosclerosis and its associated diseases[9]. Atorvastatin is a synthetic analogue of HMG-CoA, highly effective in reducing LDL-C levels and triglycerides[10]. It is one of the most effective statin, in lowering LDL-C and triglyceride levels. It has longer plasma half-life of 14 hours and metabolized extensively by cytochrome P450 3A4 (CYP3A4) enzymes, producing two active metabolites ortho-hydroxy atorvastatin and para-hydroxy atorvastatin both of which inhibit HMG-CoA reductase activity[11]. Prolonged inhibition of HMG-CoA reductase activity by atorvastatin and its active metabolites are responsible for longer duration of action and greater efficacy of this drug in reducing plasma level of LDL-C as compared to other statins with the exception of rosuvastatin[8]. It is rapidly absorbed after oral administration and after absorption, it is extensively metabolized in liver and in gastrointestinal mucosa, so it has low bioavailability of about 14 percent[12]. Due to its low bioavailability, the quantification of atorvastatin for pharmacokinetic and bioequivalence analysis needs specific and sensitive methods.

Almost all the methods, which are used for pharmacokinetic and bioequivalence studies requires analytical techniques of gas chromatography (GC) or high performance liquid chromatography (HPLC)[13]. The method used here for pharmacokinetic study is simple, precise and accurate reversed phase HPLC method. Pharmacokinetics and pharmacodynamics properties of all available statins are different in different population groups; hence, they have small differences in respect of their clinical efficacy and adverse effects profile[14]. Polymorphism in genetic makeup and ethnic factors in different population groups may affect the pharmacokinetics of atorvastatin. Keeping these factors in mind, this study was designed to evaluate the pharmacokinetic parameters of atorvastatin particularly in people of Pakistan. The basic aim of this study project was to assess the pharmacokinetic of a national brand of atorvastatin tablet (Orvastin) in healthy male subjects. Knowledge of pharmacokinetic parameters of this new brand of atorvastatin, will help us to use it when required, with confidence as we have on use of its generic product.

MATERIAL AND METHODS

Study was carried out at the Centre for Research in Experimental and Applied Medicine (CREAM) Army Medical College, Rawalpindi and it was conducted in accordance with the requirements of the current Good Clinical Practices. Study design was Quasi experimental, sampling technique was convenient and duration of study was about ten months. Study was initiated after taking approval from Ethical Committee of CREAM at Army Medical College, Rawalpindi, Pakistan. All the participants were

well explained clearly about duration, purpose, consequences of the study and investigational procedures. Volunteers who gave written consent were then allowed to take part in study. Sample size was calculated according to guidelines for bioavailability and bioequivalence studies, according to which the minimum number of subjects should not be less than 16. Twenty-four healthy adult male volunteers were finally selected after getting medical fitness through a comprehensive medical history, medical examination and baseline laboratory tests. Females and all the males having age below 18 and above 40 years with history of smoking, drug addiction, hypersensitivity,tuberculosis and with any evidence of heart, hepatic and renal disease, or gastrointestiual tract problems were excluded. The selected participants had an average age, body weight and height of about 25.75 ± 4.17 years, 64.41 ± 5.33 Kg and 171.76 ± 4.22 cms respectively. Atorvastatin tablets introduced by Amson pharmaceutical limited were used. Two tablets of orvastin, each containing atorvastatin 40mg, were given to every participant of the study. Sixteen samples having 5ml blood of each subject, were taken periodically over the period of 48 hours after passing intravenous cannula, first sample taken prior to administration of drug (0 hours) and 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. Blood samples were immediately transferred to heparinized tubes and centrifuged for 5 min, collected plasma frozen at –80C° until analysis.

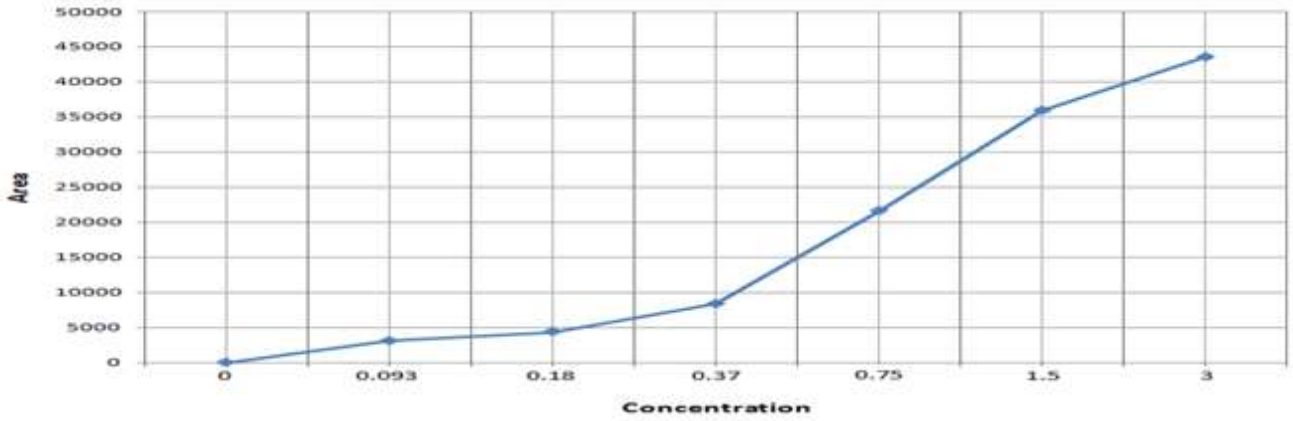

Figure-1: Calibration curve of atorvastatin.

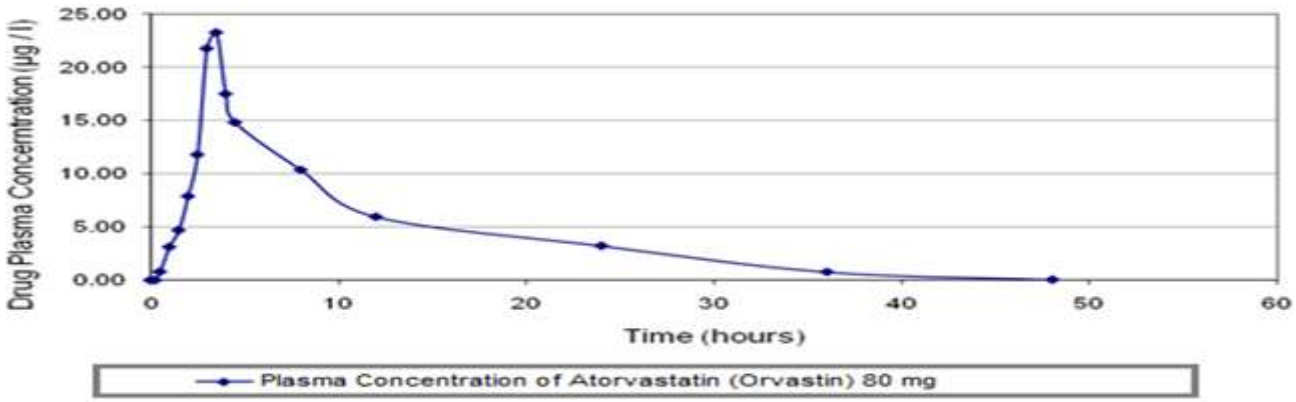

Figure-2: Plasma concentration-time curve of atorvastatin.

Drug Administration

The study subjects were given a single dose of two tablets of Orvastin orally, with a glass of water after an overnight fast of 10 hours. Subjects were allowed to take liquid after one hour and

standardized food at least four hours after dosing. No adverse reactions seen during and after the study in any of the volunteers.

Chemical reagents required in this study were calcium salt of atorvastatin, progesterone, HPLC grade methanol, HPLC grade water, sodium dihydrogen phosphate, di-sodium hydrogen phosphate (Appli Chem), acetonitrile (Sigma-Aldrich). sodium hydroxide, O-phosphoric acid (Merck) and sodium chloride (Riedel-de Haen).

Atorvastatin level in human plasma was detected by using HPLC methodology. Gradient HPLC system with UV detector and autosampler (Perkin-Elmer) along with column oven and vacuum degasser (Series 200) were used. C18-RT analytical column and guard column were for chromatographic separation of atorvastatin. Wavelength of UV–V detector was set at 247 nm. The column oven temperature was maintained at 62C°[13]. Mobile phase was made by 60% acetonitrile and 40%, 0.05M sodium phosphate buffer. The flow rate was 1.5ml/min and 5.5 was pH, adjusted for mobile phase in HPLC system.

Atorvastatin and progesterone stock solution were prepared by dissolving atorvastatin in methanol and progesterone in acetonitrile with target concentration of 1mg/ml for each stock solution. In 500 µl of plasma, 20 µl of progesterone used as internal standard, 750 µl of acetonitrile and 200 µl saturated solution of NaCl were added. Vortex mixer mixed all reagents for 30 seconds; the centrifugation of mixture was done at 6000 rpm for 15 min. 20 µl of supernatant, obtained after centrifugation, was injected into the HPLC gradient system by the autosampler[15].

Table-I: Statistical analysis of pharmacokinetic profile of orvastin.

Pharmacokinetic Profile	Statistical Analysis				
	Orvastin 80 mg				
	Mean	SD	SEM	MIN	MAX
AUC PolyexponentialAUC(0-∞) [h.µg/l]	208.74	71.51	14.60	93.85	358.70
AUC Trapezoidal Rule (t=48) [h.µg/l]	208.77	67.49	13.78	95.69	368.18
Clearance (CL) [l/h]	420.87	170.64	34.84	158.50	831.10
Volume of distribution (V) [l]	3244.84	1237.36	252.63	1262.00	5533.00
Elimination Rate Constant (k) [l/h]	0.15	0.10	0.02	0.04	0.55
Elimination Half Life (t1/2) [h]	6.14	3.47	0.71	1.26	16.71
Mean Residence Time (MRT) [h]	8.86	5.01	1.02	1.81	24.11
Mean Input Time (MIT) [h]	3.89	1.69	0.34	1.37	8.11
Absorption rate constant (ka) [l/h]	0.41	0.33	0.07	0.13	1.77
Absorption half life (t1/2_a) [h]	2.35	1.14	0.23	0.39	5.39
Lag time t0 [h]	0.50	0.29	0.06	0.08	1.46
Time to peak (Tmax) [h]	3.33	0.41	0.08	3.00	4.50
Peak concentration (Cmax) [µg/l]	26.69	6.67	1.36	14.60	38.70

Chromatograms were obtained by running samples in HPLC system and interpretation of chromatographic data was done.

By using standard solutions of atorvastatin and progesterone (IS), calibration curves were made. The calibration curve was generated by plotting the ratio of the peak area of atorvastatin and progesterone (IS) against the atorvastatin concentration in solution. Least square regression equation used for the calibration curve of plasma atorvastatin and it was found linear within the range of 0-3.0 µg/ml as shown in (fig-1). The time at which maximum concentration occurred (Tmax) and the maximum plasma concentration (Cmax) were obtained directly from concentration time data (fig-2). The area under the curve (AUC_{0-t}h/µg/L) was the plasma concentration of atorvastatin from zero time upto

the time t (48 hours). AUC_{0-t} was measured by applying the trapezoidal rule up to the last data point. Pharmacokinetic analysis of plasma atorvastatin concentrations was done by using the software (APO, MW PHARM, Ver. 3.60). The type of model applied for pharmacokinetic profile analysis was one compartment. Statistical analysis of pharmacokinetic data of all volunteers was made with the use of SPSS Ver. 15.0 and Microsoft excel with statistical data analysis tool pack Ver. 2007. Mean and standard deviation were calculated for the continuous variables.

RESULTS

The study was conducted in twenty-four adult male subjects who were declared healthy after routine medical examination. The average age, body weight and height of the volunteers were 25.75 ± 4.17 years, 64.41 ± 5.33 Kg and 171.76 ± 4.22 cms respectively. No adverse drug reactions were seen in any participants of the study. The pharmacokinetic profile of a new atorvastatin formulation have been studied after administration of single oral dose of tablet atorvastatin (80mg). The pharmacokinetic data obtained was analyzed by using software APO MWPHARM version 3.60.

Atorvastatin given orally was found to be absorbed after a mean lag time of 0.50 ± 0.29 hours from the gastrointestinal tract. It was detected in plasma of all subjects after 1 hour of drug administration, except one. The absorption rate constant (ka) for atorvastatin was 0.41 ± 0.33 l/hr. The plasma concentration of atorvastatin gradually increased with time reaching Cmax. The mean peak concentration in plasma was 26.69 ± 6.67 µg/l. Tmax was 3.33 ± 0.41 hours. The mean apparent volume of distribution (Vd) of atorvastatin, on the basis of one-compartment model, was 3244.84 ± 1237.36 liters. The elimination half-life and elimination rate constant after oral administration of atorvastatin was calculated as 6.14 ± 3.47 hours and 0.15 ± 0.10 l/hr respectively.

AUC is the mean value of area under the curve, was obtained for atorvastatin by applying trapezoidal rule (t=48) and it was found to be 208.77 ± 67.49 h/µg/l. The mean area under the plasma concentration-time curve from time zero to infinity ($AUC_{0-\infty}$) was calculated to be 208.74 ± 71.51 h/µg/l. Clearance of atorvastatin in Pakistani volunteers was 420.87 ± 170.64 liters/hour. Mean Residence Time (MRT), an important pharmacokinetic parameter, shows the time for which drug stays in body. In present study, it was calculated on basis of oral administration of 80mg tablets of atorvastatin, followed by plasma sampling extending up to forty-eight hours. MRT was observed to be 8.86 ± 5.01 hours for atorvastatin. The pharmacokinetic profile of orvastin was analyzed statistically and results obtained are shown in table-I.

DISCUSSION

This pharmacokinetic study was carried out in human volunteers, according to guidelines of the current good clinical practices and the Declaration of Helsinki. Twenty-four healthy Pakistani male volunteers participated in this study. Orvastin, a new formulation of atorvastatin, was administered as a single dose of 80 mg of atorvastatin in tablet form. Pharmacokinetic profile of atorvastatin was studied after administration of a single oral dose of 80mg of atorvastatin in tablet form in local population, under indigenous conditions. Pharmacokinetic studies play an important role in drug development. Single dose pharmacokinetic studies are usually preferred by FDA because according to FDA "single dose pharmacokinetic study are more sensitive to quantify the released drug substance from the product into the systemic circulation"[16]. A validated, precise and specific method was used for quantification of atorvastatin in human plasma. After 1 hour of drug administration, atorvastatin was detected in plasma of all participants except one, this is also shown in other studies[13,17]. The mean lag time for atorvastatin was found to be 0.50 hours and Ka was 0.41 hours with absorption half-life of 2.35 hours. Both these pharmacokinetic parameters are not sufficiently available in literature, so

could not be compared. The apparent volume of distribution (Vd) was 3244.84 liters in Pakistani volunteers. Cmax and Tmax were obtained directly from the original data and it was 26.69 µg/l and 3.33 hrs respectively. A pharmacokinetic study after administration of 40 mg of atorvastatin in humans also showed the comparable data[18]. In a study performed in European population Cmax was 23.2 ± 10.4 µg/l and Tmax was 0.25–3 hours[19] and Tmax was 1.36 ± 0.68 hrs with Cmax 8.54 ± 5.06 ng/ml with 20mg of atorvastatin, in another study done in Chinese[17]. The values for elimination rate constant, elimination half-life, and MRT were 0.105 ± 0.003l/hrs, 6.654 ± 0.164 hrs and 8.403 ± 0.127 hrs respectively[20] in a study performed in Pakistani population with 40mg of atorvastatin, which are also in accordance to the values in present study. The AUC was estimated by use of the linear trapezoidal method[21]. The $AUC_{0-\infty}$ was 208.74 ± 71.51µg/l and AUC_{0-48} was calculated to be 208.77 ± 67.49µg/l in study subjects, again both these parameters are in comparison with other studies like AUC_{0-t} 102 ± 50.9 ng/ml and $AUC_{0-\infty}$ was 130 ± 54.2 ng/ml, after 40mg of atorvastatin in Indian population[22], similarly AUC_{0-48} was 54.77 ± 21.82 ng/ml and $AUC_{0-\infty}$ was 58.32 ± 23.09 ng/ml in Chinese population[17]. Therefore, it was found that pharmacokinetic data of atorvastatin collected through this study in Pakistani volunteers was within comparable limits with pharmacokinetic profile observed in other studies. There was no gross difference in pharmacokinetic parameters of atorvastatin in Pakistani population and among different ethnic groups in the present study. So the overall pharmacokinetic profile of atorvastatin in healthy male Pakistani subjects is in comparable range to other ethnic groups.

CONCLUSION

Pharmacokinetic studies play an important role in new drug development. It give us understanding about main characteristic of a drugs like absorption, distribution, metabolism and excretion. The pharmacokinetic data of a new formulation of atorvastatin (orvastin) obtained in this study are in comparable limits as obtained from different ethnic groups. This study is the first step towards the bioequivalence and if this drug (orvastin) found to be bioequivalent to innovator brand of atorvastatin, this will help us to use an efficacious and cost effective drug instead of using expensive brands of atorvastatin in treatment of hyperlipidemia.

RECOMMENDATIONS

As the pharmacokinetic data of orvastin is in comparable range with pharmacokinetic data of atorvastatin obtained from different formulations, so the bioequivalence study of orvastin should be conducted in future to use it as confidently like other international brands of atorvastatin.

CONFLICT OF INTEREST

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

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