Pharmacokinetic and Bioavailability Studies of Commercially Available Simvastatin Tablets in Healthy and Moderately Hyperlipidemic Human Subjects

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Summary: Simvastatin, an analogue of Lovastatin, is a HMG.CoA reductase inhibitor. It is widely used in the treatment of hyperlipidemia and coronary heart disease (CHD) with low incidence of myopathy and rhabdomyolysis. As these diseases may alter the pharmacokinetics of drugs, the present study was aimed to elaborate the variation in the pharmacokinetics and bioavailability of simvastatin in local healthy and moderately hyperlipidemic population. Open, single dose and parallel design was applied to study. A total of 36 male volunteers were used for healthy and moderately hyperlipidemic groups (n = 18 for each) in this study on the basis of screening procedures, body chemistry and physical examination. Simvastatin 40 mg tablets (Saista 40, Bosch, Pakistan) were administered to over-night fasted volunteers. Blood samples were collected before dosing (zero time) and at regular intervals of time. The plasma samples were processed through a liquid-liquid extraction procedure and assayed by using HPLC consisting reversed phase C_{18} column (ZORBAX, 4.6 x 150 mm, 5 µm), UV detector set at 238 nm. The mobile phase consisted of the mixture of 0.025 M sodium dihydrogen phosphate (pH 4.5): acetonitrile (35: 65, v/v) which was pumped at a flow rate of 1.5 $mL.min^{-1}$. The retention time of simvastatin was 7.5 minutes. The plasma drug concentration-time profiles of both groups were found significantly ($P < 0.05$) identical. The data was analyzed by using Kinetica® version 4.4 according to non-compartment model of pharmacokinetic analysis. There was statistically no significant ($P > 0.05$) difference between the values of following pharmacokinetic parameters in healthy and hyperlipidemic volunteers *i.e.* C_{max}, t_{max}, $AUC_{0-\infty}$, $AUMC_{0-\infty}$, MRT, $t_{1/2}$, Cl_t and K_e. This study confirmed no significant ($P > 0.05$) difference in pharmacokinetics and bioavailability parameters after the administration of a single oral dose of 40 mg simvastatin (cholesterol lowering drug) to healthy and moderately hyperlipidemic volunteers.

Introduction

Hyperlipidemia is a clinical manifestation characterized by abnormal high concentration of fats such as cholesterol, esters, triglycerides and phospholipids in the blood [1]. Primary hyperlipidemia (genetic predisposition) and secondary hyperlipidemia (diet, medication or underlying disease) are two main types due to defect in lipid metabolism or its transport, resulting in reduced LDL receptors [1, 2].

Simvastatin is an orally active cholesterol lowering agent [3]. Its chemical structure is given in Fig. 1. Simvastatin is metabolized to potent inhibitors of hepatic 3-hydroxy-3-methyl-glutryl-coenzyme A (HMG.CoA) reductase after administration, resulting in reduction of hepatic cholesterol synthesis and increase in a number of low density lipoprotein (LDL) receptors [4]. Consequently, it reduces triglycerides, low density lipoprotein (LDL) and total cholesterol levels but increases high density lipoproteins (HDL) level. Simvastatin (lactone prodrug) has bioavailability of about 5% of the orally administered dose as its active *β*-hydroxy acid metabolites. This drug has 95% plasma proteins binding capacity. Biliary excretion is the major route of Simvastatin and its metabolite as it is mainly excreted in the faeces whereas fraction of 0.1-0.15 is excreted in the urine as its inactive form. Peak plasma concentration after oral administration is reached at approximately 1 h and the mean plasma elimination half-life of Simvastatin and its active *β*-hydroxy acid metabolite is 1.8 and 1.9 h, respectively [5-7].

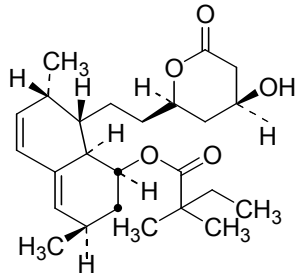

Fig. 1: Chemical structure of simvastatin.

Extremely limited information is available in literature regarding bioavailability and pharmacokinetics of simvastatin in healthy as well as in hyperlipidemic patients. Also, no similar study is available in Pakistan. Therefore, it was important to

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investigate the pharmacokinetics and bioavailability in local target population to ensure its efficacy and safety and help in devising the dosage regimen for optimum therapeutic outcome.

Results and Discussion

The study was performed on Simvastatin, an oral lipid lowering agent used for the treatment of hyperlipidemia. The purpose of current study is to observe and highlight the pharmacokinetics variability in diseased states. Standard curve of Simvastatin in concentration range of 3-18 ng.mL^{-1} is shown in Fig. 2. The mean plasma drug concentration-time profiles of 18 healthy and 18 moderately hyperlipidemic volunteers have been plotted on semi-log graph paper, shown in Fig. 3. The drug is separated on C_{18}, shown in Fig. 4. It is evident from results that no significant ($P > 0.05$) difference is observed between both profiles. The data was analyzed by using Kinetica® 4.4 according to non-compartment model of pharmacokinetic analysis. Statistical comparison of bioavailability and pharmacokinetic parameters of simvastatin in healthy and moderately hyperlipidemic volunteers is given in Table-1.

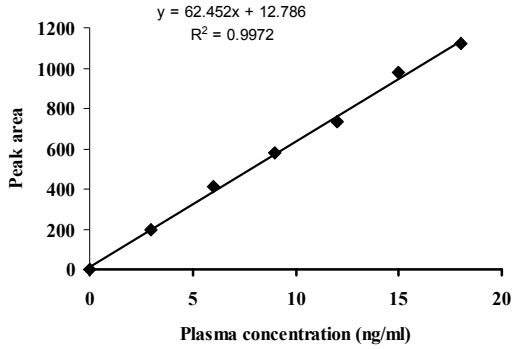

Fig. 2: Calibration curve of simvastatin in human plasma.

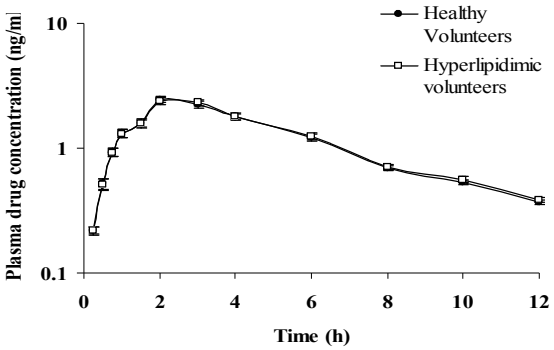

Fig. 3: Comparison of mean ± SEM concentration of simvastatin on semi-log graph, in healthy (n=18) and hyperlipidemic (n=18) volunteers, administered in an oral dose of 40 mg.

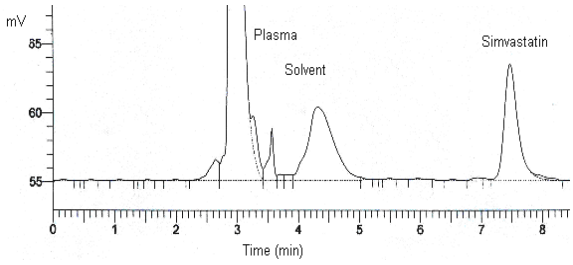

Fig. 4: Agilent technologies (Series 1100) chromatogram showing separation of simvastatin on ZORBEX C_{18} column.

Table-1: Comparison of Mean ± SEM of bioavailability and pharmacokinetic parameters of simvastatin (Saista 40) administered in an oral dose of 40 mg in healthy (n=18) and hyperlipidemic (n=18) volunteers.

Parameters	Healthy volunteers (Mean ± SEM)	Hyperlipidemic volunteers (Mean ± SEM)
C_{max} (ng.mL^{-1})	2.650 ± 0.119	2.685 ± 0.109 ^{ns}
t_{max} (h)	2.250 ± 0.131	2.333 ± 0.142 ^{ns}
$AUC_{0-\infty}$(ng.h.mL^{-1})	15.529 ± 0.754	15.844 ± 0.676 ^{ns}
$AUMC_{0-\infty}$ (ng.h^2.mL^{-1})	94.961 ± 4.272	97.386 ± 3.679 ^{ns}
MRT (h)	6.128 ± 0.057	6.160 ± 0.079 ^{ns}
K_e (h^{-1})	0.202 ± 0.003	0.203 ± 0.003 ^{ns}
$t_{1/2}$ (h)	3.519 ± 0.052	3.574 ± 0.073 ^{ns}
V_d (L.Kg^{-1})	13.101 ± 0.618	12.724 ± 0.542 ^{ns}
Cl_t (mL.min.$^{-1}$.Kg^{-1})	2.633 ± 0.107	2.568 ± 0.093 ^{ns}

ns = non significant difference ($P > 0.05$)

Maximum Plasma Concentration (C_{max})

In present study, value of C_{max} (Mean ± SEM) was 2.650 ± 0.119 ng.mL^{-1} for healthy and 2.685 ± 0.109 ng.mL^{-1} for hyperlipidemic volunteers. There is statistically no significant difference ($P > 0.05$) between values of C_{max} in healthy and hyperlipidemic volunteers. The values are in agreement with previous study conducted by Najib *et al.*, (2003) [8] where they found a value of C_{max} as 2.78 ng.mL^{-1} in test and 3.24 ng.mL^{-1} in reference product. Whereas in another study performed by Lohitnavy *et al.*, (2004) [9], mean values of C_{max} were very high in test (7.78 ± 2.82) (Mean ± SEM) and reference (7.75 ± 3.42) (Mean ± SEM) compared to the present study. This difference in values may be due to difference in excipients and manufacture process of the formulations. The difference in analytical technique is another variable as the study conducted by Lohitnavy *et al.*, (2004) [9], LC-MS/MS is used which is considered more sensitive than HPLC, which is used in present study. Ethnic diversity is another factor of variation in values of C_{max}, as this dissimilarity is observed in present study also. The C_{max} value was higher in healthy subjects 3 and 4 compared to other subjects of the same group.

Time of Peak Plasma Concentration (t_{max})

In current study, value of t_{max} (Mean ± SEM) was 2.250 ± 0.131 h and 2.333 ± 0.142 h for healthy and hyperlipidemic volunteers, respectively. The range 2-3 h in healthy and hyperlipidemic volunteers is seen in this study. There is statistically no significant difference ($P > 0.05$) between values of t_{max} in healthy and hyperlipidemic volunteers. These values for t_{max} are in good agreement with previously reported values of t_{max} as 1.73 ± 1.18 h [8] after administration of a single oral dose of 40 mg of simvastatin. It has a difference when head to head comparisons are performed between the mean values of t_{max} with another study [9] as 1.6 ± 0.6 (Mean ± SD) in test and 1.9 ± 0.08 (Mean ± SEM) in reference, respectively. The subjects in these studies belong to different races as well as different countries. A possible explanation of the difference in these values is the dissimilarity in their hepatic metabolism [10]. The findings of a study performed by Kim *et al.*, (2007) [11] clearly showed the effects of polymorphic CYP3A5 genotype on the pharmacokinetics of simvastatin in healthy subjects. They also suggested that polymorphic CYP3A5 genotype affects the disposition of simvastatin only but not other pharmacokinetic parameters like peak plasma concentration and half life. These findings provide an explanation for inter-individual variability of simvastatin.

Area Under Curve ($AUC_{0-\infty}$)

In this study, value of $AUC_{0-\infty}$ (Mean ± SEM) for healthy volunteers was 15.529 ± 0.754 $ng.h.mL^{-1}$and for hyperlipidemics 15.844 ± 0.676 $ng.h.mL^{-1}$. There is statistically no significant difference ($P > 0.05$) between the values of $AUC_{0-\infty}$ in healthy and hyperlipidemic volunteers.

In previous study, $AUC_{0-\infty}$ (Mean ± SEM) was found to be 13.59 ± 5.57 $ng.h.mL^{-1}$and for healthy volunteers [4] which in agreement with values of $AUC_{0-\infty}$ in present study. A bioequivalence study [9] was performed on Thai population and mean values of $AUC_{0-\infty}$ were 37.94 ± 16.82 (Mean ± SD) and 37.45 ± 17.90 (Mean ± SD) in test and reference products, respectively. The higher values were due to the fact that sampling time in that study was 0-24 h while in the present study, it was 0-12 h. The different value of $AUC_{0-\infty}$ (22.2 ± 16.6) was seen in another study [8] having the same sampling time from 0-12 h. This difference may be due to inter-subject variability. Other factors may be that simvastatin has high first pass effect and high hepatic clearance which is the key factor in greater variability of this drug [12].

Area under the First Moment Curve ($AUMC_{0-\infty}$)

The $AUMC_{0-\infty}$ (Mean ± SEM) was 94.961 ± 4.272 and 97.386 ± 3.679 $ng.h^2.mL^{-1}$ for healthy and hyperlipidemic volunteers, respectively. There is statistically no significant difference ($P > 0.05$) between the values of $AUMC_{0-\infty}$ in healthy and hyperlipidemic volunteers. Although mean $AUMC_{0-\infty}$ value in hyperlipidemic volunteers is slightly greater than that of healthy volunteers. For some drugs *i.e.* simvastatin, variation in pharmacokinetic parameters can occur over time caused primarily by changes in clearance. They may be because of variation in enzyme levels, inhibitors, inducers and biliary as well as renal functions [12].

Mean Residence Time (MRT)

In present study, MRT (Mean ± SEM) for healthy volunteers was 6.128 ± 0.057 h and 6.160 ± 0.079 h for hyperlipidemic volunteers. There is statistically no significant difference ($P > 0.05$) in MRT values of both groups. The slightly greater mean values of MRT in hyperlipidemic than healthy volunteers may be due to the fact that average weight of the individuals of hyperlipidemic groups was greater than healthy group. The lipid levels were also higher in diseased groups. As simvastatin is lipophilic in nature so it resides more in obese persons. Clearance of drug also affects the MRT values. In present study, the total clearance values are less in diseased group than healthy.

Elimination Rate Constant (K_e)

In this study, value of K_e (Mean ± SEM) for healthy and hyperlipidemics was 0.202 ± 0.003 h^{-1} and 0.203 ± 0.003 h^{-1}, respectively. There is statistically no significant difference ($P > 0.05$) between values of K_e in healthy and hyperlipidemic volunteers. The value of elimination rate constant was reported by Najib *et al.*, (2003) [8], found in almost same range of 0.2234 ± 0.0522 h^{-1} (Mean ± SEM) in 24 adult male healthy volunteers. So value of K_e in the present study is very close to the previously conducted study. The values of present study are slightly different to values previously reported by Lohitnavy *et al.*, (2004) [9] which were 0.1386 h^{-1} and 0.126 h^{-1} in test and reference products, respectively, in 18 Thai male volunteers. This variation usually exists due to ethnic diversity, renal functioning and high hepatic ratio of simvastatin [13].

Half Life ($t_{1/2}$)

In present study, value of $t_{1/2}$ (Mean ± SEM) for healthy and hyperlipidemic volunteers was 3.519 ± 0.051 h and 3.574 ± 0.073 h, respectively. There is statistically no significant difference ($P > 0.05$) between values of $t_{1/2}$ in healthy and hyperlipidemic volunteers. The values of half life are in accordance with previously reported values of half life *i.e.* 3.26 ± 0.75 h [8] and 3.45 ± 0.094 [12] after single dose of 40 mg simvastatin. While Lohitnavy *et al.*, (2004) [9] reported slightly different values of half life as 5 ± 2.9 h (Mean ± SD). This difference with the present study may be due to inter-subject variability, ethnic diversity, physico-chemical properties of the drug and the formulations.

Volume of Distribution (V_d)

In this study, the volume of distribution (V_d) (Mean ± SEM) for healthy volunteers was 13.101 ± 0.618 L/Kg and 12.724 ± 0.542 L/Kg for hyperlipidemic volunteers. There is statistically no significant difference ($P > 0.05$) between values of V_d in healthy and hyperlipidemic volunteers. The values of V_d calculated in present study are slightly different from previously reported values of 18.48 L/Kg and 15.204 L/Kg in test and reference products, respectively [8]. The volume of distribution depends upon many factors such as blood flow rate in different tissues, lipid solubility of the drug, partition coefficient of drug and different types of tissues, pH, binding to biological materials and obesity [14]. The difference may be due to one of the above mentioned factors.

Total Body Clearance (Cl_t)

In current study, value of clearance (Mean ± SEM) for healthy and hyperlipidemic volunteers was 2.633 ± 0.107 $mL.min^{-1}.Kg^{-1}$ and 2.568 ± 0.093 $mL.min^{-1}.Kg^{-1}$, respectively. There is statistically no significant difference ($P > 0.05$) between values of Cl_t in healthy and hyperlipidemic volunteers. The values calculated in present study are in agreement with values 2.943 $mL.min^{-1}$ reported by Najib *et al.*, (2003) [8]. In present study, values of total clearance of hyperlipidemics are less than values of healthy subjects. It may be due to renal functioning which is usually altered in diseased states [12].

Experimental

Apparatus and Chemicals

The Agilent Quaternary system (1100 Series, USA) equipped with pump, micro vacuum degasser and diode-array detector (DAD) set at 238 nm. Agilent ChemStation was used for taking chromatograms. A reversed phase C_{18} column (ZORBAX, 4.6 x 150 mm, 5 μm) was used to separate simvastatin from other eluents (Fig. 4). Simvastatin was supplied by Artemis Biotech, India. HPLC grade Sodium dihydrogen phosphate, perchloric acid and toluene and acetonitrile were purchased from Merck, Germany.

Experimental Design

The study was an open, single dose and parallel design. Eighteen healthy and 18 moderately hyperlipidemic human male volunteers were included in the study. All volunteers signed a written informed consent form before commencement of the study. Each volunteer received a single dose of Simvastatin 40 mg (Saista 40 mg, Bosch, Pakistan, Batch no. E4059) orally 30 minutes before breakfast. The tablets were administered with 240 mL of water. The volunteers remained seated in either a bed or a chair for next four hours. Standard lunch and dinner, not exceeding 2000 K Cal, were served five hours and twelve hours, respectively after taking the tablet.

Sample Collection

Blood samples were collected before dosing (zero time) and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 10.0 and 12.0 hours in heparinized glass test tubes. Test tubes were centrifuged at 2200 rpm for 10 minutes at 5 $^{\circ}$C. The plasma was separated and stored at -20 $^{\circ}$C until assay.

Preparation of Mobile Phase

The mobile phase consisted of a mixture of 0.025 M sodium dihydrogen phosphate (pH 4.5) and acetonitrile (35: 65, v/v) which was pumped at a flow rate of 1.5 $mL.min^{-1}$. The column was maintained at ambient temperature (20 $^{\circ}$C). UV detector was operated at 238 nm. Each analysis required a maximum of 20 minutes.

Preparation of Standard Solutions

Initial stock solution (1 $mg.mL^{-1}$) of Simvastatin was prepared by dissolving 100 mg in 100 mL acetonitrile. Standard solutions were obtained by diluting initial stock solution with acetonitrile to give concentrations over the range of 0-100 $mg.mL^{-1}$. Solutions were protected from light and stored in refrigerator. Solutions were stable for at least 3 to 4 months when stored at 4 $^{\circ}$C.

Extraction Procedure and Preparation of Standard Curve

Standard curve was constructed to encompass anticipated range of plasma Simvastatin concentration found in healthy and moderately hyperlipidemic volunteers. Blank plasma (0.5 mL) was spiked with Simvastatin drug solutions to give concentrations of 3, 6, 9, 12, 15 and 18 $ng.mL^{-1}$ in a 10 mL centrifuge glass tube. In 0.5 mL sample, 30 μL of perchloric acid (50% of 70%) was added, vortexed at 2200 rpm for 10 sec. Then 1 mL of toluene was added, vortexed at 2200 rpm for 40 seconds and centrifuged at 3000 rpm for 10 min. The supernatant was transferred to a second tube; 1 mL fresh toluene was added and the procedure was repeated. The supernatant was pooled and centrifuged at 3000 rpm for 10 sec. This pooled fraction was finally evaporated to dryness with nitrogen stream under vacuum. The samples were re-constituted with mobile phase. Aliquots of each sample (20 μL) were chromatographed. The interday and intraday precision and accuracy were also determined.

Preparation of Sample

Plasma sample (0.5 mL) containing Simvastatin was taken in 10 mL centrifuge tube and 30 μL of perchloric acid were added in each 10 mL centrifuge tube.

High Performance Liquid Chromatography Conditions

High Performance Liquid Chromatographic system of Agilent Technologies (series 1100) describes in apparatus and chemical section was primed and utilized for separation and quantification of simvastatin. The mobile phase was pumped at a rate of 1.5 $mL.min^{-1}$. An aliquot of 20 μL reconstituted sample was injected into HPLC system.

Calibration Curve and Quantification

The calibration curve was constructed by plotting peak-area of calibration curve standards versus concentrations of Simvastatin expressed in $ng.mL^{-1}$ plasma. The curve was constructed with at least six different concentrations in the range of 3-18 $ng.mL^{-1}$.

Safety Analysis

Health assessment including vital signs, physical examinations and clinical laboratory testing was performed before and seven days after study. The volunteers were interviewed at the beginning and end of each study period and were monitored through out the study period to determine any adverse events potentially related to simvastatin.

Pharmacokinetic and Statistical Analysis

Kinetica® 4.4 was applied for the pharmacokinetic analysis using non-compartmental method of analysis. SPSS version 12 was employed using independent t-test to calculate the difference whether significant or insignificant between the values of bioparameters of two different groups. Mean values and their standard error of means (SEM) were calculated for each parameter.

Conclusion

The results of present study have shown no significant difference ($P > 0.05$) in pharmacokinetic parameters of simvastatin between healthy and hyperlipidemic subjects. The study revealed potential results in healthy and moderate hyperlipidemic conditions. It is recommended that bioavailability and pharmacokinetic studies should be conducted in healthy versus severe hyperlipidemic conditions. Moreover, it is recommended to perform polymorphic CYP3A5 genotype based study in local population to avoid inter-individual variability of simvastatin disposition in polymorphic CYP3A5 genotype population.

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