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Pharmacokinetics and Bioequivalence Evaluation of Two Simvastatin 40 mg Tablets (*Simvast* & *Zocor*) in Healthy Human Volunteers

Naji M. Najib[a], Nasir Idkaidek[a], Ayman Adel[a], Isra' Admour[a], Rafel E. B. Astigarraga[b], Gliberto De Nucci[b], S. Mahmood Alam[c], Ruwayda Dham[c,*] and Qumaruzaman[c]

[a] *International Pharmaceutical Research Centre (IPRC), Amman – Jordan*

[b] *Cartesius Analytical Unit, Institute of Biomedical Sciences – USP, Sao Paulo, Brazil*

[c] *Gulf Pharmaceutical Industries – Julphar, UAE*

ABSTRACT: The pharmacokinetics of two brands of simvastatin 40 mg tablets were compared in 24 healthy human volunteers after a single oral dose in a randomized cross-over study, conducted at IPRC, Amman, Jordan. Reference (*Zocor*, MSD, Netherlands) and test (*Simvast*, Julphar, UAE) products were administered to fasted volunteers; blood samples were collected at specified time intervals, plasma separated and analyzed for simvastatin and its active metabolite (*β*-hydoxy acid) using a validated LC–MS/MS method at Cartesius Analytical Unit, Institute of Biomedical Sciences – USP, Sao Paulo, Brazil. The pharmacokinetic parameters AUC_{0-t}, $AUC_{0-\infty}$, C_{MAX}, T_{MAX}, $T_{1/2}$ and elimination rate constant were determined from plasma concentration–time profile for both formulations and were compared statistically to evaluate bioequivalence between the two brands, using the statistical modules recommended *by FDA*. The analysis of variance (*ANOVA*) did not show any significant difference between the two formulations and 90% confidence intervals fell within the acceptable range for bioequivalence. Based on these statistical inferences it was concluded that the two brands exhibited comparable pharmacokinetic profiles and that Julphar's *Simvast* is bioequivalent to *Zocor* of MSD, Netherlands. Copyright © 2003 John Wiley & Sons, Ltd.

Key words: simvastatin; bioequivalence; pharmacokinetics; HPLC; Julphar

Introduction

Simvastatin is an antilipaemic agent used in the primary treatment of primary hyperchloesterolemia. It is administered as an inactive lactone pro-drug, which needs conversion by esterase to become an active competitive inhibitor of HMG-CoA reductase [1–3]. It has an extremely high affinity for this enzyme. Simvastatin is synthesized from a fermentation product of *Aspergillus terrus* [4].

Chemically it is butanoic acid, 2,2-dimethyl-, 1,2,3,7,8,8a – hexahydro-3,7-dimethyl-8-(2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)-ethyl)-1-naphthalenyl ester, (IS*-(1 alpha, 3 alpha, 7β, 8β (2S*, 4S*),-8 aβ)). The empirical formula of simvastatin is $C_{25}H_{38}O_5$ and its molecular weight is 418.57 [5,6]. The chemical structure is shown below.

Simvastatin is a pro-drug, which is activated in the liver to generate simvastatin acid [7–10]. The latter is an active metabolite structurally similar to HMG-CoA. Simvastatin acid competes with HMG-CoA reductase, a hepatic microsomal enzyme [10]. Interference with the activity of this enzyme reduces the quantity of mevalonic acid, a precursor of cholesterol, therefore inhibiting *de*

*Correspondence to: Gulf Pharmaceutical Industries, Julphar 1201, Twin Towers, P.O. Box 42040, Dubai, UAE.
E-mail: julphard@emirates.net.ae (R. Dham)

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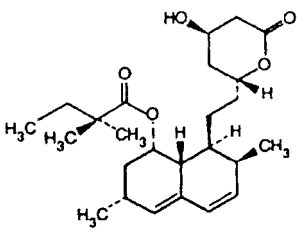
novo synthesis of cholesterol. This augments the synthesis of cholesterol from LDL, resulting in the clearance of LDL and so reduction of plasma cholesterol [10].



The chemical structure of simvastatin[6]

Simvastatin has several unique pharmacokinetic characteristics. Following an oral dose of ^{14}C-labeled simvastatin in man, 13% of the dose was excreted in urine and 60% in feces. The latter represents absorbed drug equivalents excreted in bile, as well as any unabsorbed drug. Plasma concentrations of total radioactivity (simvastatin plus ^{14}C-metabolites) peaked at 4 h and declined rapidly to about 10% of peak by 12 h postdose [10]. In a single-dose study in nine healthy subjects, it was estimated that less than 5% of an oral dose of simvastatin reaches the general circulation as active inhibitors [10], even though absorption was about 85%.

Both simvastatin and its (beta)-hydroxy acid metabolite are highly bound (approximately 95%) to human plasma proteins. Absorption is not significantly reduced if taken before a low fat meal [10]. The lipophilic nature allows simvastatin to penetrate the CNS [10]. The elimination half-life is 1.9 h [5].

Objectives

The purpose of this study was to determine the bioequivalence of a new tablet formulation of simvastatin (*Simvast* 40 mg tablets) produced in United Arab Emirates by Gulf Pharmaceutical Industries-Julphar, in comparison with *Zocor* from MSD, Netherlands.

Materials and Methods

Study products

Test Product:	*Simvast* – Simvastatin 40 mg tablets.
Batch No.:	0004 Manufacturing date: 11/01; Expiry date: 11/03.
Manufacturer:	Gulf Pharmaceutical Industries – Julphar, U.A.E.
Reference Product:	*Zocor* – Simvastatin 40 mg Tablets.
Batch No.:	HP 33390 Manufacturing date: 07/01; Expiry date: 07/03.
Manufacturer:	Merck Sharp & Dhome (MSD), Netherlands.

Study design

Twenty-four healthy adult male volunteers participated in this comparative study at Al-Mowasah Hospital, Amman, Jordan, as joint venture with International Pharmaceutical Research Center (IPRC), Amman, Jordan. Their mean age was 22.92 ± 4.59 years with a range of 18–37 years; mean body weight was 70.25 ± 8.22 kg with a range of 54–88 kg and mean body height was 172.08 ± 4.68 cm with a range of 163–180 cm.The volunteers did not have any significant cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal or haematological diseases, as determined by their medical history, physical examination, and routine laboratory tests (haematology, blood biochemistry, and urine analysis). All subjects were negative for hepatitis B antigen and were instructed to abstain from taking any drug including over the counter (OTC) for 2 weeks prior to and during the study period. They were informed about the aim and risks of the study by the clinical investigator, based on which they signed a written informed consent statement before entering the study. The study was performed according to the revised Declaration of Helsinki for bio-medical research involving human subjects and the rules of Good Clinical Practices. Before the start of the study the protocol was approved by Institutional Review Board (IRB) of Al-Mowasah Hospital, Amman, Jordan.

Drug administration and sample collection

The study was designed as a single dose, randomized, two treatment, two periods cross over design. In the morning of phase I, after an overnight fasting (10 h) volunteers were given

single dose of either formulation (reference or test) of simvastatin 40 mg with 240 ml of water. No food was allowed until 5 h after dose administration. Water intake was allowed after 2 h of dose; water, lunch and dinner were given to all volunteers according to a time schedule. The volunteers were continuously monitored by Al-Mowasah Hospital Staff throughout the confinement period of study. They were not permitted to lie down or sleep for the first 5 h after the dose. Approximately 10 ml of blood samples for simvastatin and active metabolite assay were drawn into heparinized tubes through indwelling cannula before (0 h) and at 0.33, 0.66, 1.0, 1.33, 1.66, 2.0, 2.33, 2.66, 3.0, 3.5, 4.0, 5.0, 6.0,8.0, 10, 12, 16, and 24 h after dosing. Blood samples were centrifuged at 3500 rpm for 10 min, plasma was separated and kept frozen at -20°C until assayed. After a washout period of 7 days the study was repeated in the same manner to complete the crossover design.

Analysis for parent drug – simvastatin

Sample preparation for HPLC injection. Fifty microliters of formic acid was added to 200 µl of plasma sample and vortexed for 10 s, then 50 µl internal standard (lovastatin 10 ng/ml) was added and vortexed for 10 s. Four milli liters of extraction mixture (diethyl ether/hexane 80/20) was added and vortexed for 40 s then was frozen for 5 min at –70°C freezer and then organic layer was transferred to another tube and evaporated to dryness in a water bath at 37°C under a gentle stream of nitrogen. Residue was reconstituted with 200 µl of acetonitrile/water (10/90) and vortexed for 30 s and then centrifuged at 13200 rpm for 2 min; 60 µl was injected to the column, where simvastatin and the internal standard were separated from endogenous plasma substances.

Chromatographic conditions. Plasma samples were analyzed for simvastatin by a validated LC–MS–MS method. All solvents used were of HPLC grade and were purchased from Merck, Germany; while other chemicals and reagents were of analytical grade; simvastatin and lovastatin were obtained from Julphar.

The LC–MS–MS was from Hewlett Packard (HP), USA and it consisted of liquid chromatograph (G1311A), degasser (G1322A), an autoinjector (G1329A), and an ion trap mass spectrometer with head nebulizer source (AP12000) from PE-Sciex, USA; integration was done using Analyst software (PE-Sciex, USA).

Chromatographic separation was performed using Alltech C18 (3 µm) (150 × 4.6 mm) column from Alltech Chromatography, UK. The mobile phase consisted of 90% of acetonitrile, 10% water+10 mM formic acid and eluted at a flow rate of 1.0 ml/min at ambient temperature. Detection was done at MRM of 436.2>198.8 and MRM of 405.3>199.1 for simvastatin and lovastatin, respectively. The peak area was measured, and the peak area ratio of drug to internal standard and the concentration were calculated by Analyst software. The method was validated by following international guidelines [11].

Analysis for active metabolite – β-hydroxy simvastatin acid

Sample preparation for HPLC injection. Fifty microliters internal standard solution (OH-lovastatin 10 ng/ml) was added to 200 µl plasma sample and vortexed for 15 s, then 20 µl of formic acid was added and vortexed for 15 s. Four milliliters of extraction mixture (diethyl ether/hexane 70/30) was added and vortexed for 60 s then centrifuged at 2000 rpm for 5 min. Organic layer was transferred to another tube and evaporated to dryness in a water bath at 40°C under a gentle stream of nitrogen. Residue was reconstituted with 200 µl of acetonitrile/water (20/80) and vortexed for 10 s and then centrifuged at 13200 rpm for 2 min; 80 µl was injected to the column, where OH-simvastatin and the internal standard were separated from endogenous plasma substances.

Chromatographic conditions. Plasma samples were analyzed for OH-simvastatin by a validated LC–MS–MS method. All solvents used were of HPLC grade and were purchased from Merck, Germany; while other chemicals and reagents were of analytical grade.

The LC–MS–MS was from Hewlett Packard (HP), USA and it consisted of liquid chromato-

graph (G1311A), degasser (G1322A), an autoinjector (G1329A), and a Micromass Quattro LC with electroscopy source mass spectrometer (Micromass, UK); integration was done using Masslynx software version 3.5 (Micromass, UK). Chromatographic separation was performed using Genesis C18 (5 μm) (150 × 4.6 mm) column from Jones Chromatography, UK. The mobile phase A consisted of 2.28 mM ammonium hydroxide solution and mobile phase B consisted of 2.28 mM ammonium hydroxide solution in acetonitrile eluted following gradient program. Detection was done at MRM of 435.46>115.06 and MRM of 421.44>319.17 for OH-simvastatin, and OH-lovastatin, respectively. The peak area was measured, and the peak area ratio of drug to internal standard and the concentration were calculated by Masslynx software. The method was validated by following international guidelines [11].

Pharmacokinetic analysis. Pharmacokinetic analysis was performed by means of model independent method using Kinetica™ 2000 computer program [12]. The elimination rate constant (λ_Z) was obtained as the slope of the linear regression of the log-transformed concentration values versus time data in the terminal phase. Elimination half-life ($T_{1/2}$) was calculated as $0.693/\lambda_Z$. Area under the curve to the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal rule. Area under the curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-t}+C_t/\lambda_Z$, where C_t is the last measurable concentration.

Statistical analysis. For the purpose of bioequivalence analysis AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} were considered as primary variables. Two-way analysis of variance (ANOVA GLM procedure; KineticaTM 2000 Computer program [12]) for crossover design was used to assess the effect of formulations, periods, sequences and subjects on these parameters. Difference between two related parameters was considered to be statistically significant for p-value equal to or less than 0.05. Parametric 90% confidence intervals [13] based on the ANOVA of the mean test/reference (T/R) ratios of *AUCs* and C_{max} were computed.

Results and Discussion

Simvastatin was well tolerated by all volunteers; unexpected incidents that could have influenced the outcome of the study did not occur. There was no drop-out and all volunteers who started the study continued to the end and were discharged in good health.

Under the described conditions, the lower limit of quantitation from 200 μl plasma was 0.10 ng/ml for simvastatin and active metabolite. The relationship between concentration and peak area ratio was found to be linear within the range of 0.10–8.0 ng/ml for simvastatin and 0.10–4.0 ng/ml for active metabolite. The intra-day accuracy of the method for simvastatin ranged from 96.33 to 108.0%, while the intra-day precision ranged from 5.17 to 8.30%. The inter-day accuracy for simvastatin ranged from 97.33 to 106.0%, while the inter-day precision ranged from 4.08 to 8.49%. The intra-day accuracy for active metabolite ranged from 92.60 to 101.20%, while the intra-day precision ranged from 2.34 to 13.86. The inter-day accuracy for active metabolite ranged from 97.93 to 100.80%, while the inter-day precision ranged from 2.53 to 13.00%. Absolute recovery were 63.83 and 71.79% for parent drug and active metabolite respectively; relative recovery ranged from 97.78 to 100.45% for parent drug, and from 90.94 to 102.00% for active metabolite. Stability study showed that both parent drug and active metabolite were stable in plasma for 6 months when stored at −20°C.

Both formulations were readily absorbed from the gastrointestinal tract and simvastatin was measurable at the first sampling time (0.33 h) in majority of the volunteers, while active metabolite was detectable after 1 h samples in almost all volunteers. The mean concentration–time profile of simvastatin for the two formulations is shown in the Figure 1, while Figure 2 shows mean concentration–time profile of active metabolite. Peak concentration of 2.78 and 3.24 ng/ml for simvastatin were attained at 1.73 and 1.80 h after drug administration and then declined rapidly and was detectable up till 12 h. Peak concentration of 0.64 and 0.73 ng/ml for active metabolite were attained at 4.85 and 4.24 h after drug administration and then declined rapidly but was still detectable up till 12 h.

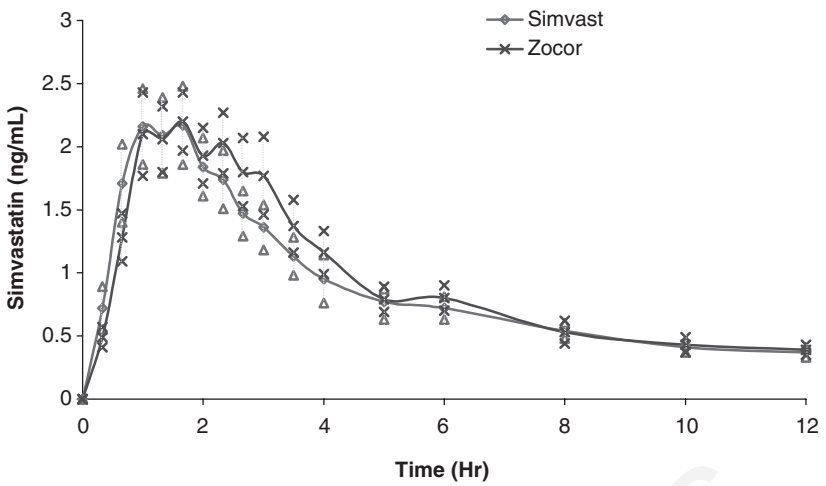


Figure 1. Mean plasma concentration (± SEM) of simvastatin after oral administration of single dose of two brands to 24 healthy human volunteers

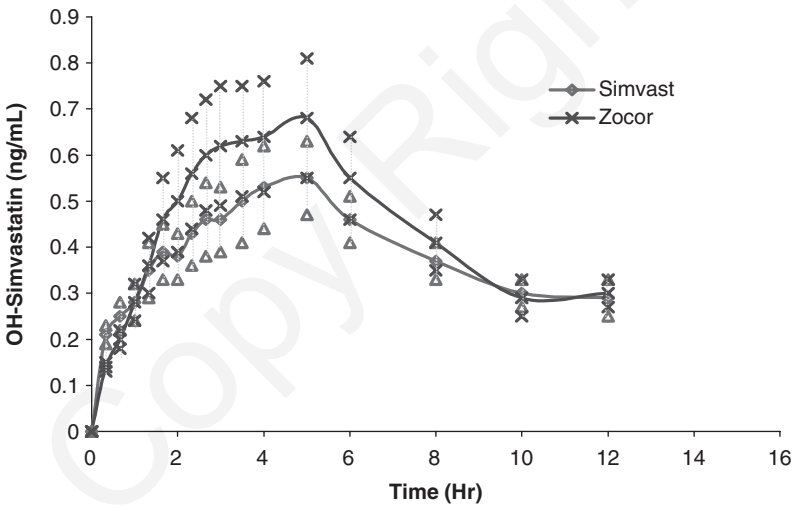


Figure 2. Mean plasma concentration (± SEM) of OH-simvastatin after oral administration of single dose of two brands to 24 healthy human volunteers

Table 1 shows the pharmacokinetic parameters of simvastatin for the two brands, and Table 2 shows pharmacokinetic parameters of active metabolite. The extent of absorption is a key characteristic of drug formulation and, therefore *AUC* is an important parameter for comparative bioavailability (bioequivalence) studies [14]. However, the other two parameters, C_{max} and T_{max}, are also important features and could affect the therapeutic behavior of a drug [15] and hence were also considered in the study. The relative bioavailability of *Simvast* on the basis of parent drug was $101.7 \pm 36.42\%$ for AUC_{0-t}, $103.99 \pm 33.85\%$ for $AUC_{0-\infty}$, and $92.14 \pm 43.83\%$ for C_{max}. On the basis of active metabolite the relative bioavailability was $107.21 \pm 42.21\%$ for AUC_{0-t}, $104.23 \pm 30.34\%$ for $AUC_{0-\infty}$, and $112.54 \pm 52.31\%$ for C_{max}.

The most important objective of bioequivalence testing is to assure the safety and efficacy of generic formulations. When two formulations of the same drug are equivalent in the rate and

extent to which the active drug becomes available to the site of drug action, they are bioequivalent and thus considered therapeutically equivalent [16]. To demonstrate bioequivalence certain limits should be set depending on the nature of drug, patient population, and clinical end points. It is generally accepted that for basic pharmacokinetic characteristics, such as AUC_{0-t}, and $AUC_{0-\infty}$ the standard equivalence range is 0.8–1.25 [13], while C_{max} range is 70–143% for highly variable drugs such as simvastatin [17]. The results of statistical analysis are shown in Tables 3 and 4.

Table 1. Pharmacokinetic parameters of simvastatin for two brands (mean ± standard deviation, $n = 24$)

Pharmacokinetic parameter	*Simvast* 40 mg tablets (test)	*Zocor* 40 mg tablets (Reference)
AUC_{0-t} (ng/ml h)	10.18 ± 4.98	10.63 ± 5.15
$AUC_{0-\infty}$ (ng/ml h)	13.59 ± 5.57	11.67 ± 5.64
C_{max} (ng/ml)	2.78 ± 1.64	3.24 ± 1.73
T_{max} (h)	1.73 ± 1.18	1.80 ± 1.24
$T_{1/2}$ (h)	3.26 ± 0.75	2.80 ± 0.99
λ_Z (/h)	0.2234 ± 0.0522	0.2831 ± 0.1140

Values are given as ± standard deviation.

Table 2. Pharmacokinetic parameters of active metabolite for two brands (mean ± standard deviation, $n = 24$)

Pharmacokinetic parameter	*Simvast* 40 mg tablets (test)	*Zocor* 40 mg tablets (Reference)
AUC_{0-t} (ng/ml h)	4.38 ± 2.69	4.95 ± 3.74
$AUC_{0-\infty}$ (ng/ml h)	6.04 ± 2.57	7.18 ± 4.77
C_{max} (ng/ml)	0.64 ± 0.43	0.73 ± 0.63
T_{max} (h)	4.85 ± 2.62	4.24 ± 1.33
$T_{1/2}$ (h)	4.88 ± 1.27	4.42 ± 1.45
λ_Z (/h)	0.1511 ± 0.0379	1766 ± 0.01719

Table 3. Statistical analysis of Ln-transformed data of simvastatin

Statistical analysis	AUC_{0-t}	$AUC_{0-\infty}$	C_{max}
ANOVA GLM (*p*-value)	0.5335	0.9276	0.0639
90% CI	82.81–109.04%	83.72–117.33%	72.32–97.59

For parent drug and active metabolite, mean and standard deviation of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} of the two formulations did not differ significantly, suggesting that the blood profiles generated by *Simvast* are comparable to those produced by *Zocor*. Analysis of variance (ANOVA) for these parameters, after log-transformation of the data, showed no statistically significant difference between the two formulations, with p value greater than 0.05. Ninty percent confidence intervals also demonstrated that the ratios of AUC_{0-t}, $AUC_{0-\infty}$ of the two formulations lie within the *FDA* acceptable range of 80–125% and for C_{max} the ratio was found within 70–143% acceptance criteria [17].

In case of simvastatin absolute difference in T_{max} (test – reference) was –0.07 h, and found to be within the acceptance limits ± 20% of reference mean; for active metabolite this T_{max} difference was 0.61 h (within ± 20% of reference mean).

Plasma levels may be used as surrogate parameters for clinical activity; therefore, results of this study suggest equal clinical efficacy of the two brands of simvastatin.

Summary and Conclusion

Statistical comparison of the AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} for simvastatin and OH-simvastatin clearly indicated no significant difference between *Simvast* and *Zocor* tablets in any of the calculated pharmacokinetic parameters. The confidence intervals for the ratios of mean AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} indicated that these values were entirely within the bioequivalence acceptance range (using log-transformed data). Based on the above we can conclude that *Simvast*, manufactured by Gulf Pharmaceutical

Table 4. Statistical analysis of Ln-transformed data of active metabolite

Statistical analysis	AUC_{0-t}	$AUC_{0-\infty}$	C_{max}
ANOVA GLM (*p*-value)	0.7778	0.9733	0.8105
90% CI	81.91–115.99%	84.74–118.72%	77.56–121.83%

Industries, UAE is bioequivalent to *Zocor* manufactured by MSD, Netherlands, and that both products can be considered equally effective in medical practice.

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