Journal of Food and Drug Analysis, Vol. 15, No. 1, 2007, Pages 15-19 藥物食品分析 第十五卷 第一期

Bioequivalence Assessment of Two Simvastatin Tablets in Healthy Taiwanese Volunteers

CHIH-MENG TSENG[1], CHUN-CHEN HUANG[1], MENG-CHUN HO[1], YEN-AN CHEN[1], YING-HUA SHIEH[2] AND I-KAI CHEN[1]

[1] Protech Pharmaservices Corporation, 4F, 360, Ruiguang Rd., Neihu District, Taipei City 114, Taiwan, R.O.C.
[2] Taipei Medical University / WangFang Hospital, 111, Sec. 3, Hsinglong Rd., Taipei City 110, Taiwan, R.O.C.

(Received: June 16, 2006; Accepted: October 4, 2006)

ABSTRACT

The pharmacokinetics and bioequivalence of two tablets of simvastatin, Zolotin and ZOCOR®, were evaluated in 26 healthy male Taiwanese volunteers who reside in Taiwan. The experiments were designed as a randomized, two-sequence, two-period and single-dose crossover study. Blood samples were obtained at 0 (pre-dose), 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 14 and 24 hr after oral dosing of one tablet. β-hydroxyacid simvastatin concentrations in plasma were analyzed by a validated LC/MS/MS method. The pharmacokinetic parameters were analyzed by non-compartmental analysis. The analysis of variance was carried out using log-transformed AUC_{0-t}, $AUC_{0-\infty}$ and C_{max}. The results revealed that the C_{max} of Zolotin and ZOCOR® were 4.78 ± 2.75 ng/mL and 4.52 ± 2.01 ng/mL; the T_{max} were 3.80 ± 1.63 hr and 4.31 ± 1.73 hr; the $T_{1/2}$ were 4.32 ± 1.82 hr and 5.11 ± 2.49 hr; the AUC_{0-t} were 35.6 ± 21.7 ng×hr/mL and 36.5 ± 20.0 ng×hr/mL; and the $AUC_{0-\infty}$ were 38.1 ± 24.3 ng×hr /mL and 40.3 ± 23.6 ng×hr/mL, respectively. The ratios of log-transformed AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} values of the plasma β-hydroxyacid simvastatin between two tablets were within the range of 80-125% as judged by 90% confidence intervals and satisfied the bioequivalence criteria. The generic simvastatin tablets formulation, Zolotin, was shown to be bioequivalent to the ZOCOR® tablets.

Key words: bioequivalence, pharmacokinetics, simvastatin, β-hydroxyacid simvastatin

INTRODUCTION

Simvastatin is a specific inhibitor of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. In clinical application, the inhibition of this enzyme will result in the lowering of plasma cholesterol by inhibiting the biosynthesis of cholesterol to a small extent, and, more importantly, by increasing the number of low-density lipoprotein (LDL) receptors on hepatic and extrahepatic tissues. These LDL receptors bind circulating LDL and remove them from the circulation. Simvastatin has been shown to be effective in lowering the total cholesterol, LDL cholesterol and apolipoprotein B in hypercholesterolemic patients. In addition, simvastatin reduces triglycerides and increases high-density lipoprotein cholesterol[1-4].

Simvastatin is an inactive prodrug which is readily hydrolyzed *in vivo* to the corresponding β-hydroxyacid, a potent inhibitor of HMG-CoA reductase. Simvastatin undergoes extensive first-pass extraction in the liver, its primary site of action, with a low consequence of low general circulation[2-5].

Although simvastatin and its β-hydroxyacid metabolite are highly bound (approximately 95%) to human plasma proteins, long-term administration did not result in an accumulation of drug[5]. Simvastatin was metabolized by the cytochrome P450 system to at least 5 intermediates of which the structures were elucidated for 4: β-hydroxyacid simvastatin, 6'β-hydroxy simvastatin, 3'-hydroxy simvastatin and 6'-exomethylene simvastatin. The relative inhibitory activities on HMG CoA reductase of β-hydroxyacid simvastatin, 6'β-hydroxy simvastatin and 3"-hydroxy simvastatin were 100, 50 and 20%, respectively[7]. Regardless of being administered orally or parenterally, simvastatin tends to be eliminated in feces. Following the oral administration of a single radiolabelled dose, 100 mg, of simvastatin to healthy subjects, 60% of the radioacitivity was recovered in the feces (which also included unabsorbed parent drug) and 13% was recovered in the urine (of which < 0.5% was active metabolites)[3]. The elimination half-life of β-hydroxyacid simvastatin is 1.9 hr and the total body clearance is 31.8 L/hr[7].

This study was aimed to evaluate the bioequivalence between two different formulations of simvastatin tablets (Zolotin V.S. ZOCOR®) in healthy male Taiwanese volunteers.

MATERIALS AND METHODS

I. *Drugs*

Zolotin (simvastatin 40 mg tablet, Pharmosa Ltd.) and ZOCOR® (simvastatin 40 mg tablet, Merck Sharp & Dohome Ltd.) were obtained from the manufacturers.

* Author for correspondence. Tel: +886-2-26577777; Fax: +886-2-27993366; E-mail: cmt@ppccro.com

Before dosing, the analysis of these two preparations by HPLC indicated that Zolotin and ZOCOR® contained 98.63% and 99.62% of the labeled amount respectively.

II. *Subjects*

This study was performed in accordance with the Taiwan Law of Pharmaceutical Affairs, Good Clinical Practices, Good Laboratory Practices, local regulatory requirements, and the principles enunciated in the Declaration of Helsinki. A total of 26 healthy male Taiwanese volunteers participated in this study after signing an informed consent form. The subjects of ages 20 to 40 years old, with a body weight within 20% of ideal weight, were enrolled. All subjects were in good physical conditions as determined by complete physical and clinical examinations before the study. These subjects were instructed to abstain from any drugs for at least one week prior to and during the study.

III. *Study Design*

This study was designed by a randomized, two-sequence, two-period and crossover study under fasting conditions. The subjects were randomly assigned to one of the treatment sequences as following one week of wash-out period.

Plasma was obtained from the antecubital vein of the forearm using evacuated tubes containing potassium oxalate and sodium fluoride (Vacutainer, Becton Dickinson). Eight milliliter of each blood sample was collected before dosing and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 14 and 24 hr after the administration of one simvastatin formulation. Blood samples were centrifuged within 30 min at 1900 × g for 10 min at 4°C to collect the plasma. Plasma were transferred to an appropriately labeled tube and stored at -20°C until subsequent assays.

IV. *Assay Method*

β-hydroxyacid simvastatin concentration in plasma were measured by a validated liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) method. The preparation and extraction of plasma are summarized as follows. Each 0.5 mL of plasma sample was added to 0.1 mL KH_2PO_4 (1M) solution containing internal standard (1 mg/mL topiramate solution). After vortexing thoroughly for 10 sec, the mixture was extracted with 4 mL of diethylether. The ether mix was vortexed for 1 min and centrifuged at 3,000 rpm for 10 min. The ether layer was transferred to another tube and evaporated under a stream of nitrogen gas. Zero point two milliliter of of MeOH/acetonitrile (ACN)/H_2O = 60/20/20 (v/v/v) was then added to solublize the residue, and 10 μL was injected automatically into the LC/MS/MS system for analysis.

The chromatographic system is consisted of an Agilent 1100 HPLC system coupled to an Applied Biosystems API 4000 mass spectrometer. The separation was achieved using a 4.6 × 150 mm, 5 μm Zorbax Ecilpse XDB-C8 column with the mobile phase consisting of MeOH/ACN/H_2O = 60/20/20 (v/v/v) with 1 mM CH_3COONH_4. The mobile phase was delivered into the LC/MS/MS system at a flow rate of 0.8 mL/min. Detection was carried at Multiple Reaction Monitoring of 435.40 > 115.10 and 338.40 > 77.70 for β-hydroxyacid simvastatin and topiramate, respectively.

V. *Pharmacokinetic Analysis*

A non-compartmental pharmacokinetic method was employed to determine the pharmacokinetic parameters of β-hydroxyacid simvastatin. C_{max}, the maximum observed concentration, and T_{max}, the time to observe the peak concentration, were determined for each subject and for each treatment. The area under the concentration-time curve from time zero to the last quantifiable concentration (AUC_{0-t}) was determined by trapezoidal rule. $AUC_{0-\infty}$, the area under the concentration-time curve from time zero to infinity, was determined by the trapezoidal rule and extrapolated to infinity as estimated by the last quantifiable concentration divided by the elimination rate constant (K_{el}). K_{el} was determined by simple linear regression based on the terminal phase of plasma concentration. Plasma half-life ($T_{1/2}$) was estimated by ($0.693/ K_{el}$)

VI. *Statistical Analysis*

Analysis of Variance (ANOVA), 90% confidence intervals, power analysis and two one-sided test were used to make statistical evaluation of pharmacokinetic data and the assessment of bioequivalence with the acceptable limits of 0.8 and 1.25.

RESULTS

Both simvastatin formulations were well tolerated by all the subjects; unexpected incidents that could have influenced the outcome of the study did not occur. There was no drop-out. All volunteers stayed in the study until the end and were discharged in good health.

I. *Validation of the Analytical Method*

The LC/MS/MS method for measuring of β-hydroxyacid simvastatin in human plasma sample was validated with a calibration range from 0.1 to 20 ng/mL. The between-run accuracy (%RE; the relative error) of the method for the calibration standard ranged from -5.0 to 5.0%, while the between-run precision (%CV; the coefficient of variation) ranged from 2.7 to 5.1% (Table 1). Quality control (QC) samples at three different concentrations were 0.3 ng/

Journal of Food and Drug Analysis, Vol. 15, No. 1, 2007

Table 1. Precision and accuracy of between-run (n = 6) for β-hydroxyacid simvastatin calibration standards in plasma determined by the LC/MS/MS method

	Within-run			Between-run		
Known Conc. (ng/mL)	Conc. found (ng/mL)	Coefficient of variation (%)	Relative error (%)	Conc. found (ng/mL)	Coefficient of variation (%)	Relative error (%)
0.3	0.281 ± 0.012	4.3	-6.3	0.292 ± 0.013	4.5	-2.7
2	2.02 ± 0.06	3.0	1.0	2.00 ± 0.11	5.5	0.0
16	16.5 ± 0.2	1.2	3.1	15.9 ± 0.6	3.8	-0.6

mL, 2 ng/mL and 16 ng/mL. The between-run accuracy (%RE) for QC samples ranged from -2.7 to 0.0%, while the between-run precision (%CV)-ranged from 3.8 to 5.5%. The within-run accuracy (%RE) for QC samples ranged from -6.3 to 3.1%, while the within-run precision (%CV) ranged from 1.2 to 4.3%. These results indicated that the method was precise and accurate.

II. *Pharmacokinetics of β-hydroxyacid Simvastatin*

The mean plasma concentration-time curves of β-hydroxyacid simvastatin after single oral administration of each simvastatin formulation in 26 subjects are shown in Figure 1. Pharmacokinetic parameters for each treatment are presented in Table 2. Maximal β-hydroxyacid simvastatin levels were observed after 3.80 ± 1.63 hr for Zolotin and 4.31 ± 1.73 hr for ZOCOR®. The peak concentration was 4.78 ± 2.75 ng/mL after receiving Zolotin and 4.52 ± 2.01 ng/mL after receiving ZOCOR®. The AUC_{0-t} was 35.6 ± 21.7 ng×hr/mL for Zolotin and 36.5 ± 20.0 ng ×hr/mL for ZOCOR®. The $AUC_{0-\infty}$ was 38.1 ± 24.3 ng× hr/mL for Zolotin and 40.3 ± 23.6 ng×hr/mL for ZOCOR®. The mean half-life was calculated to be 4.32 ± 1.82 hr for Zolotin and 5.11 ± 2.49 hr for ZOCOR®.

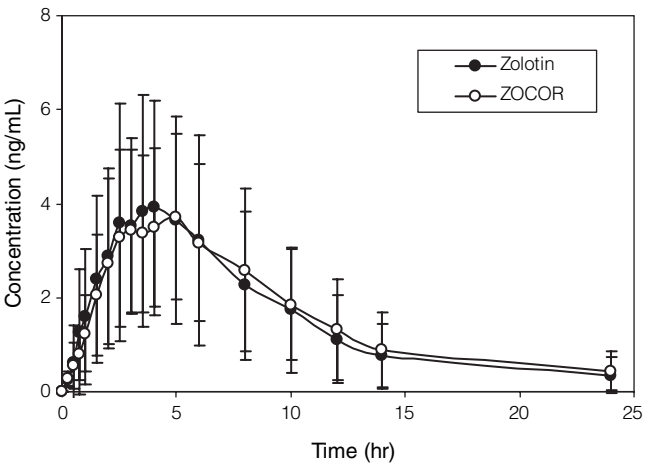

Figure 1. Time profile of mean plasma concentration of β-hydroxyacid simvastatin obtained after a single oral administration of 40 mg simvastatin tablet.

III. *Statistical Analysis of β-hydroxyacid Simvastatin*

The bioequivalence analysis of the three pharmacokinetic parameters (AUC_{0-t}, $AUC_{0-\infty}$ and C_{max}) are shown in Table 3. After log-transformation of the data, the ratios of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} for Zolotin to those of ZOCOR® were 0.951 (90% C.I. 84.0-108%), 0.926 (90% C.I. 81.8-105%) and 1.03 (90% C.I. 90.3-117%). ANOVA analysis among these parameters showed no significant difference between the two formulations ($P > 0.05$). The parametric 90% confidence intervals of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} lie entirely within the bioequivalence acceptance limits of 80%-125%.

Table 2. Pharmacokinetic parameters of β-hydroxyacid simvastatin for Zolotin and ZOCOR® simvastatin formulations obtained from 26 healthy volunteers after a single oral administration of 40 mg simvastatin tablet

Pharmacokinetic parameters	Zolotin	ZOCOR®
AUC_{0-t} (ng×hr/mL)	35.6 ± 21.7[a]	36.5 ± 20.0
$AUC_{0-\infty}$ (ng×hr/mL)	38.1 ± 24.3	40.3 ± 23.6
C_{max} (ng/mL)	4.78 ± 2.75	4.52 ± 2.01
T_{max} (hr)	3.80 ± 1.63	4.31 ± 1.73
$T_{1/2}$ (hr)	4.32 ± 1.82	5.11 ± 2.49

[a]Values are given as arithmetic mean ± standard deviation.

Table 3. Statistical evaluation of logarithmically transformed data for the comparison of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} of β-hydroxyacid simvastatin for Zolotin and ZOCOR® obtained from 26 healthy volunteers after a single oral administration of 40 mg simvastatin tablet

Pharmacokinetic parameters	Zolotin geometric mean	ZOCOR® geometric mean	Ratio (T/R)	90% C.I.
AUC_{0-t} (ng×hr/mL)	30.9	32.5	0.951	84.0-108
$AUC_{0-\infty}$ (ng×hr/mL)	32.8	35.4	0.926	81.8-105
C_{max} (ng/mL)	4.20	4.10	1.03	90.3-117

DISCUSSION

Simvastatin is an inactive lactone prodrug, which undergoes rapid hydrolysis after absorption from the gastrointestinal tract to generate the major active metabolite, β-hydroxyacid simvastatin[3,7]. A linear increase in the inhibitory activity of simvastatin occurs as the dose is elevated from 5 to 120 mg and the clinical pharmacologic effects of simvastatin are predominantly attributed to the actions of the β-hydroxyacid simvastatin[7]. The peak inhibition of HMG-CoA reductase activity occurs within 2 to 4 hours and the T_{max} of β-hydroxyacid simvastatin is reached approximately at the same time[7]. Hence, it is the usual practice to monitor the β-hydroxyacid simvastatin concentrations to predict the therapeutic effect. Therefore, a validated LC/MS/MS method was employed in this study to measure plasma β-hydroxyacid simvastatin and to compare the pharmacokinetic profiles of β-hydroxyacid simvastatin for the test and reference formulations, Zolotin and ZOCOR®.

The pharmacokinetic parameters of β-hydroxyacid simvastatin obtained in this study differ from previously reported data[8-9]. In the present study, after a single oral dose of 40 mg simvastatin tablet under fasting conditions, C_{max} values (4.52 ± 2.01 ng/mL) were higher than those reported in the Najib *et al*. study (0.73 ± 0.63 ng/mL) but lower than those observed by the Lohitnavy *et al*. study (20.18 ± 17.59 ng/mL); the same trend was seen for the corresponding $AUC_{0-\infty}$ values (40.3 ± 23.6 vs 7.18 ± 4.77 vs 132.16 ± 113.50 ng×hr/mL, respectively)[8-9]. The absolute bioavailability of simvastatin is less than 5% in humans and the low systemic availability is attributed to the extensively first-pass metabolism in the liver[7]. Vree's study indicated that there is highly variable hydrolysis of simvastatin to yield the active metabolite[10]. About 29% subjects have a high yield of β-hydroxyacid simvastatin but 18% subjects show an extremely low level of β-hydroxyacid simvastatin[10]. Most of subjects (about 53%) have a low-to-intermediate yield of β-hydroxyacid simvastatin[10]. The variation of inter-subject may explain the difference between our study and the others. In additional, ethnic-related differences in simvastatin pharmacokinetics may play a role in these results; further study may be warranted.

Manufacturing information from Merck indicated a $T_{1/2}$ of 1.9 hr for β-hydroxyacid simvastatin, which is significantly different from the value in our study and those in the studies by Najib *et al*. and Lohitnavy *et al*. Notably, the study by Vree *et al*. demonstrated a two-phase elimination of β-hydroxyacid simvastatin, a fast elimination $T_{1/2}$ of 2.7 hr and a slow elimination $T_{1/2}$ of 80 hr[10]. Therefore, $T_{1/2}$ values may differ, depending on the blood sampling duration or LLOQ (lower limit of quantification). However, similar values were observed for the corresponding $T_{1/2}$ values in these studies (5.11 ± 2.49 vs 4.42 ± 1.45 and 5.3 ± 1.4 hr, respectively)[8-9]. This may be explained by that our study conditions were similar to those in the Najib *et al*. and Lohitnavy *et al*. studies, including the same dosage form (tablet), strength (40 mg), LLOQ (0.1 ng/mL), blood sampling duration (24 hr) and single dosing under fasting status.

Among pharmacokinetic parameters, AUC is considered to be an indicator of the extent of absorption, whereas C_{max} is regarded to be an estimation of the rate and the extent of absorption. In this study, no significant difference was observed in the mean AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} values obtained for each formulation, Zolotin and ZOCOR®, as assessed by the 90% C.I. of the geometric means. Thus, the obtained 90% C.I. for these parameters fitted well within the criterion of 80-125%. No safety concerns were identified during the study.

CONCLUSIONS

Based on the results of our study, it can be stated that the two simvastatin formulations are bioequivalent both in terms of the rate and extent of absorption. Notably, this is the first investigation to provide valuable information concerning the pharmacokinetic properties of β-hydroxyacid simvastatin in Taiwanese people.

ACKNOWLEDGEMENTS

The authors would like to thank the Pharmosa Ltd. for providing a research grant, and the Tai-An Hospital for providing the clinical trial unit.

REFERENCES

1. Plosker, G. L. and McTavish, D. 1995. Simvastatin: a reappraisal of its pharmacology and therapeutic efficacy in hypercholesterolaemia. Drugs 50: 334-363.
2. Todd, P. A. and Goa, K. L. 1990. Simvastatin: A review of its pharmacological properties and therapeutic potential in hypercholesterolaemia. Drugs 40: 583-607.
3. Mauro, V. F. and MacDonald, J. L. 1991. Simvastatin: a review of its pharmacology and clinical use. DICP 25: 257-264.
4. Hsu, I., Spinler, S. A. and Johnson, N. E. 1995. Comparative evaluation of the safety and efficacy of HMG-CoA reductase inhibitor monotherapy in the treatment of primary hypercholesterolemia. DICP 29: 743-759.
5. Vickers, S., Duncan, C. A., Chen, I. W., Rosegay, A. and Duggan, D. E. 1990. Metabolic disposition studies on simvastatin, a cholesterol-lowering prodrug. Drug Metab. Dispos. 18: 138-145.
6. Pentikainen, P. J., Saraheimo, M., Schwartz, J. I., Amin, R. D., Schwartz, M. S., Brunner-Ferber, F. and Rogers, J. D. 1992. Comparative pharmacokinetics of lovastatin, simvastatin and pravastatin in humans. J. Clin.

Pharmacol. 32: 136-140.
7. Mauro, V. F. 1993. Clinical pharmacokinetics and practical applications of simvastatin. Clin. Pharmacokinet. 24: 195-202.
8. Najib, N. M., Idkaidek, N., Adel, A., Admour, I., Astigarraga, R. E., Nucci, G. D., Alam, S. M., Dham, R. and Qumaruzaman. 2003. Pharmacokinetics and bioequivalence evaluation of two simvastatin 40 mg tablets (Simvast and Zocor) in healthy human volunteers. Biopharm. Drug Dispos. 24: 183-189.
9. Lohitnavy, M., Lohitnavy, O., Chaijittiprasert, K., Taytiwat, P. and Polnok, S. 2004. Bioequivalence study of two formulations of simvastatin tablets in healthy Thai volunteers. Arzneimittelforschung 54: 31-34.
10. Vree, T. B., Dammers, E., Ulc, I., Horkovics-Kovats, S., Ryska, M. and Merkx, I. 2001. Variable plasma/liver and tissue exterase hydrolysis of simvastatin in healthy volunteers after a single oral dose. Clin. Drug Invest. 21: 643-652.