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조코 정에 대한 엘바스타 정의 생물학적 동등성 평가

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Evaluation of the Bioequivalence of Simvastatin 20mg Tablets in Healthy Volunteers

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심바스타틴은 cholesterol 생합성 과정에서 속도 조절 효소인 HMG-CoA reductase의 강력한 상경적 길항약으로서 고지혈증 치료에 널리 쓰이는 약물이다. 심바스타틴 제제인 MSD 사의 조코 20 mg정을 대조약으로 하여 시험약인 유영 제약의 엘바스타 20 mg정의 생물학적 동등성 평가를 하기 위해 22명의 건강한 지원자를 모집하였다. 지원자를 두 군으로 나누어 2정씩 투여하였고 2 × 2 교차시험을 실시하였다. 심바스타틴의 혈장 중의 농도를 정량하기 위하여 발리데이션된 LC/MS/MS를 사용하였다. 채혈 시간은 투약 전 및 투약 후 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12 시간에 걸쳐 총 12시점에 걸쳐 시행하였다. 생물학적 동등성을 판정하기 위한 파라미터로 12시간까지의 혈장 중 농도 곡선 하 면적(AUC_{12hr})과 최고 혈중 농도(C_{max})를 사용하였다. 12시간 까지의 혈중 농도 곡선 하 면적의 기하 평균은 17.30 ng·ml/hr (시험약)과 17.35 ng·ml/hr(대조약)으로 나타났다. 최고 혈중 농도의 경우 각 각 5.08 ng/ml(시험약)과 5.20 ng/ml(대조약)으로 관찰 되었다. AUC_{12hr}의 경우 로그변환한 평균치 차의 90% 신뢰구간이 log0.8510 - log1.1694이었고, C_{max}의 경우 log0.8176 - log1.1649로 계산되어 두 항목 모두 log0.8-log1.25이어야 한다는 식품의 약품 안전청과 FDA의 기준을 모두 만족시켰다. 이상의 결과를 종합하면 시험약 엘바스타 정 20mg은 대조약 조코 정 20 mg에 대하여 생물학적으로 동등한 것으로 판정되었다.

☐ Key words - simvastatin, bioequivalence, LC/MS/MS, elvasta, Zocor®

Simvastatin is the lactone form of 1', 2', 6', 7', 8' 8a' -hexahydro-3,5-dihydroxy-2', 6'-dimethyl-8'(2'', 2''-dimethyl-1''-oxo-butoxy)-1'-naphthaleneheptanoic acid[1]. This compound is a highly effective cholesterol-lowering agent, which is widely used in the treatment of hypercholesterolemia. The statin are reversible inhibitors of the microsomal enzyme HMG-CoA reductase, which converts HMG-CoA to mevalonate. This is an early rate-limiting step in cholesterol biosynthesis.[2] Simvastatin, a semisynthetic analogue of lovastatin, is aborbed from the gastrointestinal tract and is hydrolysed to its active β-hydroxy acid from. Absorption rate is approximately 60%, while the absolute bioavailability of the β-hydroxy acid is only 5 %.[3] Both simvastatin and its β-hydroxy acid metabolite are approximately 95% bound to plasma protein. Simvastatin is mostly excreted in the feaces via the bile as metabolites, with 10 to 15% being recovered in the urine, mainly in the active forms. The half-life of the active metabolite is approximately 1.9 hours.[4]

Yooyoung Pharm. Co., Ltd. (Seoul, Korea) have developed a new formulation of simvastatin tablet: Elvasta 20mg this study assessed, hence the bioequivalence of this newerly developed formulation with a refernce formulation, Zocor 20mg (MSD Co., Inc.) in 22 healthy Korean volunteers. Typical bio-availability, including AUC_t (the area under the plasma concentra-

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tion-time curve from 0 until the last sampling time, 12 hr) and C_{max} (the maximum plasma concentra-tion) parameters were compared.

Materials and Methods

test and Reference Products

The test product, Elvasta 20 mg (20 mg of simvastatin, lots no. 03254001, Yooyoung Pharm. Co., Ltd.) and the reference produt, Zocor 20 mg (20 mg simvastatin, lots no. 04084, MSD Co., Inc.) were supplied by tablets.

Subjects and Methods

The 20 mg simvastatin bioequivalence study involved 22 healthy Korean volunteers with the age from 20 to 30 years (24.1±3.4 years), in weight from 45 to 85 kg (65.8±10.6 kg), and height from 158 to 187 cm (172.2±5.7 cm). All the volunteers were enrolled after passing a clinical examination, including a physical examination and laboratory tests (blood analysis: hemoglobin, hematocrit, WBC, platelets, WBC differential, blood urea nitrogen, total bilirubin, cholesterol, total protein, albumin, alkaline phosphatase, glucose fasting, ALT, and AST, and urine analysis: specific gravity, color, pH, sugar, albumin, bilirubin, RBC, WBC, and casts). Any with potential hypersensitivity to this type of medication, a history of the hepatic, renal, or cardiovascular disease, or chronic alcohol consumption or other medications was excluded. This criteria was applied to elimination the source of variation which can influence the pharmacokinetics of the drug. All the volunteers were retricted not to take using other drugs from at least one week before the study and until the completion of the study. They also refrained from alcoholic beverages and xanthine-containing foods and beverages 48 hr before the study, until the last sampling time. Each volunteer received an oral dose of 40 mg (20 mg × 2 tablets) of simvastatin in a standard 2 × 2 cross-over design, in randomized order. There was a 6-day washout period. The study was approved by a local ethics committee. All the participants signed a written informed consent, in accordance with the Korea Guidelines for Bioequivalence Tests (KGBT 1998).

The subjects were hospitalized (Sun Obstetrics Hospital, Daejeon, Korea) at 7:00 p.m. the day before drug administration. At 7:00 a.m., the median cubital vein was cannulated and 1 ml of heparinized injectable normal saline solution was flushed into the cannula to prevent blood clotting. The doses were taken at 8:00 a.m. on each dosing day with 240 ml of drinking water. Four hours after oral administration, all the subjects were given standard meals. The subjects were not allowed to take a supine position or to sleep until 4 hr after oral administration. Approximately 7-ml blood samples were collected before and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 12 hr after drug administration. The cannula was flushed with 1 ml of heparinized injectable normal saline solution after each blood sampling. The blood sample was centrifuged immediately, and the plasma was frozen at -70°C until the LC/MS/MS analysis.

LC/MS/MS Assay of Simvastatin in Plasma

The simvastatin concentration in plasma was analyzed using a reported LC/MS/MS method,[5] with slight modification. Briefly, 100 μl of internal standard (lovastatin, 100 ng/ml) and 50 μl of formic acid were added to 1 ml of plasma, followed by a one-minute liquid-liquid extraction with 5 ml of diethylether: hexane mixture (8:2). The organic layer was transfered and evaporated to dryness under a gentle nitrogen stream. The residue was reconstituted in 100 μl of methanol: water mixture (9; 1) and 5 μl was injected onto the column. The mobile phase was a mixture of 0.1% formic acid: acetonitrile (5:95, v/v).[6] Analytes were eluted using an HP 1100 series pump (Agilent, Wilmington, DE, USA) at 0.2 ml/min. The turbo-ion spray interface was operated in positive ion mode at 5,500 V and 350°C. The operating conditions were optimized by flow injection of a mixture of all analysis: nebulizing gas flow, 1.04 L/min; collision gas flow, 5.0 L/min; curtain gas flow, 1.6 L/min; declustering potential voltage, 106 V; entrance potential voltage 10V; collision cell exit potential voltage 38V. Quantitation was performed by multiple reaction monitoring (MRM) of the protonated precursor ion and the related product ion for benidipine using the internal standard method with the peak area ratio. The

mass transition used for simvastatin and the internal standard was m/z 442.2→325.0 and 427.0→ 325.0, respectively (collision energy, 31 eV). Quadruples Q1 and Q3 were set on unit resolution. The analytical data were processed using Analyst Software (Version 1.2).

Pharmacokinetic Analysis

The non-compartmental pharmacokinetic parameters were derived using standard methods. The maximum plasma concentration, C_{max}, was obtained from the concentration-time data. The AUC_t was calculated using the logarithmic trapezoidal rule and was extrapolated to infinity using the relationship:

$$AUC_{inf} = AUC_t + (C_t/k_{el}) \quad \text{(Eq.1)}$$

where AUC_{inf} is the area under the plasma concentration-time curve from zero to time infinity, C_t is the concentration of the last plasma sample(greater than the limit of quantification LOQ), and k_{el} is the elimination rate constant of the terminal phase.[7]

Statistical Analysis

The following tests or procedures were carried out for AUC_t, and C_{max}. ANOVA was performed using logarithmic transformed AUC_t and C_{max}. Schuirmann's two one-sided *t*-test (*i.e.*, for logarithmic transformed AUC_t and C_{max}) approach was used to test the bioequivalence of the pharmacokinetic characteristics between the products. The range of bioequivalence for the parametric analysis was set to the 80-125%, and the range of equivalence for the non-parametric analysis was set to 20% of the reference mean.[8] All statistical comparisons were made using EquivTest version 1.0 (Statistical Solution Ltd., Sangus, MA, USA).

Results and Discussion

LC/MS/MS Analysis

With the LC/MS/MS method, no interference was observed in human plasma. The respective retention times for simvastatin and the internal standard (lovastatin) were approximately 1.96 and 1.78 min (Fig. 1). The quantification limit for simvastatin in human plasma was 0.2 ng/ml, based on a signal-to-noise ratio of 5.0. The intra- and inter-day coefficients of variation were

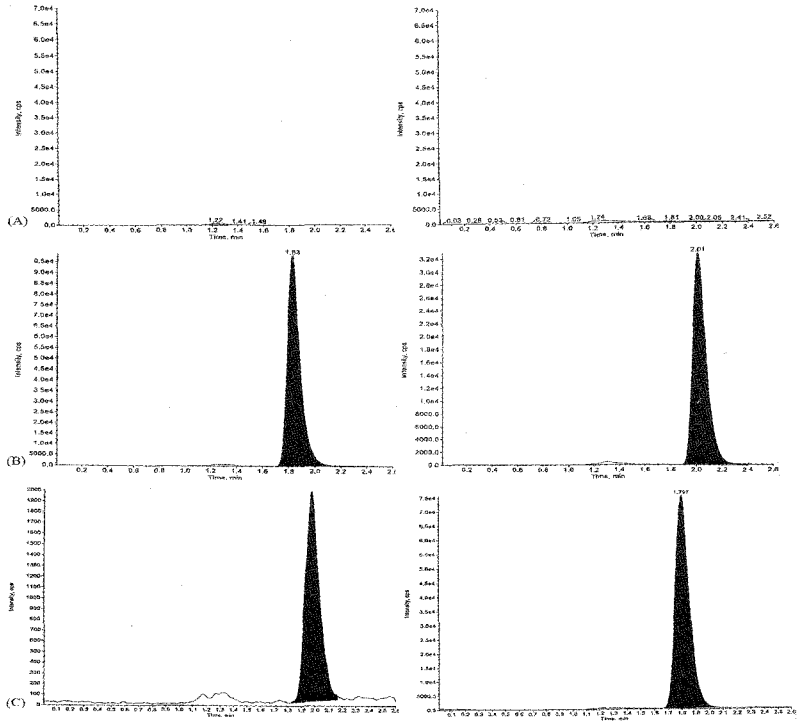

Fig. 1. Chromatogram of simvastatin (left) and lovastatin (right). (A) blank plasma, (B) plasma spiked with 20 ng/ml simvastatin and 100 ng/ml lovasatin, (C) plasma sample equivalent to 1.37 ng/ml from a volunteer 1 hr after the oral dose.

less than 9.0 and 14.5%, respectively, for the concentration range from 0.2 to 20 ng/ml.

Clinical Observations

The tolerability of simvastatin 20mg medication was acceptable. Clinically relevant or drug-related side effects were not observed in any of the 22 volunteers.

Pharmacokinetic Characteristics

The plasma simvastatin (reference and test products) concentration-time profiles are shown in Fig. 2. Table 1 shows the pharmacokinetic parameters of simvastatin for the two brands. The mean terminal half-life of simvastatin of reference and test brands was 2.35±3.04 and 3.26±2.52, respectively (mean terminal half-life of two products 2.81±2.80), which were very similar to the results of other previous studies

Table 1. Pharmacokinetic parameters of simvastatin for two brands (mean ± standard deviation, n=22)

Pharmacokinetic parameter	Elvasta 20mg × 2tablets (Test)	Zocor 20mg × 2tablets (Reference)
AUC_t (ng·hr/ml)	20.99 ± 13.09	22.48 ± 17.74
AUC (ng·hr/ml)	27.17 ± 19.92	24.43 ± 18.47
C_{max} (ng/ml)	6.33 ± 2.95	5.37 ± 2.84
T_{max} (hr)	2.84 ± 1.95	2.23 ± 1.70
k_{el} (hr^{-1})	0.19 ± 0.10	0.22 ± 0.09
Cl_{total}/F (L/hr)	1596 ± 1145	1628 ± 1207

Table 2. Analysis of variance test (α=0.05) for AUC_t (log-transformed) and C_{max} (log-transformed) for the simvastatin tablets

ANOVA	log-transformed AUC_t (F-value)	log-transformed C_{max} (F-value)
Group or Sequence	7.107(4.351)	2.208(4.351)
Subjects/Group	7.496(2.124)	3.758(2.124)
Period	0.002(4.351)	2.521(4.351)
Drug	0.001(4.351)	0.056(4.351)

Standard Bioequivalence Analysis

No significant sequence effect was found for any of the bioavailability parameters, indicating that the cross-over design was properly performed. Significant F-test values were found between subjects and the subjects' nested sequence' (SEQ) for AUC_t and C_{max}, indicating substantial inter-subject variation in the pharmacokinetics of simvastatin from the two formulations (Table 2). No significant period effect in AUC_t or C_{max} was detected in this study.

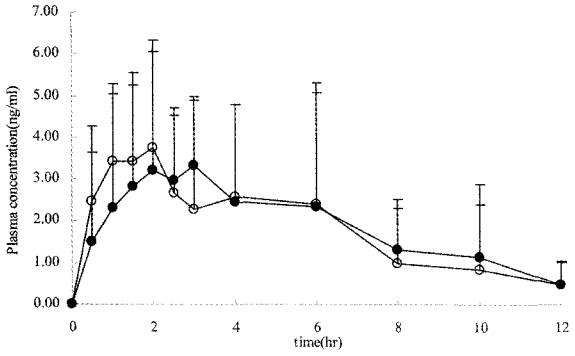

Fig. 2. Mean plasma concentration-time profiles of 40 mg simvastatin (20 mg×2 tablets) after Zocor® 20 mg (reference tablet: ●) or Elvasta 20mg (test tablet: ○) administration to 22 volunteers. The vertical bars represent the standard deviation.

The detailed statistical and bioequivalence analyses of simvastatin for AUC_t and C_{max} under the assumptions of the multiplicative model are given in Table 4. The geometric means of the parameters are given for the test and reference formulations of simvastatin, separately and as combined estimates. The parametric point estimates of the ratio of geometric mean of test and reference products for AUC_t and C_{max} were 0.997 and 0.975, respectively (Table 4), and the parametric 90% confidence intervals for AUC_t and C_{max} were 0.8510-

Table 3. The 90% confidence intervals and results of Schuirmann's test on the target pharmacokinetic parameters of simvastatin

	Geometric means			90% C.I	Result of Schuirmann's test			
	Test (T)	Reference (R)	T/R		Side I		Side II	
					t	P	t	P
C_{max}	5.076	5.201	0.975	0.81-1.16	13.08	<0.01	8.20	<0.01
AUC_t	17.303	17.346	0.997	0.85-1.17	15.46	<0.01	9.80	<0.01

1.1694 and 0.8176-1.1649 (Table 3), respectively, which were within the commonly accepted bioequivalence range of 0.80-1.25.

In conclusion, the results indicate that the two forms of simvastatin 20mg is bioequivalent.

Acknowledgments

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