Pharmacokinetic and Safety Profiles of a Fixed-Dose Combination of Amlodipine, Valsartan, and Atorvastatin: A 3-Period Replicate Crossover Study

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Abstract

The objective of study was to compare the pharmacokinetic and safety profiles of a fixed-dose combination (FDC) formulation of 5/160/20 mg amlodipine/valsartan/atorvastatin with those of separate formulations of a 5/160-mg amlodipine/valsartan tablet and a 20-mg atorvastatin tablet. This was a randomized, open-label, single-dose, 3-sequence, 3-period replicate crossover study with 42 subjects. Serial blood samples for pharmacokinetic assessment were collected up to 72 hours postdose. For establishing bioequivalence (BE) for amlodipine, valsartan, and atorvastatin, a reference-scaled average BE approach was used if applicable, as well as the conventional limit of 0.80-1.25. The 90% confidence intervals (CIs) for the geometric mean ratios (GMRs) for the maximum plasma concentration (C_{max}) and the area under the curve to the last measurable concentration (AUC_t) between the FDC and separate formulations were within the 0.80-1.25 limit for all analytes but atorvastatin. The estimated within-subject standard deviation of the log-transformed values of the separate formulations, the reference intervention, was 0.3804 for the C_{max} of atorvastatin, being set at 0.7489-1.3352 for the BE acceptance limit. For both the C_{max} and AUC_t for atorvastatin, the GMRs lay within 0.80-1.25, and the 90%CIs for the GMRs were within the BE acceptance limit. This 3-period replicate crossover study demonstrated the BE of the FDC formulation of amlodipine, valsartan, and atorvastatin and the separate formulations of an amlodipine/valsartan tablet and an atorvastatin tablet. A similar incidence of treatment-emergent adverse events (TEAEs) was observed in both interventions, and headache was the most common TEAE.

Cardiovascular disease (CVD) is an important cause of morbidity and mortality worldwide,[1] and the burden of CVD, including the cost and health loss, has increased in the past several decades.[2] These burdens have been a major concern for individual countries, and relevant guidelines about CVD are regularly published. Epidemiological studies, including the Framingham Heart Study, have played an important role in the elucidation of predisposing factors for CVD,[3] and the association of hypertension (HTN) and dyslipidemia with CVD is well known.[4–6] Regarding these factors, guidelines related to CVD, HTN, and dyslipidemia highly recommend properly managing blood pressure and cholesterol levels.[1,7,8] Furthermore, the comorbidity of HTN and dyslipidemia is also common.[8,9] Thus, angiotensin II receptor blockers (ARBs) and inhibitors of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase

(statins) may also influence atherosclerotic disease progression and CVD progression.[10]

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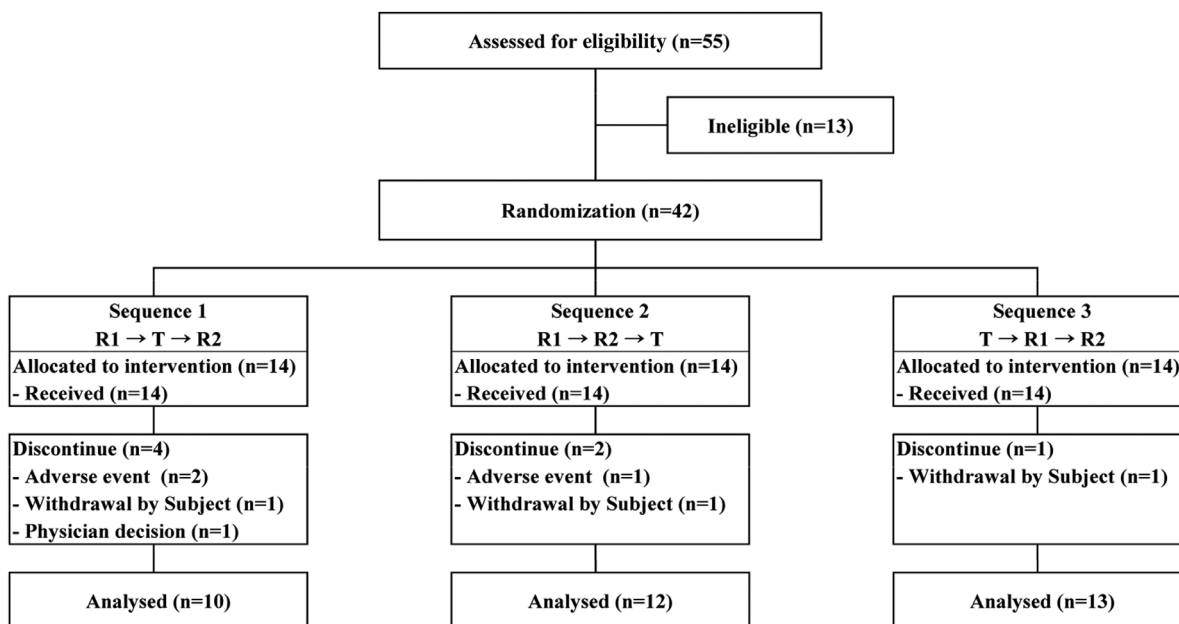(e-mail: jungryul.kim@samsung.com)

Figure 1. Study design and subject disposition. T, administration of a single dose of the fixed-dose combination tablet of 5/160/20 mg of amlodipine/valsartan/atorvastatin; R1, the first administration of a single dose of the separate formulations of a 5/160-mg amlodipine/valsartan tablet and a 20-mg atorvastatin tablet; R2, the second administration of a single dose of the separate formulations.

However, polypharmacy leads to decreased patient compliance, and this poor adherence to treatment is translated into worsened conditions and increased mortality to a significant extent.[11,12] In a meta-analysis, a fixed-dose combination (FDC) decreased the risk of medication noncompliance and improved clinical outcomes.[13] Thus, an FDC formulation should be considered to reduce the pill burden for patients with chronic diseases. Based on these observations, the CJ HealthCare Corp. has developed an FDC formulation of amlodipine, valsartan, and atorvastatin.

Amlodipine, a dihydropyridine calcium channel blocker, is used to treat HTN. Peak amlodipine concentrations occur between 6 and 8 hours after oral dosing, and the majority of amlodipine absorbed is metabolized in the liver and then excreted in urine.[14] There is no established evidence of pharmacokinetic drug interactions with amlodipine.

Valsartan, an ARB with selectivity for the type 1 receptor subtype, is eliminated mainly through biliary excretion in an unchanged form.[15] Moreover, it is metabolized to a small extent, approximately 8% of the dose administered.

Atorvastatin is rapidly absorbed and then extensively metabolized to 2-OH atorvastatin, which is the most dominant and pharmacologically active metabolite.[16] This metabolism is mediated by the intestinal and hepatic cytochrome P450 (CYP) 3A4 enzyme. Thus, atorvastatin is well known to interact with drugs that inhibit or induce this enzyme, such as itraconazole or rifampin.

In general, a bioequivalence (BE) limit of 0.80-1.25 for the 90% confidence interval (CI) of the geometric mean ratio (GMR) has been adopted for an average BE criterion.[17] However, it is difficult to demonstrate BE for highly variable drugs (HVDs) unless very many subjects are included in a clinical trial.[18] Thus, regulatory authorities published guidelines for HVDs with a within-subject coefficient of variation (CV) of more than 30% based on a replicate crossover design.[17] The objective of this study was to compare the pharmacokinetic (PK) and safety profiles of an FDC formulation of 5/160/20 mg amlodipine/valsartan/atorvastatin with those of separate formulations of a 5/160-mg amlodipine/valsartan tablet and a 20-mg atorvastatin tablet.

Subjects and Methods

Study Design

This study was conducted in accordance with the Declaration of Helsinki and the Korea Good Clinical Practice guideline. Prior to the initiation of the study, the study protocol was reviewed and approved by the Ministry of Food and Drug Safety of the Republic of Korea and the Institutional Review Board of the Samsung Medical Center.

This was a randomized, open-label, single-dose, 3-sequence, 3-period replicate crossover study (ClinicalTrials.gov identifier NCT03657472), and the study design is depicted in Figure 1. The FDC tablet of 5/160/20 mg of amlodipine/valsartan/atorvastatin (CJ-30061; CJ HealthCare Corp., Seoul, Republic of

Korea) was used as the test intervention, and the separate formulations of a 5/160-mg amlodipine/valsartan tablet (Exforge; Novartis, Basel, Switzerland) and a 20-mg atorvastatin tablet (Lipitor; Pfizer, New York, New York) were used as the reference intervention. Each subject received the test in 1 period, and the reference in the other 2 periods. Furthermore, this study was carried out in 3 groups of subjects because only a small number of subjects could be studied at one time.

All the study subjects gave written informed consent before any study-related procedures. Healthy men aged 19-55 years with a body mass index of 19-27 kg/m^2 were eligible. Major exclusion criteria were: a history of major illness; a history of drug hypersensitivity; a positive status for HIV, hepatitis B and/or C virus, or syphilis; and abnormal laboratory and 12-lead electrocardiography (ECG) findings. Eligible subjects were randomly allocated to 1 of 3 sequence groups with the same ratio. A total of 42 subjects were enrolled, and they had an arithmetic mean ± standard deviation (SD) age of 30.8 ± 7.5 years and body mass index of 23.0 ± 1.8 kg/m^2.

According to a previous study conducted with amlodipine, the within-subject CVs of the maximum plasma concentration (C$_{max}$) and the area under the curve to the last measurable concentration (AUC$_t$) were 8%-10% for 10 mg amlodipine.[19] In a replicate crossover study for a tablet containing 320 mg of valsartan, the corresponding value of C$_{max}$ was 26%.[20] As for 40 mg atorvastatin, a replicate crossover study reported that the within-subject CV of C$_{max}$ and AUC$_t$ were 44% and 23%, respectively.[21] Assuming a 40% within-subject CV of the C$_{max}$ for the separate formulations and a 5% difference between the 2 interventions, a sample size of 42 subjects was planned for the replicate crossover study to have 80% statistical power after accounting for a 28% dropout rate.[22]

Dosing and Assessment Schedules

In each period, subjects were hospitalized at the Clinical Trial Center of the Samsung Medical Center for 1 day before the administration of the study drug. Subjects took the study drug orally with 150 mL of water after an overnight fast of at least 10 hours and fasted for 4 hours afterward. Water was prohibited during 2 hours after drug administration. The study procedures were performed over the next 30 hours of admission. After discharge, the subjects visited the Clinical Trial Center 48 and 72 hours postdosing for the collection of PK samples.

Serial blood samples for PK assessment were collected in heparinized tubes at the scheduled times: before drug administration (0 hour) and then 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16, 24, 30, 48, and 72 hours after amlodipine administration; 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 8, 12, 16, 24, and 30 hours after valsartan administration; and 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 5, 8, 12, 16, 24, 30, and 48 hours after atorvastatin administration. Blood samples were centrifuged at approximately 1800g for 10 minutes at 4°C. Aliquots of plasma for amlodipine, valsartan, atorvastatin, and 2-OH atorvastatin analyses were then placed in polypropylene tubes. The plasma samples were transferred to a deep freezer and stored at less than −70°C until the assays.

Bioassays

Amlodipine. In the amlodipine assay, LC-20AD chromatography (Shimadzu, Japan) was performed with a Unison UK-C18 3-μm (75-mm length × 2.0 mm) column (Imtakt, Portland, Oregon), and the column temperature was maintained at 40°C. The mobile phase for amlodipine consisted of a mixture of (A) 10 mM ammonium acetate (v/v, with 0.1% formic acid) and (B) acetonitrile (v/v, with 0.1% formic acid) — A:B = 6:4, v/v — with a flow rate of 0.35 mL/min. The detection of amlodipine was conducted by an API 4000 mass spectrometer (AB Sciex, Framingham, Massachusetts) with the positive electrospray ionization multiple reaction monitoring mode set to transmit at m/z 409.30 → 238.10 and 413.20 → 298.20 for amlodipine and amlodipine-d4, respectively. The assay was linear in the concentration range of 0.1-20 μg/L, and the lower limit of quantification (LLOQ) was 0.1 μg/L. Intrabatch and interbatch precision for amlodipine in plasma samples was less than 5.4% and 3.7%, respectively. Intrabatch and interbatch accuracy was between 96.7% and 106.7% and between 100.3% and 101.0%, respectively.

Valsartan. In the valsartan assay, LC-30AD chromatography (Shimadzu) was performed with a Kinetex 2.6 μm C18 100A (100-mm length × 2.1 mm) column (Phenomenex, Torrance, California), and the column temperature was maintained at 40°C. A mixture of water, acetonitrile, and formic acid (50:50:0.1, v/v/v) was used for the mobile phase, with a flow rate of 0.3 mL/min. The detection of valsartan was conducted by an API 4000 mass spectrometer with the positive electrospray ionization multiple reaction monitoring mode set to transmit at m/z 436.279 → 235.000 and 439.294 → 207.100 for valsartan and valsartan-d3, respectively. The assay was linear in the concentration range of 20-20 000 μg/L, and the LLOQ was 20 μg/L. Intrabatch and interbatch precision for valsartan in plasma samples was less than 4.3% and 3.7%, respectively. Intrabatch and interbatch accuracy was between 94.2% and 110.8% and between 96.8% and 104.8%, respectively.

Atorvastatin and 2-OH Atorvastatin. In the atorvastatin and 2-OH atorvastatin assay, LC-20AD chromatography was performed with a Unison UK-C18 3-μm (75-mm length × 2.0 mm) column, and the column temperature was maintained at 40°C. A mixture

of water, acetonitrile, and formic acid (40:60:0.1, v/v/v) was used for the mobile phase, with a flow rate of 0.3 mL/min. The detection of atorvastatin and 2-OH atorvastatin was conducted by an API 5000 mass spectrometer (AB Sciex) with the positive electrospray ionization multiple reaction monitoring mode set to transmit at m/z 559.282 → 440.300 and 564.379 → 445.300 for atorvastatin and atorvastatin–d5, respectively, and at m/z 575.287 → 440.400 and 580.410 → 445.300 for 2-OH atorvastatin and 2-OH atorvastatin–d5, respectively. The assay was linear in the concentration ranges of 0.1-50 μg/L for atorvastatin and 0.05-25 μg/L for 2-OH atorvastatin. The LLOQ of the atorvastatin and 2-OH atorvastatin assay was 0.1 and 0.05 μg/L, respectively. Intrabatch and interbatch precision for atorvastatin in plasma samples was less than 6.1% and 4.8%, respectively. Intrabatch and interbatch accuracy for atorvastatin was between 92.2% and 108.0% and between 100.0% and 103.1%, respectively. Intrabatch and interbatch precision for 2-OH atorvastatin in plasma samples was less than 7.3% and 3.7%, respectively. Intrabatch and interbatch accuracy for 2-OH atorvastatin was between 93.3% and 102.4% and between 97.7% and 99.6%, respectively.

Assessment. The PK parameters for amlodipine, valsartan, atorvastatin, and 2-OH atorvastatin were determined by noncompartmental analysis with a Phoenix WinNonlin (version 7.1; Certara, Princeton, New Jersey). The C_{max} and the time to reach the C_{max} (t_{max}) were directly determined from the observed values. The terminal elimination half-life ($t_{1/2}$) was determined by the linear regression of the log-linear part of the concentration-time curve. The AUC_t and the area under the curve extrapolated to infinity (AUC_{inf}) were estimated.

Physical examinations, vital signs, ECGs, and clinical laboratory tests (hematology, clinical chemistry, coagulation, and urinalysis) were performed at predefined, regular intervals throughout the study. Adverse events (AEs) were recorded on investigators' questionnaires or subjects' spontaneous reports. Safety was assessed by evaluating treatment-emergent adverse events (TEAEs) as well as physical examinations, vital signs, ECGs, and clinical laboratory tests. Particularly, blood pressure deceases that were greater than expected in healthy subjects following the study drug administration were documented as TEAEs.

Statistical Analysis
Statistical analysis was performed by using SAS Enterprise Guide (version 7.1; SAS Institute Inc., Cary, North Carolina). The descriptive statistics of the PK parameters for each analyte were summarized by intervention, and the comparison of PK parameters was performed on C_{max}, AUC_t, and AUC_{inf}.

Log-transformed C_{max}, AUC_t, and AUC_{inf} values were assessed by a mixed-effects model with the sequence, period within the group, intervention, and group as fixed effects and the subject within the sequence*group as a random effect, where the cluster of subjects studied at one time was reflected in the group effect. The 2 interventions were said to be bioequivalent if the 90%CI for the ratio of the FDC to the separate formulations fell within the range of 0.80-1.25 for both the C_{max} and AUC_t of all analytes but 2-OH atorvastatin. However, for the C_{max}, an expanded BE limit was allowed according to the regulation in the Republic of Korea if the estimated within-subject SD of the log-transformed values of the reference intervention (S_{WR}) was greater than 0.2936. When the estimated S_{WR} was greater than 0.2936, the expanded acceptance limit was calculated as exp ($\pm$0.760*S_{WR}), with a maximum of 0.6984-1.4319. The S_{WR} was estimated from the residual variance in the mixed-effects model on the separate formulations' data only. For comparison, we also estimated the S_{WR} by using 2 methods based on the Food ad Drug Administration guidance. The first method was to calculate the value without fitting a model,[23] and the other method was to use the mixed-effects model with the covariance structure of no diagonal factor analytic of grade 1, FA0(1), rather than FA0(2), for obtaining stable estimates.[24]

Results
Subject Disposition
Among 42 subjects enrolled, a total of 35 subjects completed the study (Figure 1). Three subjects withdrew their informed consent, and 3 subjects discontinued as a result of AEs (2 subjects from "blood creatine phosphokinase [CK] increased" and 1 subject from "alanine aminotransferase increased"). Furthermore, the physician discontinued the participation of 1 subject, who had a plan to undergo hemorrhoid surgery during the study.

Pharmacokinetics. The mean plasma concentration-time profiles of amlodipine, valsartan, atorvastatin, and 2-OH atorvastatin following the administration of the FDC and separate formulations are shown in Figure 2. A summary of the PK parameters for amlodipine, valsartan, atorvastatin, and 2-OH atorvastatin are presented in Table 1, and a comparison of the PK parameters between the FDC and separate formulations for each analyte is presented in Table 2. Table 3 summarizes the estimated S_{WR} values of the separate formulations based on 3 different methods.

Amlodipine. The median t_{max} was approximately 6 hours for the FDC and 5-6 hours for the separate formulations, with a range of 4.97-12.00 hours. The
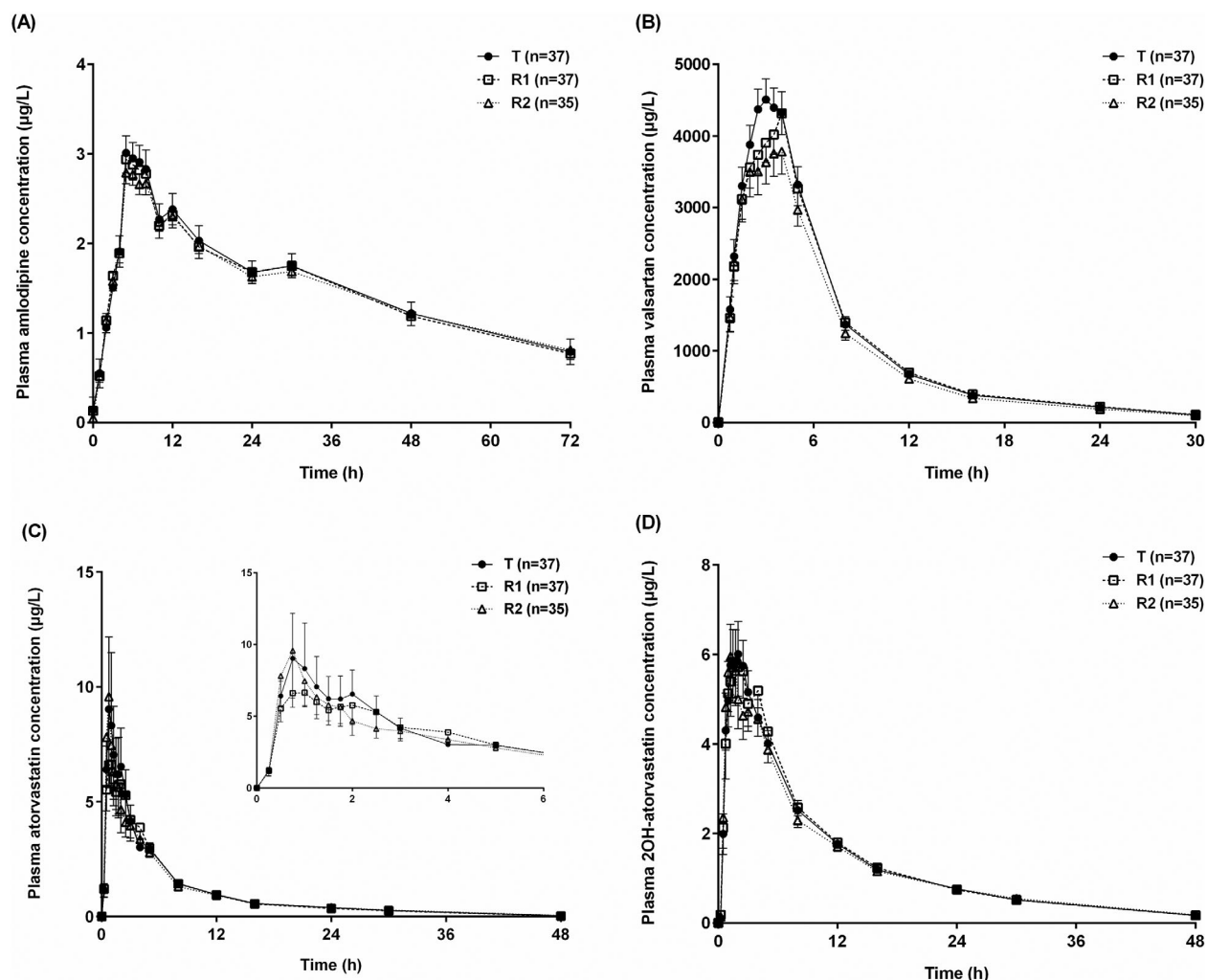
Figure 2. Mean plasma concentration-time profiles of (A) amlodipine, (B) valsartan, (C) atorvastatin, and (D) 2-OH atorvastatin following intervention T (administration of a single dose of the fixed-dose combination tablet of 5/160/20 mg of amlodipine/valsartan/atorvastatin), R1 (the first administration of a single dose of the separate formulations of a 5/160-mg amlodipine/valsartan tablet and a 20-mg atorvastatin tablet), or R2 (the second administration of a single dose of the separate formulations). Bars represent standard deviations.

mean value of the $t_{1/2}$ was approximately 36 hours, and the plasma concentration-time curves showed a similar parallel decline in the distribution and elimination phases for both interventions. The mean C_{max} and AUC_t for the FDC were comparable to those for the separate formulations. Moreover, the GMRs and their 90%CIs for both the C_{max} and AUC_t were within 0.80-1.25.

Valsartan. The median t_{max} was 3.00 for the FDC and 3.00-3.52 for the separate formulations. The mean values of the C_{max} and AUC_t for the FDC seemed slightly higher than those of the separate formulations. However, the 90%CIs for the GMRs of the 2 interventions for both the C_{max} and AUC_t were within 0.80-1.25. The estimated S_{WR} of the C_{max} was 0.2447, with the BE acceptance limit remaining unchanged.

Atorvastatin. The median t_{max} was 1.50 hours for the FDC and approximately 1.00 hours for the sep-

arate formulations; both the FDC and separate formulations had the same ranges of 0.48-5.00 hours. Moreover, a similar mean $t_{1/2}$, of approximately 12 hours, was observed in both interventions. The GMRs (90%CIs) for the C_{max} and AUC_t were 0.9137 (0.7978-1.0466) and 1.0257 (0.9846-1.0685), respectively. The estimated S_{WR} of the C_{max} was 0.3804, and based on this value, the BE acceptance limit for the C_{max} was set at 0.7489-1.3352. For both the C_{max} and AUC_t, the GMRs were between 0.80 and 1.25, and the 90%CIs for the GMRs were within the BE acceptance limit.

2-OH Atorvastatin. The median value of the t_{max} was 2.47 hours for the FDC and 1.50-1.97 hours for the separate formulations. Both the FDC and separate formulations showed a similar elimination profile, with a $t_{1/2}$ of approximately 12 hours. Although the

Table 1. Summary of Pharmacokinetic Parameters by Intervention

	FDC Formulation (n = 37)	Separate Formulations	
		R1 (n = 37)	R2 (n = 35)
Amlodipine			
C_{max} (µg/L)	3.28 ± 1.28	3.20 ± 1.21	3.02 ± 0.79
AUC_t (µg·h/L)	109.5 ± 61.0	107.6 ± 52.9	107.2 ± 33.2
t_{max} (h)	5.97 (4.97-12.00)	5.00 (4.98-8.00)	6.00 (4.97-12.00)
$t_{1/2}$ (h)	36.3 ± 15.1	36.5 ± 17.6	35.5 ± 5.6
AUC_{inf} (µg·h/L)	168.4 ± 219.2	166.0 ± 203.7	140.9 ± 38.0
Valsartan			
C_{max} (µg/L)	5186 ± 1817	4887 ± 1691	4701 ± 1972
AUC_t (µg·h/L)	32545 ± 12716	31594 ± 11957	28838 ± 11511
t_{max} (h)	3.00 (1.48-5.00)	3.52 (1.48-5.00)	3.00 (1.00-5.00)
$t_{1/2}$ (h)	7.4 ± 1.1	7.2 ± 1.4	7.5 ± 1.3
AUC_{inf} (µg·h/L)	33694 ± 13221	32747 ± 12381	29929 ± 11847
Atorvastatin			
C_{max} (µg/L)	12.72 ± 19.09	11.35 ± 8.28	13.74 ± 18.81
AUC_t (µg·h/L)	44.52 ± 34.99	42.47 ± 25.96	42.57 ± 31.02
t_{max} (h)	1.50 (0.48-5.00)	1.02 (0.50-5.00)	0.97 (0.48-5.00)
$t_{1/2}$ (h)	12.2 ± 6.3	11.3 ± 4.9	14.1 ± 8.8
AUC_{inf} (µg·h/L)	48.02 ± 34.78	45.87 ± 26.02	47.02 ± 30.97
2-OH atorvastatin			
C_{max} (µg/L)	8.79 ± 5.41	9.34 ± 4.29	8.34 ± 4.98
AUC_t (µg·h/L)	63.75 ± 25.59	64.73 ± 24.77	61.78 ± 25.47
t_{max} (h)	2.47 (0.72-5.00)	1.97 (0.50-5.00)	1.50 (0.50-5.00)
$t_{1/2}$ (h)	12.0 ± 3.8	11.6 ± 2.6	12.1 ± 3.4
AUC_{inf} (µg·h/L)	66.86 ± 26.26	67.68 ± 25.29	65.16 ± 26.39

FDC formulation, a fixed-dose combination tablet of 5/160/20 mg of amlodipine/valsartan/atorvastatin; separate formulations, a 5/160-mg amlodipine/valsartan tablet and a 20-mg atorvastatin tablet; R1, the first administration; R2, the second administration; C_{max}, maximum plasma concentration; AUC_t, area under the curve to the last measurable concentration; t_{max}, time to reach the C_{max}; $t_{1/2}$, terminal elimination half-life; AUC_{inf}, area under the curve extrapolated to infinity.
Data are presented as the arithmetic means ± standard deviations, except for t_{max}, which is presented as medians (min-max).

estimated SWR of the C_{max} was 0.3115, the 90%CIs for the GMRs of the FDC and separate formulations for both the C_{max} and AUC_t were all within 0.80-1.25.

Safety

No serious TEAEs were reported throughout the study, and all TEAEs were mild to moderate in severity. All TEAEs except 1 ("rhinitis") were resolved without sequelae. Among the 2 discontinued subjects because of increased CK, one was in the separate formulations period and another in the FDC formulation period. Because all the increases were observed 13 days after administration of a single dose, the causal relationships with the study drug were considered as unlikely despite levels more than 10 times the upper limit of normal. Thirty-nine TEAEs were reported by 25 subjects, and headache was the most common TEAE (Table 4). There was no clinically significant change in physical examinations, vital signs, or ECGs.

Discussion

This study showed the PK and safety profiles of the FDC tablet containing 5/160/20 mg amlodipine/valsartan/atorvastatin and the separate formulations of 2 tablets, one for 5/160 mg amlodipine/valsartan and the other for 20 mg atorvastatin. In regard to the linear PK properties, the PK parameters for each analyte were comparable to those reported earlier.[25,26] The 90%CIs for the GMRs of the C_{max} and AUC_t between the FDC and separate formulations were all within the BE acceptance limits based on the replicate crossover study design.

The most frequently reported TEAE was headache, followed by increased CK in this study. According to previous studies, headache was a common AE following administration of the amlodipine and valsartan combination,[27] and increased CK was one of the well-recognized AEs of atorvastatin.[28] There was no significant difference in terms of safety between the 2 interventions.

Table 2. Comparison of Pharmacokinetic Parameters

	Geometric LSM[a]		Geometric Mean Ratio (90% Confidence Interval)[a]
	FDC Formulation (n = 37)	Separate Formulations (n = 37, 35)	
Amlodipine			
C_{max} (µg/L)	3.26	3.11	1.0472 (0.9999-1.0968)
AUC_t (µg·h/L)	104.7	104.6	1.0004 (0.9599-1.0427)
AUC_{inf} (µg·h/L)	139.7	142.5	0.9804 (0.9397-1.0228)
Valsartan			
C_{max} (µg/L)	4773	4398	1.0852 (0.9936-1.1852)
AUC_t (µg·h/L)	29763	27472	1.0834 (1.0018-1.1716)
AUC_{inf} (µg·h/L)	30809	28482	1.0817 (1.0013-1.1685)
Atorvastatin			
C_{max} (µg/L)	9.07	9.93	0.9135 (0.7995-1.0439)[b]
AUC_t (µg·h/L)	38.84	37.81	1.0272 (0.9826-1.0738)
AUC_{inf} (µg·h/L)	42.78	41.92	1.0207 (0.9727-1.0711)
2-OH atorvastatin			
C_{max} (µg/L)	7.69	7.91	0.9723 (0.8634-1.0949)
AUC_t (µg·h/L)	57.78	56.73	1.0186 (0.9743-1.0648)
AUC_{inf} (µg·h/L)	60.76	59.78	1.0164 (0.9739-1.0608)

FDC formulation, a fixed-dose combination tablet of 5/160/20 mg of amlodipine/valsartan/atorvastatin; separate formulations, a 5/160-mg amlodipine/valsartan tablet and a 20-mg atorvastatin tablet; LSM, least-squares mean.
[a] Transformed back to the original scale after the linear mixed-model analysis using log-transformed data.
[b] Bioequivalence acceptance limit is expanded to 0.7489-1.3352 because the estimated within-subject standard deviation of the separate formulations for C_{max} is 0.3804, being greater than 0.2936.

Table 3. Within-Subject Standard Deviation of the Separate Formulations

	Method 1[a]	Method 2[b]	Method 3[c]
Amlodipine			
$Log(C_{max})$	0.1596	0.1626	0.1553
$Log(AUC_t)$	0.1349	0.1372	0.1331
Valsartan			
$Log(C_{max})$	0.2431	0.2447	0.2476
$Log(AUC_t)$	0.2371	0.2387	0.2449
Atorvastatin			
$Log(C_{max})$	0.3804	0.3832	0.3764
$Log(AUC_t)$	0.1548	0.1521	0.1510
2-OH atorvastatin			
$Log(C_{max})$	0.3115	0.3155	0.3148
$Log(AUC_t)$	0.1405	0.1423	0.1459

[a] The same as "Method B" in the European Medicines Agency guideline.
[b] Calculating based on the Food and Drug Administration (FDA) guidance without fitting a model.
[c] Using the mixed-effects model with the covariance structure of FA0(1), the simplified version of FA0(2) suggested in the FDA guidance

Table 4. Summary of Treatment-Emergent Adverse Events Occurring in 2 or More Subjects by Intervention

Symptoms and Signs	FDC Formulation (n = 37)	Separate Formulations	
		R1 (n = 42)	R2 (n = 35)
Anemia	1 (1)		1 (1)
Neutropenia		2 (2)	1 (1)
Pyuria	1 (1)	1 (1)	1 (1)
Blood creatine phosphokinase increased	1 (1)	2 (2)	2 (2)
Glucose urine			2 (2)
Headache	2 (2)	4 (4)	1 (1)

FDC formulation, a fixed-dose combination tablet of 5/160/20 mg of amlodipine/valsartan/atorvastatin; separate formulations, a 5/160-mg amlodipine/valsartan tablet and a 20-mg atorvastatin tablet; R1, the first administration; R2, the second administration.
Data are presented as number of subjects (number of events).

Approximately 66 subjects are required to establish BE in a conventional 2 × 2 crossover study with 80% statistical power at a 5% level of significance, with the assumption of a 40% within-subject CV of the C_{max} for atorvastatin and a 5% true difference between the test and reference interventions. However, we performed a 3-period replicate crossover study with 42 subjects, although the minimum sample size for that study design was 30 under the same conditions as the 2 × 2 crossover study. If the reference-scaled average BE approach is applied according to the regulations of regulatory authorities, a replicate crossover design can

reduce the required sample size without compromising the statistical power.

The 3 different methods resulted in very similar estimates of the S_{WR}. Moreover, no overall trend was observed among the methods. The S_{WR} estimates in this study were similar to those reported in previous studies,[19–21] particularly the highest one of the C_{max} for atorvastatin. A previous study revealed that extensive first-pass metabolism was probably the most important factor for high within-subject variability.[29] As expected, atorvastatin is extensively metabolized in both the gut and liver, resulting in low oral bioavailability.[16] Furthermore, it should be noted that the S_{WR} of the C_{max} for the metabolite of atorvastatin, 2-OH atorvastatin, was considerably lower than that of atorvastatin. This is in line with the understanding that it is more appropriate to assess BE in terms of the parent drug whose PK is more sensitive to changes than its metabolite.

A replicate crossover design with 3 or 4 periods would be appropriate to assess the BE of HVDs, which have a relatively short elimination half-life. Theoretically, a 4-period replicate crossover design would require fewer subjects than a 3-period replicate crossover design given the same conditions.[22] It is considered ethical that healthy subjects participate in a clinical study with a short duration and low exposure to drugs. Moreover, as the study duration becomes longer, it becomes more likely that subjects will be lost. Thus, a 3-period replicate crossover design can be the most efficient way when multiple aspects of obtaining evaluable data are considered.[30]

There are a few things to consider when the results of this study are extrapolated to patients with HTN and dyslipidemia. These data were collected after administration of a single dose only in relatively young and healthy men. In view of patients being likely to be old and that the drugs used for this study are administered regardless of sex, the results may be interpreted with caution. Also, it should be noted that it is difficult to delicately adjust the dose of each component in FDCs, although dose titration is quite important in some patients with HTN. Furthermore, a previous study reported that FDCs were associated with increased risk of therapeutic duplication.[31]

In conclusion, this 3-period replicate crossover study demonstrated the BE of the FDC formulation of amlodipine, valsartan, and atorvastatin and the separate formulations of an amlodipine/valsartan tablet and an atorvastatin tablet by using a reference-scaled average BE approach.

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Conflicts of Interest

The authors declare no conflicts of interest.

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