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ORIGINAL RESEARCH

Pharmacokinetic Drug Interactions Between Amlodipine, Valsartan, and Rosuvastatin in Healthy Volunteers

Sook Jin Seong · Boram Ohk · Woo Youl Kang · Mi-Ri Gwon · Bo Kyung Kim · Seungil Cho · Dong Heon Yang · Hae Won Lee · Young-Ran Yoon

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ABSTRACT

Introduction: Amlodipine, valsartan, and rosuvastatin are among the medications widely coadministered for the treatment of hyperlipidemia accompanied by hypertension. The aim of this study was to investigate the possible pharmacokinetic drug–drug interactions between amlodipine, valsartan, and rosuvastatin in healthy Korean male volunteers.

Methods: In this phase 1, open-label, multiple-dose, two-part, two-period, fixed-sequence study, the enrolled subjects were randomized into two parts (A and B). In part A (n = 32), each subject received one fixed-dose combination (FDC) tablet of amlodipine/valsartan 10 mg/160 mg alone for 10 consecutive days in period I, and the same FDC for 10 days with concomitant 7-day administration of 20 mg rosuvastatin in period II. In part B (n = 25), each subject received rosuvastatin alone for 7 days in period I, and the FDC for 10 days with concomitant 7-day administration of rosuvastatin in period II. In both parts, there was a 12-day washout between periods. Serial blood samples were collected for up to 72 h for amlodipine and rosuvastatin, and for up to 48 h for valsartan after the last dose of each period. The plasma concentrations of amlodipine, valsartan, and rosuvastatin were determined by using liquid chromatography–tandem mass spectrometry.

Results: Fifty-seven subjects were enrolled; 30 and 25 subjects completed part A and part B, respectively. The geometric mean ratios and 90% confidence intervals for the maximum plasma concentration at steady state ($C_{max,ss}$) and the area under the plasma concentration–time curve over the dosing interval at steady state ($AUC_{\tau,ss}$) were 0.9389 (0.9029–0.9763) and 0.9316 (0.8970–0.9675) for amlodipine, 0.7698 (0.6503–0.9114) and 0.7888 (0.6943–0.8962) for valsartan, and 0.9737 (0.8312–1.1407) and 0.9596 (0.8826–1.0433) for

Sook Jin Seong and Boram Ohk contributed equally to this work.

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S. J. Seong · B. Ohk · W. Y. Kang · M.-R. Gwon · B. K. Kim · S. Cho · H. W. Lee (✉) · Y.-R. Yoon (✉)
Department of Molecular Medicine, School of Medicine, Kyungpook National University, Daegu, Republic of Korea
e-mail: haewonbbc@gmail.com

Y.-R. Yoon
e-mail: yry@knu.ac.kr

S. J. Seong · B. Ohk · W. Y. Kang · M.-R. Gwon · B. K. Kim · S. Cho · H. W. Lee · Y.-R. Yoon
Department of Clinical Pharmacology, Kyungpook National University Hospital, Daegu, Republic of Korea

D. H. Yang
Division of Cardiology, Department of Internal Medicine, Kyungpook National University Hospital, Daegu, Republic of Korea

rosuvastatin, respectively. Of the 57 subjects enrolled in this study, 10 subjects experienced 13 adverse events (AEs); no severe or serious AEs were reported.

Conclusion: When amlodipine, valsartan, and rosuvastatin were coadministered to healthy volunteers, the pharmacokinetic exposure to valsartan was decreased, but no change in exposure to amlodipine and rosuvastatin occurred. All treatments were well tolerated.

Clinical Trial Registration: https://cris.nih.go.kr CRIS KCT0001660.

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Keywords: Amlodipine; Drug–drug interaction; Rosuvastatin; Valsartan

INTRODUCTION

The concurrence of two major risk factors for cardiovascular disease (CVD), hypertension and dyslipidemia, has been reported to be frequent and to synergistically increase the risk of CVD [1]. The effective and simultaneous management of hypertension and dyslipidemia can reduce the risk of CVD by more than 50% [2, 3].

According to the 2013 guidelines for the treatment of hypertension from the Eighth Joint National Committee On Prevention, Detection, Evaluation, and Treatment Of High Blood Pressure (JNC 8), the initial choice of treatment should be from any of four different classes of antihypertensive drugs: thiazide-type diuretics, calcium channel blockers (CCB), angiotensin-converting enzyme inhibitors (ACEI), or angiotensin receptor blockers (ARBs) [4, 5]. As monotherapy does not lower blood pressure (BP) to target levels in almost 70% of hypertensive patients, combination therapies, which have been shown to provide greater and more prompt BP reduction at lower doses and with fewer adverse effects than higher doses of a single drug, are required; at least two drugs should be from different classes [6]. ARB/CCB combination therapy is one of the preferred combinations of antihypertensive drugs from different classes, as the incidence of some adverse effects of CCBs, such as peripheral edema or tachycardia, was found to be reduced [7, 8].

Amlodipine, a third-generation dihydropyridine CCB, inhibits the transmembrane calcium ion influx into vascular smooth muscle cells, and lowers blood pressure through the relaxation of the smooth muscle in the arterial wall and a decrease in total peripheral resistance [9]. Amlodipine is administered at a dose of between 5 and 10 mg once daily. Following oral administration, amlodipine has a long time to reach maximum concentration (t_{max}) of 6–12 h, and a long elimination half-life ($t_{1/2}$) of 30–50 h. In total, 90% of absorbed amlodipine is converted to inactive metabolites, mainly by the CYP3A4 pathway; and 10% of the parent compound and 60% of the metabolites are eliminated via renal excretion [9–12]. The transporter involved in the efflux of amlodipine is multidrug resistance 1 (MDR1) and amlodipine is a moderate inhibitor of breast cancer-resistance protein (BCRP) [13–15].

Valsartan, the most commonly prescribed angiotensin II type 1 (AT_1) receptor blocker, selectively blocks the binding of angiotensin II to the AT_1 receptor in many tissues, including vascular smooth muscle and the adrenal gland, and lowers BP through the inhibition of the vasoconstriction and aldosterone-secreting effects of angiotensin II [16]. The usual starting dose of valsartan is 80–160 mg once daily, adjusted to 320 mg as required [16]. The maximum plasma concentration (C_{max}) of valsartan was observed to occur between 2 and 4 h after dosing, with a $t_{1/2}$ of 6–9 h [17]. When orally administered, most (83%) of the dose was excreted in feces, and to a lesser extent in urine (13%), mainly as unchanged drug, with only approximately 20% of the dose recovered as metabolites [16, 17]. The major metabolite, valeryl-4-hydroxy valsartan, is formed by the isoenzyme CYP2C9 [16]. The majority (73%) of the administered valsartan is transported to the liver by the action of organic anion-transporting polypeptide (OATP) 1B3, and a lesser amount by OATP1B1, and then excreted to the bile by multidrug resistance-associated protein 2 (MRP2) [18].

The first-line drug therapy used in most patients with dyslipidemia is statins, which are

inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase [19]. Six weeks of 10–80 mg rosuvastatin treatment showed superior effectiveness to atorvastatin, simvastatin, and pravastatin in lowering the low-density lipoprotein cholesterol levels [20]. The t_{max} of rosuvastatin occurred between 3 and 5 h after dosing, with a $t_{1/2}$ of approximately 19 h [21]. After oral administration, rosuvastatin is not extensively metabolized: 10% of a radiolabeled dose was recovered as the metabolite, and was mainly excreted into bile, primarily (76.8%) as the parent form [21, 22]. The metabolism of rosuvastatin mediated by the cytochrome P450 (CYP) is minimal; metabolism occurs primarily by the CYP2C9 pathway, with lesser involvement of CYP2C19 [21, 23]. The hepatic uptake and biliary excretion of rosuvastatin were associated with OATP1B1/1B3 and 2B1, and BCRP, MDR1, and MRP2, respectively [24].

In practice, rosuvastatin is often coadministered with amlodipine and valsartan for the treatment of hyperlipidemia accompanied by hypertension. However, to the best of our knowledge, there has been no study of the pharmacokinetic interactions of the concomitant administration of the three drugs (amlodipine, valsartan, and rosuvastatin). Therefore, the present study sought to investigate the potential pharmacokinetic drug–drug interactions of amlodipine, valsartan, and rosuvastatin in healthy Korean male subjects.

METHODS

Subjects

The subjects eligible to participate in this study were healthy male volunteers, between 20 and 55 years of age, with a body weight of at least 50 kg, and within ± 20% of their ideal body weight. All subjects were considered healthy on the basis of their medical history, and a physical examination, routine clinical laboratory tests (serology, hematology, clinical chemistry, and urinalysis), and 12-lead electrocardiography performed in the 3 weeks prior to the administration of study drugs. The following exclusion criteria were applied: (1) medical history of clinically significant hypersensitivity to study drug (major ingredient or any other ingredient) or any other drug or additives; (2) medical history that may affect the absorption, distribution, metabolism, or excretion of a drug [hepatobiliary, renal, cardiovascular, endocrine (e.g., hypothyroidism), respiratory, gastrointestinal, hemato-oncology, central nervous system, psychiatric, and musculoskeletal system history]; (3) medical history or family history of hereditary muscular disease; (4) hypotension (systolic BP ≤ 115 mmHg or diastolic BP ≤ 65 mmHg) or hypertension (systolic BP ≥ 150 mmHg or diastolic BP ≥ 100 mmHg), measured at screening; (5) active liver disease, or levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), or total bilirubin > 1.5 × the upper limit of normal (ULN); (6) creatinine clearance < 80 mL/min (calculated by the Cockcroft-Gault formula using serum creatinine); (7) history of gastrointestinal disease (e.g., Crohn's disease or active peptic ulcer) or gastrointestinal surgery that may affect the absorption of the study drug (excluding simple appendectomy or herniorrhaphy); (8) history of major injury, surgery, or suspected symptoms of acute illness (such as severe infection, trauma, diarrhea, or vomiting) in the 4 weeks prior to the first administration of study drug; (9) history of excessive alcohol use (> 21 units/week, 1 unit = 10 g = 12.5 mL of pure alcohol), or subjects who could not abstain from drinking for at least 3 days prior to the start of the study and during the study period, or excessive smoking (> 10 cigarettes/day); (10) use of any prescribed drugs or herbal remedies within 2 weeks, or use of any over-the-counter medication within 1 week, prior to the first administration of study drug; (11) participation in any other study within 3 months of the first administration of study drug (where the completion date of the previous study was the day of the final administration of the study drug); (12) donation of whole blood in the 2 months prior to the first administration of the study drug or donation of any blood component in the 1 month prior to the first administration of the study drug; (13) abnormal diet that may affect the absorption, distribution, metabolism, and excretion of

drugs (e.g., grapefruit juice ≥ 1 L/day within the 7 days prior to administration of study drug); (14) positive serologic tests (HBsAg, HCV Ab, HIV Ag/Ab, or VDRL); (15) subjects that were not eligible to participate at the discretion of the study investigator.

Subjects who participated in this study were asked to avoid any prescribed drugs, or over-the counter medication, or any food that could affect the metabolism of drugs (e.g., grapefruit juice, grape, and broccoli), 7 days prior to the first administration of the study drug to the end of the study. Alcohol, tobacco, xanthine-containing substances, and strenuous exercise were also restricted 3 days prior to the first administration of the study drug until the last blood sample was collected in the respective study period.

The study protocol was approved by the Institutional Review Board at Kyungpook National University Hospital (KNUH, Daegu, Republic of Korea), and the study (CRIS registry no.: KCT0001660) was performed in accordance with the ethical standards for studies in humans set out in the Declaration of Helsinki and its amendments, and the applicable guidelines for Good Clinical Practice. Written informed consent was obtained from all subjects before their enrollment in the study.

Study Design and Procedure

This study consisted of two separate parts: part A and B. Each was an open-label, multiple-dose, two-period, fixed-sequence study conducted at the KNUH Clinical Trial Center (CTC). After enrollment, the subjects were randomly assigned into either part A or part B by using a computer-generated randomization scheme in Visual Fortran software version 11.1 and the IMSL Fortran library (Compaq Computer Corporation, Houston, Texas, USA).

In part A of the study, each subject received the fixed-dose combination (FDC) tablet of amlodipine/valsartan 10 mg/160 mg (Exforge®, Novartis Korea, Seoul, Republic of Korea) alone, once daily for 10 consecutive days in period I, and then received the same FDC tablet for 10 days in period II, coadministered with 20 mg rosuvastatin (Crestor®, AstraZeneca Inc., London UK) once daily for 7 days, starting on day 4 of period II. The effect of multiple doses of rosuvastatin on the steady-state pharmacokinetic properties of amlodipine/valsartan administered as the FDC tablet was evaluated. To determine the plasma concentration of amlodipine and valsartan, serial blood samples were collected at 0 (predose), 1, 2, 3, 4, 5, 6, 8, 9, 10, 12, 24, 48, and 72 h after the final dose on days 10 and 31 for amlodipine, and at 0, 1, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12, 24, and 48 h after the final dose on days 10 and 31 for valsartan, respectively.

In part B of the study, each subject received 20 mg rosuvastatin alone, once daily for 7 consecutive days in period I, and then received the same FDC tablet for 10 days in period II, coadministered with 20 mg rosuvastatin once daily for 7 days, starting from day 4 of period II. The effects of multiple doses of amlodipine/valsartan on the steady-state pharmacokinetic properties of rosuvastatin administered concomitantly were evaluated. To determine the plasma concentration of rosuvastatin, serial blood samples were collected at 0 (predose), 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 8, 12, 24, 48, and 72 h after the final dose on days 7 and 28.

The study design for parts A and B are illustrated in Fig. 1. In both parts of the study, there was a 12-day washout between the final dose of period I and the first dose of period II. In both parts of the study, all subjects came to the study center every morning (part A, days 1–9 and days 22–30; part B, days 1–6 and days 19–27) for the assessment of vital signs, followed by study drug administration under the supervision of the investigator. Subjects were admitted to the center 12 h before the final dose of each period; after a 10-h overnight fast, subjects received the final maintenance dose with 150 mL water on the morning of days 10 and 31 for part A, and days 7 and 28 for part B. Additional water intake was prohibited for 2 h before and after the final dosing, and food intake was restricted for 4 h after dosing. Standard meals were served for lunch and dinner at 4 and 10 h after the final dose, respectively. Subjects were confined until 24 h after dosing, and additional 48- and 72-h visits were made for pharmacokinetic sampling.

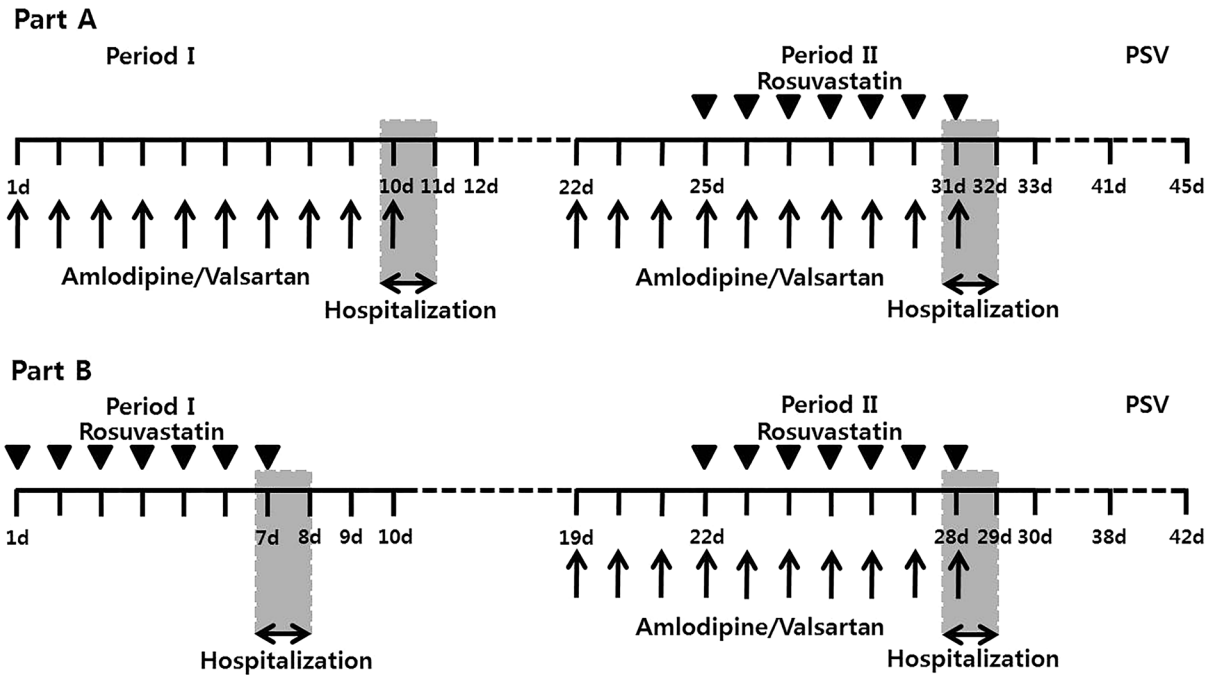

Fig. 1 Study design

Analysis of Amlodipine, Valsartan, and Rosuvastatin Concentrations in Plasma

Blood samples for pharmacokinetic analysis (each 9 mL) were collected into tubes containing sodium heparin via a saline-locked intravenous catheter inserted into each subject's forearm or the dorsum of the hand, and centrifuged at 4 °C (3000 rpm, 10 min) to separate plasma. Following centrifugation, plasma samples were transferred to Eppendorf tubes (each 1 mL) and stored below − 70 °C until analysis by International Scientific Standards, Ltd. (Chuncheon, Gangwon Province, Republic of Korea).

The plasma concentrations of amlodipine were determined by using high-performance liquid chromatography (LC-30AD, Shimadzu Corp. Kyoto, Japan) coupled with tandem mass spectrometry (MS/MS, API 5500 Triple Quadrupole, AB SCIEX, Foster City, CA, USA). Chromatographic separations were performed on a ACE 5 C18 column (Advanced Chromatography Technologies, London, UK) (2.1 × 150 mm internal diameter, 5 μm particle size) at a flow rate of 0.3 mL/min. The mobile phase consisted of a 30:70 (v/v) mixture of 10 mM ammonium acetate and methanol with 0.1% formic acid. Multiple reaction monitoring (MRM) transitions were performed at mass-to-charge ratios (m/z) of 409.2 → 237.9 and 413.2 → 237.9 for amlodipine and amlodipine-d_4 [the internal standard (IS)], respectively. Frozen plasma was thawed at room temperature and vortexed. After the addition of 20 μL IS (100 ng/mL) to 100 μL plasma, acetonitrile (v/v, with 0.1% formic acid) was added and vortexed for 1 min. The mixture was centrifuged at 13,200 rpm for 10 min. Subsequently, 100 μL of 50% methanol was added to 100 μL supernatant and the mixture was vortexed for 10 s, and a 5-μL aliquot of this solution was injected into the LC–MS/MS system for analysis. The lower limit of quantification was 0.3 ng/mL, and calibration curves ranged between 0.3 and 50 ng/mL for amlodipine ($r \geq 0.9996$). The overall intra-day and inter-day accuracy was between 95.3% and 99.5%, and between 98.9% and 100.9%, respectively. The intra-day and inter-day precision (%CV) was between 1.4% and 4.9%, and

between 1.7% and 3.4%, respectively, at concentrations of 0.3, 0.9, 5, and 40 ng/mL.

The plasma concentrations of valsartan were determined by using high-performance liquid chromatography (LC-20AD, Shimadzu Corp. Kyoto, Japan) coupled with MS/MS (API 4000 Triple Quadrupole, AB SCIEX, Foster City, CA, USA). Chromatographic separations were performed on a Hypersil GOLD column (Advanced Chromatography Technologies, London, UK) (2.1 × 150 mm internal diameter, 5 μm particle size) at a flow rate of 0.35 mL/min. The mobile phase consisted of a 50:50 (v/v) mixture of water and acetonitrile (with 0.1% formic acid). MRM transitions were performed at *m/z* of 436.3 → 235.0 and 439.4 → 207.0 for valsartan and valsartan-d_3 (the IS), respectively. Frozen plasma was thawed at room temperature and vortexed. After 50 μL IS (5000 ng/mL) was added to 50 μL plasma, 800 μL acetonitrile (v/v, with 0.1% formic acid) was added and vortexed for 1 min. The mixture was centrifuged at 13,200 rpm for 10 min, and a 3-μL aliquot of this solution was injected into the LC–MS/MS system for analysis. The lower limit of quantification was 20 ng/mL, and the calibration curves ranged between 20 and 20,000 ng/mL for valsartan ($r \geq 0.9978$). The overall intra-day and inter-day accuracy was between 92.4% and 100.7%, and between 94.9% and 103.2%, respectively. The intra-day and inter-day precision (%CV) was between 3.8% and 7.6%, and between 3.4% and 8.8%, respectively, at concentrations of 20, 60, 600, and 16,000 ng/mL.

The plasma concentrations of rosuvastatin were determined by using high-performance liquid chromatography (LC-20AD, Shimadzu Corp. Kyoto, Japan) coupled with MS/MS (API 5000 Triple Quadrupole, AB SCIEX, Foster City, CA, USA). Chromatographic separations were performed on a Symmetry C_{18} column (Advanced Chromatography Technologies, London, UK) (2.1 × 150 mm internal diameter, 5 μm particle size) at a flow rate of 0.35 mL/min. The mobile phase consisted of a 45:55 (v/v) mixture of water and acetonitrile (with 0.1% formic acid). MRM monitoring transitions were performed at mass-to-charge ratios (*m/z*) of 482.2 → 258.3 and 488.3 → 264.3 for rosuvastatin and rosuvastatin-d_6 (the IS), respectively. Frozen plasma was thawed at room temperature and vortexed. After the addition of 15 μL IS (160 ng/mL) and 10 mM ammonium acetate (pH 4.0, with acetic acid) to 100 μL plasma, 0.75 mL of a 70:30 (v/v) mixture of ethyl ether and dichloromethane was added, mixed, and extracted by shaking for 15 min at 130 rpm. The solution was centrifuged at 13,000 rpm for 5 min and kept frozen for 20 min. The organic layer was transferred to a clean glass test tube, and then evaporated to dryness under a stream of nitrogen (40 °C). The dried extract was reconstituted in 200 μL of the mobile phase, vortex mixed for 20 s, and centrifuged for 5 min at 13,000 rpm. A 5-μL aliquot was then injected into the LC–MS/MS system for analysis. The lower limit of quantification was 0.2 ng/mL, and the calibration curves ranged between 0.2 and 100 ng/mL for rosuvastatin ($r \geq 0.9997$). The overall intra-day and inter-day accuracy was between 93.0% and 98.1%, and between 94.4% and 99.1%, respectively. The intra-day and inter-day precision (%CV) was between 0.4% and 4.8%, and between 1.1% and 5.6%, respectively, at concentrations of 0.2, 0.6, 6, and 80 ng/mL.

Pharmacokinetic Evaluation

The pharmacokinetic parameters for amlodipine, valsartan, and rosuvastatin in plasma were calculated by non-compartmental methods using WinNonlin Pro 5.3 (Pharsight Corporation, Mountain View, CA, USA). The PK parameters of $C_{max,ss}$ (the maximum concentration of drug in plasma at steady state) and $T_{max,ss}$ (the time to reach $C_{max,ss}$) were determined on the basis of the amlodipine, valsartan, and rosuvastatin concentrations in individual subjects by using the actual sampling times after the administration of the final maintenance dose and were estimated directly from the observed plasma concentrations over time. The terminal elimination rate constant (λ_z) was determined by linear regression of the logarithmic-linear decline of the final data points; a minimum of three values were required. The value of $t_{1/2}$ was calculated by using $\ln(2)/\lambda_z$. The area under the plasma concentration–time

curve over the dosing interval (τ) after multiple-dose administration at steady state ($AUC_{\tau,ss}$) was calculated by using the linear trapezoidal method.

Assessment of Safety

Safety was assessed in all subjects who received at least one dose of the study drugs throughout the study period on the basis of clinical and laboratory adverse events (AEs), including all subjective symptoms reported by subjects and objective signs observed by the investigators. Values were collected and compared pre- and post-dosing of amlodipine, valsartan, and rosuvastatin to evaluate differences. Vital signs (BP and pulse rate) were monitored at screening, pre-dose on each dosing day, on the day of the final dose (predose, 2, 4, 6, 12, and 24 h after the final dosing) during each period, and at the follow-up visit. Body temperature was assessed at screening and at the follow-up visit. The following clinical laboratory tests, and 12-lead electrocardiograms (ECGs), were conducted at screening, before the first dose in each period (day − 1 and day 21 (± 1 day) for part A, day − 1 and day 18 (± 1 day) for part B), and at the follow-up visit: blood hematology [hemoglobin, hematocrit, red blood cell count, platelet and white blood cell count (lymphocytes, monocytes, eosinophils, and basophils) with differential counts]; urinalysis [specific gravity, pH, protein, glucose, ketone, bilirubin, occult blood, urobilinogen, nitrite, and microscopic examination (red and white blood cells)] and serum chemistry (fasting glucose, blood urea nitrogen, creatinine, total cholesterol, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT, γ-glutamyl transferase, lactate dehydrogenase, creatine phosphokinase (CPK), uric acid, sodium, potassium, and chloride). All laboratory tests were performed at an accredited laboratory (Department of Laboratory Medicine, KNUH, Daegu, Republic of Korea).

Any unfavorable symptoms, signs, or medical conditions occurring on or after the administration of the first dose (treatment-emergent AE; TEAE) were recorded, regardless of their relationship to the study drug. TEAEs were classified by severity as mild, moderate, or severe; and by their relationship to the study medication as not related, unlikely to be related, possibly related, probably related, or certainly related.

Statistical Analyses

The sample size for part A was calculated on the basis of the intra-subject variability of the AUC for valsartan (39.0%), which was the highest value of the AUC and C_{max} values for amlodipine and valsartan in previous PK studies [25]. The sample size for part B was calculated on the basis of the intra-subject variability of the AUC for rosuvastatin (34.2%), which was the highest value of the AUC values and C_{max} values in previous PK studies [26]. Overall, 27 subjects for part A and 21 subjects for part B were considered necessary to demonstrate a 20% difference in the log-transformed values from two different treatment groups with 80% power and a 5% level of significance. Therefore, the total numbers of subjects required for enrollment into for parts A and B were 32 and 25, respectively, assuming an estimated attrition rate of 15%.

Demographic data, including age, height, and body weight, and pharmacokinetic parameters, were analyzed by using descriptive statistics. The results were expressed as the mean ± standard deviation, except for t_{max} values, which were expressed as the median (maximum and minimum values). Pharmacokinetic parameters were compared between the treatment groups (concomitant administration and individual administration) by using paired t tests, after analysis with descriptive statistics.

To assess the associated drug–drug interaction, the geometric mean ratios (GMRs) and 90% CIs of log-transformed $AUC_{\tau,ss}$ and $C_{max,ss}$ values of amlodipine and valsartan (part A) and rosuvastatin (part B) for the two treatment groups (concomitant administration or individual administration) were assessed by using a mixed-effects analysis of variance (ANOVA) model, with the random effect of subject, and fixed effects of sequence, period, and treatment.

For the safety assessment, the results from AE monitoring and the assessment of vital signs, ECGs, and laboratory tests were reviewed and tabulated. All statistical analyses were computed by using SPSS for Windows software (ver. 18.0; SPSS Korea, Seoul, Korea). A p value of less than 0.05 was deemed to indicate statistical significance.

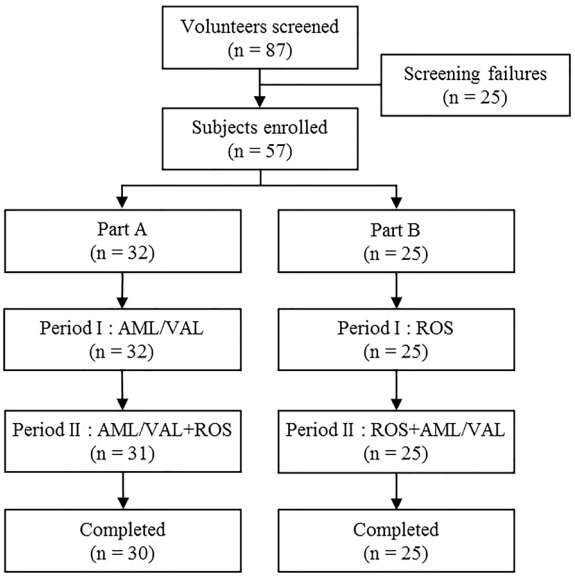

Fig. 2 Subject disposition. AML/VAL fixed-dose combination formulation of amlodipine 10 mg and valsartan 160 mg. ROS rosuvastatin 20 mg

Table 1 Demographic characteristics of the subjects who completed the study, according to the study parts

Demographic variables	Overall (n = 55)	Part A (n = 30)	Part B (n = 25)
Age, years			
Mean ± SD	26.2 ± 4.7	24.7 ± 3.4	28.0 ± 5.5
Range	19–47	19–32	23–47
Height, cm			
Mean ± SD	174.6 ± 6.0	175.5 ± 6.1	173.6 ± 5.8
Range	162.2–190.0	162.2–190.0	165.1–187.4
Weight, kg			
Mean ± SD	70.4 ± 9.9	71.5 ± 10.5	69.2 ± 9.1
Range	54.6–97.1	54.6–97.1	54.6–85.1

Data are given as the mean ± standard deviation (range) for age, height, and weight

Fig. 3 Mean (SD) plasma concentration–time profiles of ▶ **a** amlodipine and **b** valsartan after multiple oral administration of amlodipine/valsartan (10 mg/160 mg) FDC alone and in combination with rosuvastatin (20 mg), and of **c** rosuvastatin after multiple oral administration of rosuvastatin (20 mg) alone and in combination with amlodipine/valsartan (10 mg/160 mg) FDC

RESULTS

Subjects

In total, 87 volunteers were screened and 57 were enrolled in this study (32 in part A and 25 in part B). The subject disposition is illustrated in Fig. 2. In part A of the study, one subject who withdrew consent before initiation of period I was replaced with a subject from the reserve list. One subject withdrew consent during period I and one subject withdrew in period II; in total, 30 subjects completed the study. In part B of the study, two subjects who withdrew consent before initiation of period I were replaced by other subjects from the waiting list; in total, 25 subjects completed the study. The demographic characteristics of the subjects who completed the study are summarized in Table 1.

All subjects that received at least one dose of amlodipine/valsartan and/or rosuvastatin (part A, n = 32; part B, n = 25) were included in the safety analysis. In part A, 30 subjects were included in the pharmacokinetic analysis of amlodipine and valsartan, and in part B, 25 subjects were included in the pharmacokinetic analysis of rosuvastatin.

Pharmacokinetic Properties

Part A: Effect of Rosuvastatin on the Pharmacokinetic Properties of Amlodipine/Valsartan

All 30 subjects who completed the study in part A were included in the pharmacokinetic assessment. The mean plasma concentration–time profiles for amlodipine and valsartan

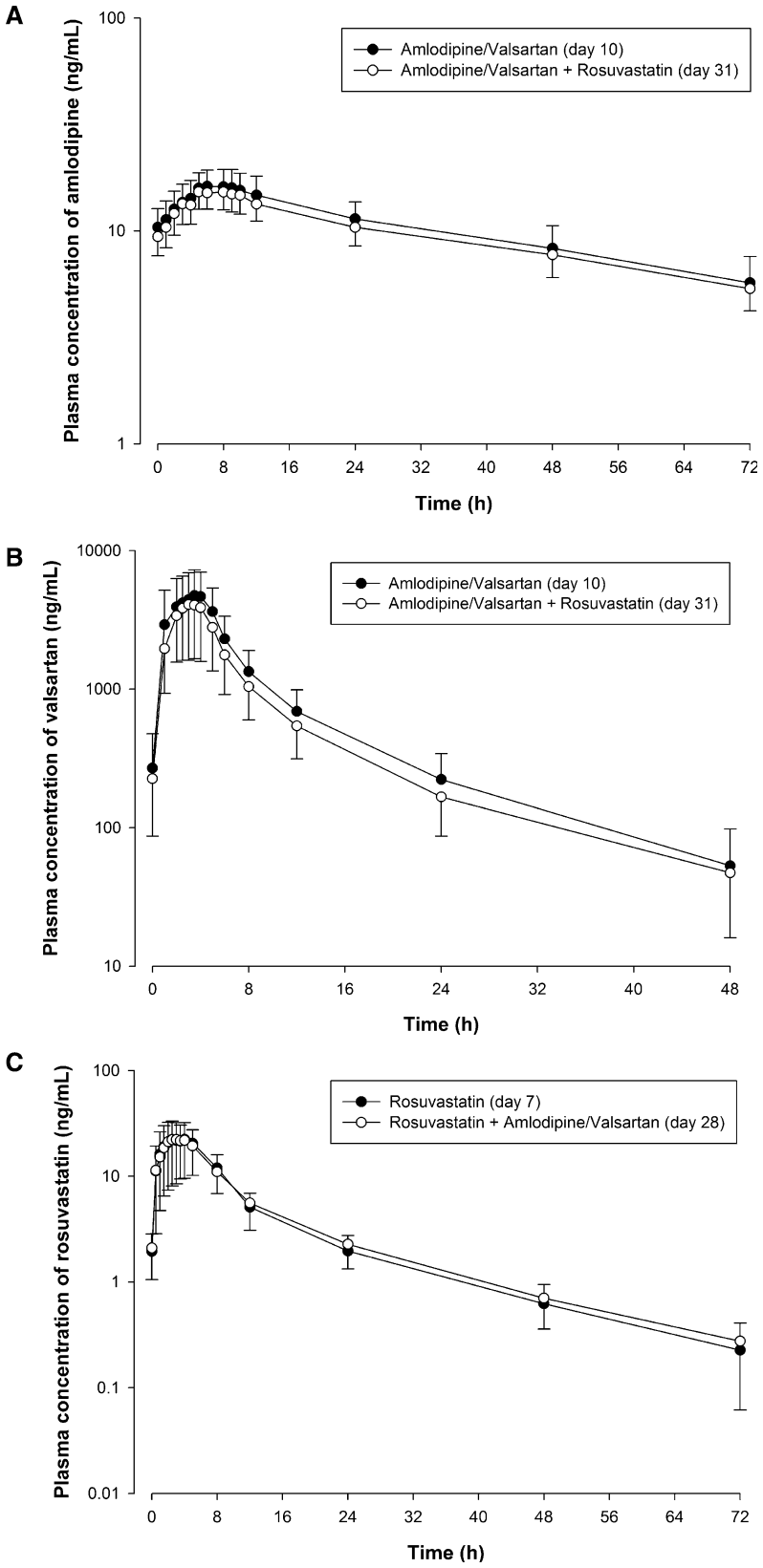
A
Plasma concentration of amlodipine (ng/mL)
100
10
1
Amlodipine/Valsartan (day 10)
Amlodipine/Valsartan + Rosuvastatin (day 31)
0
8
16
24
32
40
48
56
64
72
Time (h)
B
Plasma concentration of valsartan (ng/mL)
10000
1000
100
10
Amlodipine/Valsartan (day 10)
Amlodipine/Valsartan + Rosuvastatin (day 31)
0
8
16
24
32
40
48
Time (h)
C
Plasma concentration of rosuvastatin (ng/mL)
100
10
1
0.1
0.01
Rosuvastatin (day 7)
Rosuvastatin + Amlodipine/Valsartan (day 28)
0
8
16
24
32
40
48
56
64
72
Time (h)

Table 2 Steady-state pharmacokinetic parameters following administration of fixed-dose combination formulation of amlodipine/valsartan (10 mg/160 mg) and rosuvastatin (20 mg) as concomitant administration vs. individual administration under fasted conditions in healthy volunteers

	Part A (n = 30)	
	AML/VAL	**AML/VAL + ROS**
Amlodipine		
$C_{max,ss}$ (ng/mL)	16.9 ± 3.2	15.8 ± 2.6
$AUC_{\tau,ss}$ (ng h/mL)	331.6 ± 67.6	307.2 ± 52.3
$t_{1/2}$ (h)	49.2 ± 19.7	49.5 ± 9.1
$T_{max,ss}$ (h)[a]	6.0 (5.0–12.0)	6.0 (3.0–10.0)
Valsartan		
$C_{max,ss}$ (ng/mL)	5693.8 ± 2483.5	4553.8 ± 2390.0
$AUC_{\tau,ss}$ (ng h/mL)	34,079.6 ± 14,323.0	27,381.0 ± 12,360.2
$t_{1/2}$ (h)	8.8 ± 2.4	8.9 ± 1.7
$T_{max,\ ss}$ (h)[a]	3.0 (1.0–5.0)	3.0 (1.0–5.0)
	Part B (n = 25)	
	ROS	**ROS + AML/VAL**
Rosuvastatin		
$C_{max,ss}$ (ng/mL)	24.8 ± 10.5	26.3 ± 14.5
$AUC_{\tau,ss}$ (ng h/mL)	219.1 ± 78.4	218.5 ± 98.8
$t_{1/2}$ (h)	14.7 ± 5.0	14.4 ± 4.0
$T_{max,ss}$ (h)[a]	4.0 (1.5–5.0)	3.0 (0.5–5.0)

ROS administration of rosuvastatin 20 mg for 7 days, *AML/VAL* administration of FDC tablet of amlodipine/valsartan 10/160 mg for 10 days, $C_{max,ss}$ maximum plasma concentration at steady state, $AUC_{\tau,ss}$ area under the plasma concentration–time curve over the dosing interval at steady state, $t_{1/2}$ terminal elimination half-life, $T_{max,ss}$ time to reach $C_{max,ss}$

[a] Data are presented as mean ± SD except for $T_{max,ss}$ values as median (range)

after 10 days of once-daily administration of amlodipine/valsartan alone or 7 days of coadministration of rosuvastatin and amlodipine/valsartan are illustrated in Fig. 3a, b. The differences in PK parameters for amlodipine and valsartan after administration of amlodipine/valsartan and rosuvastatin and amlodipine/valsartan are summarized in Table 2.

The GMR (90% CI) values of the $C_{max,\ ss}$ and $AUC_{\tau,ss}$ for amlodipine were 0.9389 (0.9029–0.9763) and 0.9316 (0.8970–0.9675), respectively, which indicated that there was no significant change in amlodipine exposure when coadministered with valsartan and rosuvastatin. The GMR (90% CI) values of the $C_{max,\ ss}$ and $AUC_{\tau,ss}$ for valsartan were 0.7698 (0.6503–0.9114) and 0.7888 (0.6943–0.8962), respectively, which indicated that both the $C_{max,\ ss}$ and $AUC_{\tau,ss}$ of valsartan were slightly decreased when coadministered with amlodipine and rosuvastatin.

Part B: Effect of Amlodipine/Valsartan on the Pharmacokinetic Properties of Rosuvastatin

All 25 subjects who completed the study in part B were included in the pharmacokinetic

Table 3 Geometric mean ratio (90% CIs) for the log-transformed $C_{max,ss}$ and $AUC_{\tau,ss}$ following administration of amlodipine/valsartan (10 mg/160 mg) and rosuvastatin (20 mg) as concomitant administration versus individual administration in healthy male subjects

	Geometric mean ratio (90% CI)		
	Amlodipine (*n* = 30, part A)	**Valsartan (*n* = 30, part A)**	**Rosuvastatin (*n* = 25, part B)**
$C_{max,ss}$ (ng/mL)	0.9389 (0.9029–0.9763)	0.7698 (0.6503–0.9114)	0.9737 (0.8312–1.1407)
$AUC_{\tau,ss}$ (ng h/mL)	0.9316 (0.8970–0.9675)	0.7888 (0.6943–0.8962)	0.9596 (0.8826–1.0433)

$C_{max,ss}$ maximum plasma concentration at steady state, $AUC_{\tau,ss}$ area under the plasma concentration–time curve over the dosing interval at steady state

assessment. The mean plasma concentration–time profiles for rosuvastatin after 7 days of once-daily administration of rosuvastatin alone or 7 days of rosuvastatin and amlodipine/valsartan coadministration are illustrated in Fig. 3c. The differences in PK parameters of rosuvastatin between rosuvastatin and amlodipine/valsartan are summarized in Table 2.

After administration of rosuvastatin alone and rosuvastatin and amlodipine/valsartan, the GMR (90% CI) values of $C_{max,\ ss}$ and $AUC_{\tau,ss}$ for rosuvastatin were 0.9737 (0.8312–1.1407) and 0.9596 (0.8826–1.0433), respectively, which indicated that there was no significant change in rosuvastatin exposure when coadministered with amlodipine and valsartan (Table 3).

Safety

Multiple oral administrations of a 20-mg tablet of rosuvastatin and/or the FDC tablet were generally well tolerated by healthy adult subjects in this study. All 57 subjects (part A, $n = 32$; part B, $n = 25$) who received at least one dose of the study drug were included in the safety assessment. In total, 10 subjects (17.5% of 57 subjects: part A, $n = 8$; part B, $n = 2$) experienced at least one of 13 TEAEs reported (part A, $n = 11$; part B, $n = 2$) during this study. Of these 13 TEAEs, 11 events (amlodipine/valsartan: increased CPK, 3 events; headache, 2 events; rhinitis, 1 event; dyspepsia, 1 event; and gastroenteritis, 1 event; rosuvastatin + amlodipine/valsartan: upper respiratory infection, 1 event; increased blood bilirubin, 1 event; and headache, 1 event) were thought to be related to the study drugs. Most AEs were transient and mild in intensity, with one moderate event of increased CPK (5.7 × ULN) and one moderate event of increased blood bilirubin (1.9 × ULN). They all had no symptoms. All AEs spontaneously resolved with no specific treatment.

DISCUSSION

The present study evaluated the potential pharmacokinetic interaction between amlodipine, valsartan, and rosuvastatin following multiple administration of an FDC tablet formulation containing 10 mg amlodipine and 160 mg valsartan and a separate tablet formulation of 20 mg rosuvastatin. Multiple doses of amlodipine, valsartan, and rosuvastatin were well tolerated by all subjects enrolled in this study; no severe or serious AEs were recorded.

In part A, the 90% CI values for the $AUC_{\tau,ss}$ and $C_{max,\ ss}$ of amlodipine were 0.8970–0.9675 and 0.9029–0.9763, respectively, indicating that, when coadministered with valsartan and rosuvastatin, the pharmacokinetics of amlodipine were not significantly changed. The $AUC_{\tau,ss}$ and $C_{max,\ ss}$ of valsartan decreased by 0.7888- and 0.7698-fold, with 90% CIs of 0.6943–0.8962 and 0.6503–0.9114, respectively, when valsartan was coadministered with amlodipine and rosuvastatin. Paired *t* test or Wilcoxon signed rank test revealed no significant differences between the BP values of two treatment groups after the last dosing, except the systolic BP values 12 h after the last dose on day 10 in each period ($p = 0.0348$) in part A. Even though there was a statistically significant difference in systolic BP for 12 h after the last dosing between two treatment groups (slightly

higher systolic BP values in the treatment group that received amlodipine/valsartan with rosuvastatin than those in the group that received amlodipine/valsartan only), the values were within normal range, indicating no clinical significance. The repeated measures ANOVA test showed no significant change in the BP values between two treatment groups. In part B, the 90% CI values for the $AUC_{\tau,ss}$ and $C_{max,\ ss}$ of rosuvastatin were 0.8826–1.0433 and 0.8312–1.1407, respectively, indicating that, when coadministered with amlodipine and valsartan, the $AUC_{\tau,ss}$ and $C_{max,\ ss}$ values for rosuvastatin were not affected.

Although amlodipine and rosuvastatin are substrates of MDR1, and amlodipine has been reported to be a moderate inhibitor of BCRP, which is known to be involved in the transport of rosuvastatin, there was no significant change in the $AUC_{\tau,ss}$ and $C_{max,ss}$ values for amlodipine after the coadministration of amlodipine and rosuvastatin in the present study [13–15, 24]. This result was consistent with those of Son et al., who reported no significant changes in the pharmacokinetics of amlodipine after coadministration of rosuvastatin and telmisartan, which is transported by OATP1B3, BCRP, MDR1, and MRP2 [24]. No clinically significant pharmacokinetic interactions were observed when valsartan was orally coadministered with amlodipine [16, 27]. These results are supported by the pharmacokinetic characteristics of amlodipine, which show extensive metabolism by CYP3A4, with only 10% of the drug excreted renally.

The pharmacokinetic drug interaction between valsartan and rosuvastatin may occur through the competitive inhibition of OATP1B1/1B3-mediated hepatic uptake and MRP2-mediated efflux transport. However, the pharmacokinetics of rosuvastatin were not significantly changed after coadministration of the three drugs in our study. The pharmacokinetic exposure of valsartan was decreased when the three drugs were coadministered, with 90% CIs of the $AUC_{\tau,ss}$ and $C_{max,ss}$ values of 0.6943–0.8962 and 0.6503–0.9114, respectively. The intra-subject variabilities of the $AUC_{\tau,ss}$ and C_{max} values for valsartan (29.4–31.6% and 30.5–36.7%, respectively) in our study were lower than the value used for the sample size calculation for valsartan (39.0%). One plausible explanation for the factors that contributed to the observed decrease in the 90% CIs of the valsartan $AUC_{\tau,ss}$ and $C_{max,ss}$ values in this study is induction of the hepatic uptake transporters or the hepatic efflux transporters that are shared by valsartan and rosuvastatin after multiple-dose coadministration. The reason why there was no significant effect on pharmacokinetics of rosuvastatin may be the inhibition of BCRP, one of the transporters involved in the hepatic efflux for rosuvastatin [15]. In the study by Jung et al., the 90% CIs of the $C_{max,ss}$ values for valsartan and rosuvastatin were slightly decreased (90% CIs of 0.7946–1.0884 and 0.7873–0.9857, respectively), with those of the $AUC_{\tau,ss}$ values between 0.80 and 1.25, which indicated that there was no significant pharmacokinetic interaction between multiple-dose administration of valsartan and rosuvastatin [28].

The current study was conducted in Korea. According to Birmingham et al., significantly higher systemic exposure to rosuvastatin was observed in Asian subjects including those living in the USA. Korean subjects were included in the study [29]. It was reported that the geometric mean values for AUC and C_{max} of rosuvastatin in Korean subjects were compatible with those in Chinese or Japanese subjects enrolled in the study (64–84% higher AUC values and 70–98% higher C_{max} values, compared with Caucasians).

Multiple-dose oral administration of a 20-mg tablet of rosuvastatin and/or 10 mg/160 mg FDC tablet of amlodipine/valsartan was generally well tolerated by the healthy adult subjects enrolled in this study. The observed AEs that occurred following the multiple administrations of amlodipine, valsartan, and rosuvastatin were not severe or serious.

However, the present study has several limitations. First, the data were obtained from only healthy young male subjects, who are not representative of the target patients. Second, the sample size of this study was relatively small, which is not sufficient to detect all possible AEs. The pharmacokinetic interactions and safety profiles of the study drugs may differ from those in the target population or in older individuals.

Decreased clearance, with a resulting increase in the systemic exposure of amlodipine and valsartan, has been reported in elderly subjects [9, 16]. Accordingly, further studies conducted in a relevant population of patients with hypertension and hyperlipidemia, including older individuals, are needed.

CONCLUSIONS

When amlodipine, valsartan, and rosuvastatin are coadministered to healthy volunteers, pharmacokinetic exposure of valsartan was slightly decreased, but no changes in the pharmacokinetics of amlodipine and rosuvastatin were found. All treatments were well tolerated, with no serious AEs reported.

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Compliance with Ethics Guidelines. The study protocol was approved by the Institutional Review Board at Kyungpook National University Hospital (Daegu, Republic of Korea), and the study was performed in accordance with the ethical standards for studies in humans set out in the Declaration of Helsinki and its amendments, and the applicable guidelines for International Conference on Harmonization Good Clinical Practice, and local laws and regulations. Written informed consent was obtained from all individual subjects included in the study before their enrollment.

Data Availability. The data sets generated and/or analyzed during this study are not publicly available due to confidentiality of KyungDong Pharmaceutical Corp. Ltd., but are available from the corresponding author upon reasonable request.

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