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Pharmacokinetic Drug Interaction Between Raloxifene and Cholecalciferol in Healthy Volunteers

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Abstract

Osteoporosis is a common skeletal disorder, often leading to fragility fracture. Combination therapy with raloxifene, a selective estrogen receptor modulator, and cholecalciferol (vitamin D_3) has been proposed to improve the overall efficacy and increase compliance of raloxifene therapy for postmenopausal osteoporosis. To our knowledge, there has been no report of any study on the pharmacokinetic interaction between raloxifene and cholecalciferol. This study aimed to evaluate the possible pharmacokinetic interactions between raloxifene and cholecalciferol in healthy adult male Korean volunteers. Twenty subjects completed this open-label, randomized, single-dose, 3-period, 6-sequence, crossover phase 1 study with a 14-day washout period. Serial blood samples were collected from 20 hours before dosing to 96 hours after dosing. The plasma concentrations of raloxifene and cholecalciferol were determined using a validated method for high-performance liquid chromatography with tandem mass spectrometry. The geometric mean ratios (90%CIs) for area under the plasma concentration–time curve from time 0 to the last quantifiable time point and maximum plasma concentration of raloxifene with or without cholecalciferol were 1.02 (0.87-1.20) and 0.87 (0.70-1.08), respectively. For baseline-corrected cholecalciferol, geometric mean ratios (90%CIs) of area under the plasma concentration–time curve from time 0 to the last quantifiable time point and maximum plasma concentration with or without raloxifene were 1.01 (0.93-1.09) and 0.99 (0.92-1.06), respectively. Concurrent treatment with raloxifene and cholecalciferol was generally well tolerated. These results suggest that raloxifene and cholecalciferol have no clinically relevant pharmacokinetic drug-drug interactions when administered concurrently. All treatments were well tolerated, with no serious adverse events.

Keywords

cholecalciferol, drug-drug interaction, osteoporosis, pharmacokinetics, raloxifene

Osteoporosis is a common skeletal disease characterized by low bone mass and bone structure deterioration, often leading to bone fragility and an increased risk of fracture.[1] It is a public health concern because osteoporosis-related fractures are associated with pain, diminished quality of life, disability, and even death, imposing an enormous burden on individuals and society.[2] Nonpharmacologic interventions in osteoporosis management include calcium and vitamin D supplementation, weight-bearing exercise, smoking

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cessation, and fall-prevention strategies.[3] Current FDA-approved pharmacologic treatment options include antiresorptive agents (bisphosphonates, receptor activator of nuclear factor kappa-B ligand inhibitor denosumab, estrogens and selective estrogen receptor modulators [SERMs]), or anabolic agents (parathyroid hormone 1-34, teriparatide).[3,4]

Raloxifene hydrochloride, a SERM, exerts agonistic effects on bone and lipid metabolism and antagonistic actions on the endometrium and breast in estrogenic pathways.[5,6] It increases bone mineral density by decreasing bone resorption and bone turnover. Raloxifene has been used to prevent and treat postmenopausal osteoporosis, especially as first-line therapy for patients in whom a reduction of spine fracture is the primary aim.[3,7] In several placebo-controlled trials involving postmenopausal patients with osteoporosis, raloxifene 60 mg decreased the risk of vertebral fracture or increased bone mineral density over 36 months.[8,9] The recommended dose of raloxifene is 60 mg once daily.[10] Raloxifene undergoes rapid absorption after oral administration, with 60% absorbed. However, its bioavailability is only 2% in humans because of extensive first-pass metabolism by glucuronidation and sulfonation.[11,12] Raloxifene is not metabolized by the cytochrome P450 (CYP) system.[13] The elimination half-life of raloxifene is approximately 27 hours after oral dosing.[6] When orally administered, the majority of raloxifene is excreted in feces and to a lesser extent in the urine (6%).[11] The results of several in vitro and in vivo studies indicate that organic anion-transporting polypeptide (OATP) 1B1, OATP1B3, multidrug resistance–associated protein (MRP), P-glycoprotein (P-gp), and breast cancer resistance protein (BCRP) were important contributors to the intestinal and hepatic uptake or excretion of raloxifene, and glucuronidated and sulfated raloxifene conjugates.[11,12,14,15]

Vitamin D_3 (cholecalciferol) plays an important role in calcium absorption and bone health, and inadequate vitamin D levels have been linked to the development of osteoporosis, muscle weakness, falls, and fracture.[4,16] The recommended daily dose of vitamin D for elderly individuals varies from 400 IU to 800 IU.[17] However, dietary sources of vitamin D and adequate exposure to sunlight with efficient synthesis of vitamin D are limited, especially in older adults.[18] Accordingly, vitamin D supplementation, especially in postmenopausal women who appear to have vitamin D inadequacy, might help maintain optimal vitamin D levels and prevent fragility fractures. Vitamin D_3 from intestinal absorption or endogenous synthesis in the skin under exposure to sunlight is metabolized in the liver by 25-hydroxylases (CYP2R1, CYP27A1, and CYP3A4) to produce 25-hydroxyvitamin D_3 [$25(OH)D_3$].[19] $25(OH)D_3$ is hydroxylated in the kidney by 1α-hydroxylase (CYP27B1) into 1α, 25-dihydroxyvitamin D_3, the active form of vitamin D_3.[19] According to several in vitro studies, P-gp was involved in vitamin D efflux by enterocytes, and the expression of P-gp, MRP2, and MRP4 was activated by treatment with vitamin D_3.[20,21] In another in vitro study, vitamin D_3 showed an inhibitory effect on the transport function of P-gp, MRP1, and BCRP.[22]

In osteoporotic patients, pharmacologic treatment combined with vitamin D supplementation would be more beneficial. For either osteoporosis treatment or prevention, vitamin deficiency or hypocalcemia should be assessed and corrected, if possible, before initiation of pharmacologic therapy, with subsequent calcium and/or vitamin D supplementation.[23] As a combination tablet of a bisphosphonate and vitamin D, Fosamax Plus D (alendronate sodium/cholecalciferol) was approved by the Food and Drug Administration in 2005 to treat osteoporosis in postmenopausal women.[24] Recently, the fixed-dose combination formulation of bazedoxifene, another SERM, and cholecalciferol was manufactured, and the bioequivalence of the pharmacokinetic properties for the fixed-dose combination formulation of bazedoxifene/cholecalciferol and the coadministered individual formulations was demonstrated.[25] Combination products containing both raloxifene and vitamin D might improve the overall efficacy of raloxifene therapy for the treatment of postmenopausal osteoporosis, with increased compliance and convenience. Based on the results from several in vitro and in vivo studies, raloxifene and vitamin D_3 share the same transporter proteins like P-gp, MRP, and BCRP.[11,12,20–22] Accordingly, potential drug-drug interactions when administered concurrently might be plausible through competition for drug transporters. To our knowledge, there has been no report on the potential pharmacokinetic interaction between raloxifene and cholecalciferol. Therefore, this study sought to evaluate the potential pharmacokinetic interaction between raloxifene and cholecalciferol following coadministration as a preliminary effort in the future development of a combination tablet of raloxifene and vitamin D.

Subjects and Methods

Subjects and Design of the Study

The Ministry of Food and Drug Safety in Korea and the Institutional Review Board of Kyungpook National University Hospital (KNUH, Daegu, Republic of Korea) authorized this research (No. KNUH 2015-11-007). The research was conducted at the KNUH Clinical Trial Center in accordance with the Declaration of Helsinki and its revisions, as well as the International Conference on Harmonization's Good

Clinical Practice. Before recruitment in the trial, all individuals provided written informed consent for participation.

At screening, healthy Korean male volunteers aged >19 years with a body weight ≥50 kg and within ±20% of their ideal body mass were included if they did not have clinically significant abnormalities as determined by a detailed medical history, physical examination, routine clinical laboratory tests, and 12-lead electrocardiography. They were instructed to avoid sun exposure for 10 days before the first dose and for the duration of the trial by wearing clothes, a helmet, and sunscreen (SPF 30). The individuals were instructed to abstain from vitamin D–rich meals, vitamin D–fortified foods, and vitamin D pills for 10 days before receiving the first dose. Exclusion criteria included, but were not limited to, the following: a history of or current clinically significant medical illness; a history of hypersensitivity to raloxifene or cholecalciferol; a history of taking a high dose of vitamin D_3 (>50 000 IU) within 1 month before the first dose; and in the presence of serum $25(OH)D_3$ levels >9 ng/mL, serum phosphorus levels greater than the reference value, or serum alkaline phosphatase levels ≥2× the upper limit of normal. The ineligibility was judged at the discretion of the study investigator, based on other clinical laboratory tests.

To determine the pharmacokinetic interaction of raloxifene and cholecalciferol in healthy male volunteers, an open-label, randomized, single-dose, 3-period, tri-treatment, 6-sequence, crossover phase I research was undertaken. Each participant received 1 of the following 3 single-dose therapies during each period: 2000 IU of cholecalciferol (1000 IU/10 mg, Admin Forte®, PMG Korea, Seoul, Republic of Korea) alone (treatment A), 60 mg of raloxifene (Evista®, Eli Lilly and Company, Indianapolis, Indiana) alone (treatment B), or 2000 IU of cholecalciferol and 60 mg of raloxifene combined (treatment C). Twenty-four individuals were randomly assigned to 1 of 6 therapy sequences (ABC, ACB, BAC, BCA, CAB, and CBA), each of which included all 3 therapies, as detailed in Figure S1. At least 14 days of washout occurred between dosing intervals.

Each patient was admitted to the KNUH Clinical Trial Center 22 hours before dosing and remained restricted until 24 hours after dosing. All individuals were given the research medications with 150 mL of water in a fasting state for at least 10 hours. Food consumption was prohibited for 4 hours after dosing, with the exception of water drinking 2 hours after dosing. Subjects recruited after screening were requested to abstain from vitamin D–rich meals, vitamin D–fortified foods, and vitamin D and calcium supplementation for 10 days before the first dosage and for the duration of the research.

Raloxifene and Cholecalciferol Quantification

To determine the pharmacokinetic profiles of raloxifene and cholecalciferol, serial blood samples (8 mL each) were drawn from a catheter inserted into a forearm vein before dosing (0 hour) and at 2, 4, 5, 6, 7, 8, 10, 12, 24, 48, 72, and 96 hours after each raloxifene dose, and before dosing (–20, –16, –12, and 0 hours) and at 2, 4, 6, 8, 10, 12, 14, 16, 24, 36, 48, 72, and 84 hours after each cholecalciferol dose. Blood samples were collected in dipotassium ethylenediaminetetraacetic acid tubes and instantaneously placed on ice before centrifugation (1,600 *g* for 10 minutes at 4°C) to separate plasma. Plasma samples were dispensed into 4 Amber polypropylene tubes (0.9 mL each) and kept at 70°C until evaluated by the Biocore analytical facility in Seoul, Republic of Korea.

The plasma concentrations of raloxifene and cholecalciferol were quantified using a validated method with some modifications described in the literature. This method involved the use of ultra-fast liquid chromatography (LC; Shimadzu UFLC system, Shimadzu Corp., Kyoto, Japan) in conjunction with tandem mass spectrometry (MS/MS).[26,27]

Raloxifene chromatography was performed on a C18 column (3.0-μm particle size, 2.1-mm internal diameter × 50 mm). The mobile phase constituted a 55:45 (v/v) mixture of 10 mM of ammonium formate (0.1% formic acid) and acetonitrile (ACN), with a flow rate of 0.2 mL/min. Raloxifene detection was conducted by a TQ 5500 mass spectrometer (SCIEX, Foster City, California) with multiple reaction monitoring in positive-ion mode at mass-to-charge ratios (m/z) of 474.1 → 112.3 and 478.0 → 116.5 for raloxifene and raloxifene-d_4, the internal standard (IS), respectively. Frozen plasma was thawed and vortexed for 10 seconds at ambient temperature. After spiking 10 μL of IS (15 000 pg/mL) to 200 μL of plasma within a polypropylene tube, 1.2 mL of methyl tert–butyl ether was added and mixed for 20 minutes, followed by a 5-minute centrifugation step at 11,100 *g*. The solvent in the organic layer was evaporated to dryness using an inert stream of nitrogen gas. The residue was topped up with 150 μL of a 45:55 (v/v) blend of ACN and 10 mM of ammonium formate (0.1% formic acid), followed by a 5-minute centrifugation at 11,100 *g*. A 5-μL aliquot of this solution was then injected into the LC-MS/MS coupled system for examination. The bottom limit of quantitation was 10 pg/mL for raloxifene, while the linear calibration curves spanned from 10 to 1500 pg/mL ($r \geq 0.9950$). The overall intra- and interday accuracy at concentrations of 10, 30, 120, and 1200 pg/mL ranged from 89.6% to 111.0%, and from 99.0% to 104.3%, respectively. The intra- and interday precision (percent coefficient of variation) varied from 1.4% to 10.4% and 1.8% to 12.3%.

Cholecalciferol chromatography was performed on a C18 column (3.0-μm particle size, 2.0-mm internal diameter × 50 mm). The mobile phase consisted of a 90:10 (v/v) blend of methanol and deionized H_2O, with a flow rate of 0.4 mL/min (pump A) and 0.3 mL/min (pump B), respectively. Detection of cholecalciferol was conducted by an API 5000 mass spectrometer (SCIEX) with multiple reaction monitoring in positive-ion mode at m/z of 385.4 → 259.4 and 391.3 → 265.3 for cholecalciferol and cholecalciferol-d_6, the IS, respectively. Frozen plasma was thawed and vortexed for 10 seconds at ambient temperature. After spiking 10 μL of IS (50 ng/mL) to 300 μL of plasma within a polypropylene tube, 600 μL of ACN and 150 μL of ethyl acetate were added and mixed for 1 minute, then centrifuged at 11,100 *g* for 5 minutes. The supernatant (700 μL) was loaded on the hydrophilic lipophilic balanced solid phase extraction cartridge (30 mg), preconditioned with methanol and deionized water (1 mL each). The cartridge was rinsed with 300 μL 70% ACN and eluted with 4× 300 μL of ethyl acetate. The eluate solvent was dry evaporated under an inert stream of nitrogen. The residue was reconstituted with 100 μL of 90% methanol (0.1% formic acid) followed by a 5-minute centrifugation at 11,100 *g*. A 10-μL aliquot of the resultant solution was fed into the LC-MS/MS system. The bottom limit of quantitation was 0.1 ng/mL for cholecalciferol, while the linear calibration curves spanned from 0.1 to 10 ng/mL ($r \geq 0.9950$). The general intra- and interday accuracy at concentrations of 0.1, 0.3, 1.2, and 8 ng/mL ranged from 89.8% to 111.4%, and from 95.3% to 109.4%, respectively. The intra- and interday precision percent coefficient of variation ranged from 0.7% to 9.9%, and 2.6% to 8.3%.

Pharmacokinetic Assessments

The Phoenix WinNonlin version 6.4 (Pharsight Corporation, Certara, Princeton, New Jersey) was used to generate the pharmacokinetic parameters for raloxifene and cholecalciferol using individual subject plasma concentration-time data: the maximum plasma concentration (C_{max}); the time required to reach C_{max}; the area under the plasma concentration–time curve (AUC) from time 0 to the last quantifiable time point ($AUC_{0\text{-}t}$); the AUC from time 0 to infinity; and the elimination half-life, including total body clearance.

Due to the endogenous nature of cholecalciferol, its pharmacokinetic parameters were calculated using baseline correction.[28] Blood samples were obtained at 20, 16, 12, and 0 hours before dosing to measure the endogenous plasma concentrations of cholecalciferol. The mean of predose endogenous cholecalciferol concentrations in each sample was subtracted from the postdose plasma cholecalciferol concentrations.

Subject Safety

The safety of each individual who received at least 1 dose of raloxifene or cholecalciferol was determined by examining treatment-emergent adverse events (AEs). Throughout the trial, subjects willingly reported any subjective symptoms. Additionally, throughout the trial, vital signs, physical examinations, clinical laboratory assessments, and 12-lead electrocardiograms were performed to determine safety. All laboratory analyses were performed at a facility that was accredited (Department of Laboratory Medicine, KNUH, Daegu, Republic of Korea).

Statistical Analysis

The pharmacokinetic features of raloxifene and cholecalciferol when coadministered were compared to those when the medications were mono-administered using paired *t*-tests or the Wilcoxon signed-rank test (SPSS for Windows software version 18.0 [SPSS Korea, Seoul, Republic of Korea]). A P value $<.05$ was defined as statistically significant. To identify the possible effect of coadministration of raloxifene and cholecalciferol on the pharmacokinetic profile of each drug alone, the geometric mean ratios (GMRs) and 90%CIs of log-transformed AUC and C_{max} of raloxifene and cholecalciferol were estimated for both treatment groups (coadministration/individual administration) from the mixed-effects model, using SAS software (version 9.2; SAS Institute, Cary, North Carolina). Lack of clinically significant drug-drug interaction effect on the pharmacokinetics would be concluded if the 90%CIs of the GMRs were contained within the range of 0.80 to 1.25 for both C_{max} and AUC.

With the highest intrasubject coefficient of variation of AUC and C_{max} values (21.7%, as reported previously) of raloxifene and cholecalciferol, 18 subjects were considered to be sufficient to demonstrate a 20% difference in the log-transformed pharmacokinetic parameters of raloxifene and cholecalciferol within 80% power at a significance level of .05. With an assumed 25% dropout rate, the total number of participants was 24.

Because this trial was not meant to demonstrate bioequivalence but rather to investigate the possibility for pharmacokinetic interaction, only people who withdrew before the start of period 1 were replaced by additional waiting list participants.

Results

Subjects

Forty-five volunteers were screened, and 24 healthy Korean men participated in the study. Two subjects who withdrew consent before initiation of period 1 were replaced by other subjects from the waiting list. During

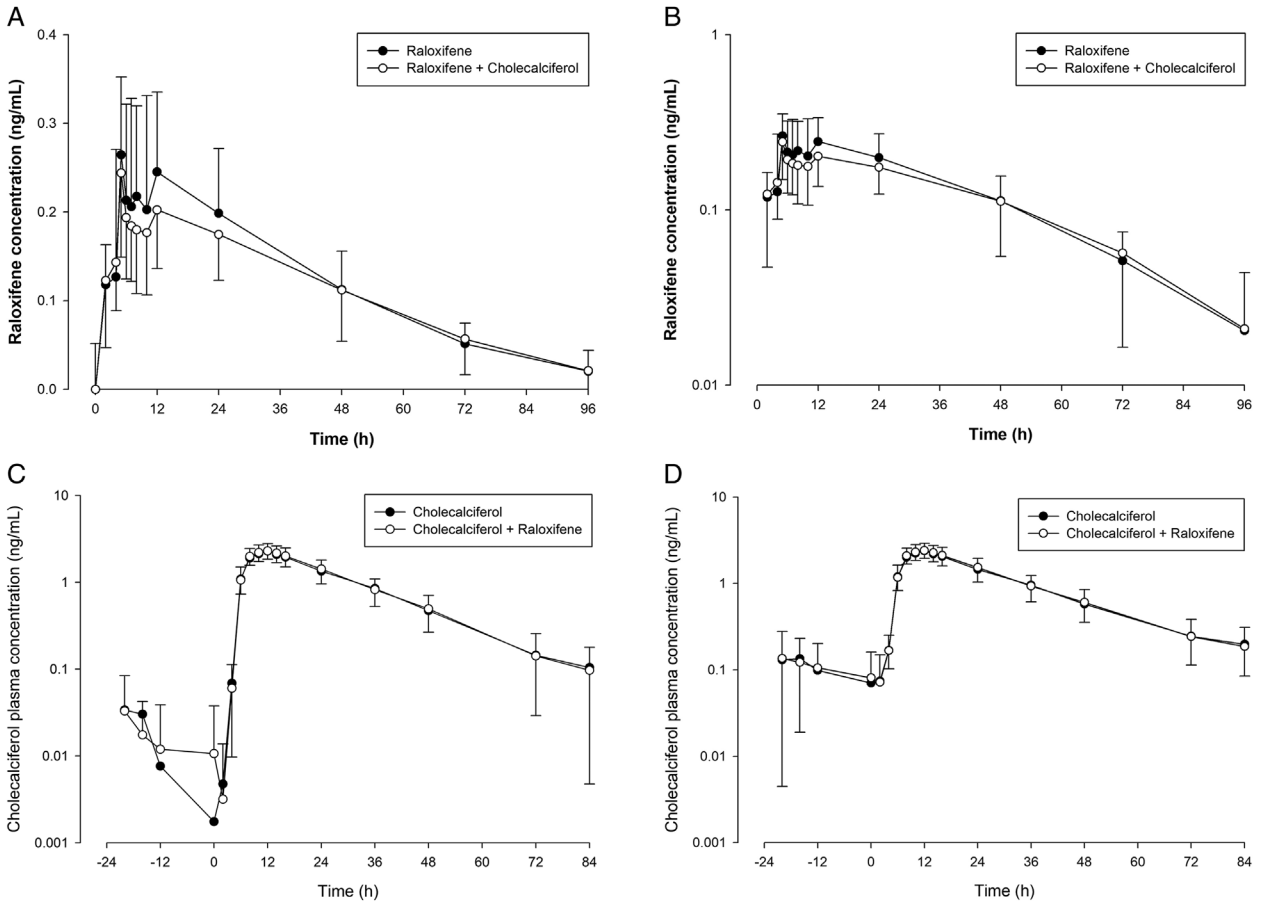

Figure 1. Mean plasma concentration-time profiles of raloxifene and cholecalciferol after administration of a single oral dose of raloxifene (60 mg) or cholecalciferol (2000 IU) alone and coadministration of raloxifene and cholecalciferol. Note: Raloxifene (A) linear scale and (B) semilog scale, and cholecalciferol; (C) baseline-corrected (semilog scale) and (D) baseline-uncorrected (semilog scale). Error bars denote the standard deviations.

the study, 4 subjects withdrew consent. Accordingly, 20 subjects aged 19 to 36 years (mean [SD], 24.0 [3.6] years), weighing 56.9 to 88.7 kg (mean [SD], 68.2 [9.1]), with body mass index of 18.4 to 26.3 kg/m^2 (mean [SD], 22.1 [2.4] kg/m^2) completed the study.

Pharmacokinetic Properties

Pharmacokinetic analysis was performed with the 20 subjects who completed the study. The mean (SD) plasma concentration–time profiles of raloxifene, baseline-corrected cholecalciferol, and baseline-uncorrected cholecalciferol after coadministration of raloxifene (60 mg) and cholecalciferol (2000 IU) and administration of each drug alone are presented in Figure 1 (A-D). A summary of the pharmacokinetic parameters for raloxifene, baseline-corrected cholecalciferol, and baseline-uncorrected cholecalciferol are shown in Table 1.

As presented in Table 1, the 90%CI for the GMR of raloxifene was 0.70 to 1.08 for C_{max} and 0.87 to 1.20 for $AUC_{0\text{-}t}$. For baseline-corrected cholecalciferol, the 90%CI for the GMR was 0.92 to 1.06 for C_{max} and 0.93 to 1.09 for $AUC_{0\text{-}t}$. For baseline-uncorrected cholecalciferol, the 90%CI for the GMR was 0.92 to 1.06 for C_{max} and 0.91 to 1.07 for $AUC_{0\text{-}t}$.

Safety

Safety was evaluated in 24 subjects who received the study medication at least once. During the study, a total of 6 treatment-emergent AEs were experienced by 4 (16.7%) subjects. No serious or severe AEs were reported in this study, and none of the subjects discontinued the study due to AEs. Of the 6 AEs, 4 were determined to be possibly related to the study medication: 1 case each of increased alanine aminotransferase and headache following administration of raloxifene alone; 1 case of hypercalciuria following administration of cholecalciferol alone; and 1 case of headache after coadministration of raloxifene and cholecalciferol. All AEs were transient, and all of the subjects with AEs recovered without any medication.

Table 1. Pharmacokinetic Properties of Raloxifene and Cholecalciferol and GMR (90%CI) for the Log-Transformed Parameters Following Single-Dose Oral Administration of Raloxifene (60 mg) and Cholecalciferol (2000 IU) as Concomitant Administration vs Individual Administration Under Fasted Conditions in 20 Healthy Male Subjects

	Variables	Arithmetic Mean ± SD		Geometric Mean		
		Raloxifene	Raloxifene + Cholecalciferol	Raloxifene	Raloxifene + Cholecalciferol	GMR (90%CI)
Raloxifene	t_{max}, h[a]	6.5 (2.0-24.0)	5.0 (2.0-48.0)	6.5 (2.0-24.0)	5.0 (2.0-48.0)	
	C_{max}, ng/mL	0.33 ± 0.16	0.27 ± 0.09	0.29	0.25	0.87 (0.70-1.08)
	AUC_{0-t}, ng · h/mL	11.2 ± 5.7	10.5 ± 3.6	9.8	9.9	1.02 (0.87-1.20)
	$AUC_{0-\infty}$, ng · h/mL	12.6 ± 6.2	11.9 ± 4.6	11.2	11.0	0.98 (0.85-1.14)
	CL/F, L/h	6052.7 ± 2932.3	5853.9 ± 2383.2	5370.3	5435.7	1.02 (0.88-1.18)
	$t_{1/2}$, h	26.5 ± 9.9	28.4 ± 13.0	24.8	25.9	1.01 (0.84-1.21)
		Cholecalciferol	Cholecalciferol + Raloxifene	Cholecalciferol	Cholecalciferol + Raloxifene	
Baseline-corrected cholecalciferol	t_{max}, h[a]	12.0 (10.0-14.0)	12.0 (8.0-14.0)	12.0 (10.0-14.0)	12.0 (8.0-14.0)	
	C_{max}, ng/mL	2.32 ± 0.43	2.35 ± 0.49	2.28	2.29	0.99 (0.92-1.06)
	AUC, ng · h/mL	63.6 ± 18.4	65.3 ± 17.7	61.0	62.7	1.01 (0.93-1.09)
	$AUC_{0-\infty}$, ng · h/mL	67.3 ± 19.5	68.4 ± 18.6	64.5	65.7	1.00 (0.93-1.08)
	CL/F, IU/(ng · h/mL)	32.4 ± 10.9	32.1 ± 12.1	31.0	30.5	1.00 (0.93-1.08)
	$t_{1/2}$, h	14.9 ± 5.4	15.0 ± 4.4	13.9	14.2	1.01 (0.84-1.22)
		Cholecalciferol	Cholecalciferol + Raloxifene	Cholecalciferol	Cholecalciferol + Raloxifene	
Baseline-uncorrected cholecalciferol	t_{max}, h[a]	12.0 (10.0-14.0)	12.0 (8.0-14.0)	12.0 (10.0–14.0)	12.0 (8.0-14.0)	
	C_{max}, ng/mL	2.42 ± 0.44	2.46 ± 0.50	2.38	2.40	0.99 (0.92-1.06)
	AUC_{0-t}, ng · h/mL	72.7 ± 20.2	74.3 ± 20.5	69.7	71.1	0.99 (0.91-1.07)
	$AUC_{0-\infty}$, ng · h/mL	79.1 ± 22.4	80.7 ± 23.1	75.8	76.9	1.00 (0.91-1.09)
	CL/F, IU/(ng · h/mL)	27.7 ± 9.8	27.7 ± 12.4	26.4	26.0	1.00 (0.92-1.09)
	$t_{1/2}$, h	19.3 ± 4.3	19.3 ± 4.2	18.8	18.8	1.00 (0.92-1.08)

AUC_{0-t}, area under the plasma concentration–time curve from time 0 to the last quantifiable time point; $AUC_{0-\infty}$, area under the plasma concentration–time curve from time 0 to infinity; C_{max}, maximum plasma concentration; CL/F, apparent clearance; GMR, geometric mean ratio; $t_{1/2}$, terminal elimination half-life; t_{max}, time to reach maximum plasma concentration.

Data are presented as arithmetic mean ± standard deviation or geometric mean, except for t_{max} values as median (range)[a], and GMR (90%CI).

Discussion

We investigated the pharmacokinetic interaction between raloxifene and cholecalciferol in healthy male volunteers. Peak and systemic exposure of raloxifene and cholecalciferol were compared when raloxifene and cholecalciferol are administered together relative to when each drug is administered alone.

The raloxifene dose in this study was the daily recommended dose (60 mg). The daily dose of vitamin D for adults aged 50 and older recommended by the National Osteoporosis Foundation is 800 to 1000 IU.[4] In a study to evaluate bioavailability and bioequivalence between a combination tablet of alendronate and vitamin D and when administered alone, the doses of vitamin D selected were 2800 IU and 5600 IU in a once-weekly dose.[18] The dose of vitamin D selected for this study was 2000 IU.

A multinational study involving 18 countries has found the high prevalence of vitamin D insufficiency (92.1%) and the lowest mean serum 25(OH)D level (17.6 ng/mL) in Korea.[29] From another study conducted in Korea, vitamin D insufficiency or deficiency was found in >98% of healthy young adolescents.[30] The predose mean $25(OH)D_3$ concentrations of the subjects in this study was 9.6 ng/mL, and the maximum value was 17.0 ng/mL. Although the vitamin D deficiency may affect the pharmacokinetic profiles of cholecalciferol, the volunteers who had vitamin D deficiency could not be excluded in our study because of the limited pool of the volunteers with a serum 25(OH)D level of >30 ng/mL. To reduce the potential bias of vitamin D activation from sun exposure or diet supplementation,

sun exposure was limited with clothing, hat, and sunblock, and foods rich in vitamin D, vitamin D–fortified foods, and vitamin D and calcium supplements were prohibited from 10 days before the first dose until study completion. Furthermore, baseline correction was performed before calculating pharmacokinetic parameters as described in the study protocol.[28]

The 90%CI values for the $AUC_{0\text{-}t}$ and C_{max} of baseline-corrected cholecalciferol were 0.9330 to 1.0888 and 0.9182 to 1.0622, respectively, indicating that the pharmacokinetics of cholecalciferol were not significantly affected when coadministered with raloxifene. For raloxifene, the 90%CIs of the GMRs for $AUC_{0\text{-}t}$ were between 0.8 and 1.25, indicating that raloxifene exposure during concomitant treatment was not significantly affected by cholecalciferol. However, the 90%CI in the C_{max} of raloxifene (0.7006-1.0795) fell slightly outside of the range of 0.80 to 1.25, and the GMRs for C_{max} of raloxifene with or without cholecalciferol in this study indicated that C_{max} of raloxifene decreased by 13% in the presence of cholecalciferol. However, considering the high intrasubject variability of raloxifene C_{max}, the 13% decrease of C_{max} was not clinically relevant.

The large intersubject variability in raloxifene C_{max} and $AUC_{0\text{-}t}$ could be explained by genetic polymorphisms in uridine 5-diphosphate–glucuronosyltransferase or the transporters required for intestinal and hepatic uptake or excretion of raloxifene, and glucuronidated and sulfated raloxifene conjugates including OATP1B1, OATP1B3, P-gp, or BCRP.[11,12,28,31]

Raloxifene has been approved for use in the treatment and prevention of osteoporosis in postmenopausal women. As raloxifene, as an estrogen agonist/antagonist, increases estrogen levels in women, premenopausal use of raloxifene should be avoided.[3] Accordingly, female volunteers were excluded from this study. When orally administered to male rats for at least 2 weeks, impairment of fertility including sperm production or reproductive performance was not affected by raloxifene (unpublished data, Alvogen Korea Co. Ltd.). However, significantly increased testosterone levels were observed with raloxifene administration in several studies conducted in healthy men or in male patients with schizophrenia.[32,33] Accordingly, instead of a multiple-dose study, a single-dose study with a special and useful type of crossover design such as Williams design in healthy male volunteers was recommended by Korea Ministry of Food and Drug Safety. Even though long-term osteoporosis treatment is required in clinical settings, only a single dose was administered in this study. According to the results reported by Fan et al, the expression of P-gp, MRP2, and MRP4 was upregulated by vitamin D_3 treatment through the nuclear vitamin D receptor.[21] Durk et al[34] reported the increased expression levels of P-gp after 2- or 3-day vitamin D_3 treatment in rats, compared to no immediate effect. According to Tan et al,[22] MRP1 mRNA and protein expression was not significantly altered with 2-day vitamin D_3 treatment at 1 μM concentration. Therefore, the inhibitory effects of vitamin D_3 on drug efflux transporters may differ when multiple doses are administered, leading to the different pharmacokinetic interactions between raloxifene and vitamin D_3 from those in a single-dose study.

Conclusions

In conclusion, the individual pharmacokinetics of raloxifene and cholecalciferol were not affected by their coadministration, and there were no serious or unexpected AEs.

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Conflicts of Interest

B.L. is an employee of Alvogen Korea Co. Ltd. The authors declare no conflict of interest regarding the content of this article. The sponsor did not participate in the execution of the study or analysis of the data.

References

1. Vandenbroucke A, Luyten FP, Flamaing J, Gielen E. Pharmacological treatment of osteoporosis in the oldest old. *Clin Interv Aging*. 2017;12:1065-1077.
2. Dempster DW. Osteoporosis and the burden of osteoporosis-related fractures. *Am J Manag Care*. 2011;17(suppl 6): S164-S169
3. Tu KN, Lie JD, Wan CKV, et al. Osteoporosis: a review of treatment options. *P.T.* 2018;43:92-104.
4. Cosman F, de Beur SJ, LeBoff MS, et al. Clinician's guide to prevention and treatment of osteoporosis. *Osteoporos Int*. 2014;25:2359-2381.
5. Avioli LV. SERM drugs for the prevention of osteoporosis. *Trends Endocrino Metab*. 1999;10(8):317-319.
6. Scott JA, Da Camara CC, Early JE. Raloxifene: a selective estrogen receptor modulator. *Am Fam Physician*. 1999;60(4):1131-1138.
7. Murthy A, Ravi PR, Kathuria H, Malekar S. Oral bioavailability enhancement of raloxifene with

nanostructured lipid carriers. *Nanomaterials (Basel)*. 2020;10(6):1085.
8. Delmas PD, Ensrud KE, Adachi JD, et al. Efficacy of raloxifene on vertebral fracture risk reduction in postmenopausal women with osteoporosis: four-year results from a randomized clinical trial. *J Clin Endocrinol Metab*. 2002;87(8):3609-3617.
9. Clemett D, Spencer CM. Raloxifene: a review of its use in postmenopausal osteoporosis. *Drugs*. 2000;60(2):379-411.
10. Snyder KR, Sparano N, Malinowski JM. Raloxifene hydrochloride. *Am J Health Syst Pharm*. 2000;57(18):1669-1675.
11. Lušin TT, Mrhar A, Stieger B, et al. Influence of hepatic and intestinal efflux transporters and their genetic variants on the pharmacokinetics and pharmacodynamics of raloxifene in osteoporosis treatment. *Transl Res*. 2012;160(4):298-308.
12. Zhou X, Wang S, Sun H, Wu B. Sulfonation of raloxifene in HEK293 cells overexpressing SULT1A3: involvement of breast cancer resistance protein (BCRP/ABCG2) and multidrug resistance-associated protein 4 (MRP4/ABCC4) in excretion of sulfate metabolites. *Drug Metab Pharmacokinet*. 2015;30(6):425-433.
13. Hochner-Celnikier D. Pharmacokinetics of raloxifene and its clinical application. *Eur J Obstet Gynecol Reprod Biol*. 1999;85(1):23-29.
14. Trontelj J, Marc J, Zavratnik A, Bogataj M, Mrhar A. Effects of UGT1A1*28 polymorphism on raloxifene pharmacokinetics and pharmacodynamics. *Br J Clin Pharmacol*. 2009;67(4):437-444.
15. Lušin TT, Stieger B, Marc J, et al. Organic anion transporting polypeptides OATP1B1 and OATP1B3 and their genetic variants influence the pharmacokinetics and pharmacodynamics of raloxifene. *J Transl Med*. 2012;10:76.
16. Gaugris S, Heaney RP, Boonen S, Kurth H, Bentkover JD, Sen SS. Vitamin D inadequacy among post-menopausal women: a systematic review. *QJM*. 2005;98(9):667-676.
17. Bouillon R. Comparative analysis of nutritional guidelines for vitamin D. *Nat Rev Endocrinol*. 2017;13(8):466-479.
18. Denker AE, Lazarus N, Porras A et al. Bioavailability of alendronate and vitamin D3 in an alendronatae/vitamin D3 combination tablet. *J Clin Pharmacol*. 2011;51(10):1439-1448.
19. Robien K, Butler LM, Wang R, et al. Genetic and environmental predictors of serum 25(OH)D concentrations among middle-aged and elderly Chinese in Singapore. *Br J Nutr*. 2013;109(3):493-502.
20. Margier M, Collet X, le May C, et al. ABCB1 (P-glycoprotein) regulates vitamin D absorption and contributes to its transintestinal efflux. *FASEB J*. 2019;33(2):2084-2094.
21. Fan J, Liu S, Du Y, Morrison J, Shipman R, Pang KS. Up-regulation of transporters and enzymes by the vitamin D receptor ligands, 1 alpha, 25-dihydroxyvitamin D3 and vitamin D analogs, in the Caco-2 cell monolayer. *J Pharmacol Exp Ther*. 2009;330(2):389-401.
22. Tan KW, Sampson A, Osa-Andrews B, Iram SH. Calcitriol and calcipotriol modulate transport activity of ABC transporters and exhibit selective cytotoxicity in MRP1-overexpressing cells. *Drug Metab Dispos*. 2018;46(12):1856-1866.
23. Lewiecki EM, Feingold KR, Anawalt B, et al. Osteoporosis: clinical evaluation. In: *Endotext [internet]*. South Dartmouth, MA: MDText.com, Inc.; 2000-2018. Accessed April 19, 2021.
24. Reynolds NA, Curran MP. Alendronate/colecalciferol. *Treat Endocrinol*. 2005;4(6):371-377.
25. Yun JN, Kan H-S, Yeun J-S, et al. Bioequivalence for a fixed-dose combination formulation of bazedoxifene and cholecalciferol compared with the corresponding single entities given together. *Clin Pharmacol Drug Dev*. 2021;10(8):850-858.
26. Zhang SW, Jian W, Sullivan S, et al. Development and validation of an LC-MS/MS based method for quantification of 25 hydroxyvitamin D2 and 25 hydroxyvitamin D3 in human serum and plasma. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2014;96:62-70.
27. Jadhav DH, Ramaa CS. Development and validation of a UPLC-MS/MS assay for simultaneous estimation of raloxifene and its metabolites in human plasma. *J Bioanal Biomed*. 2012; 4: 61-67.
28. Jeon J, Lee SY, Im Y, et al. Comparison of the pharmacokinetics, safety, and tolerability of vitamin D3 in DP-R206 (150-mg ibandronate/24,000-IU vitamin D3 tablet) as monotherapy (24,000 IU) in healthy male Korean adults. *Clin Ther*. 2014;36(1):48-57.
29. Song H-R, Kweon S-S, Choi J-S, et al. High prevalence of vitamin D deficiency in adults aged 50 years and older in Gwangju, Korea: the Dong-gu Study. *J Korean Med Sci*. 2014;29(1):149-152.
30. Shin YH, Kim KE, Lee C, et al. High prevalence of vitamin D insufficiency or deficiency in young adolescents in Korea. *Eur J Pediatr*. 2012;171(10):1475-1480.
31. Traina TA, Poggesi I, Robson M, et al. Pharmacokinetics and tolerability of exemestane in combination with raloxifene in postmenopausal women with a history of breast cancer. *Breast Cancer Res Treat*. 2008;111(2):377-388.
32. Owens SJ, Weickert TW, Purves-Tyson TD, et al. Sex-specific associations of androgen receptor CAG trinucleotide repeat length and of raloxifene treatment with testosterone levels and perceived stress in schizophrenia. *Mol Neuropsychiatry*. 2019;5(1):28-41.

33. Birzniece V, Sata A, Sutanto S, Ho KKY. Neuroendocrine regulation of growth hormone and androgen axes by selective estrogen receptor modulators in healthy men. *J Clin Endocrinol Metab*. 2010;95(12):5443-5448.
34. Durk MR, Fan J, Sun H, et al. Vitamin D receptor activation induces P-glycoprotein and increases brain efflux of quinidine: an intracerebral microdialysis study in conscious rats. *Pharm Res*. 2015;32(3):1128-1140.

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