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Effects of Red Ginseng Extract on the Pharmacokinetics of Nifedipine

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Abstract : This study investigated the impact of red ginseng extract (RGE) on the pharmacokinetics of nifedipine (NFD) and its primary metabolite, dehydronifedipine (DHNFD), in rats. A sensitive and robust analytical method was developed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the quantification of NFD and DHNFD in rat plasma. The method demonstrated high and reproducible extraction recovery rates, ranging from 84.50% to 91.06%, with no interference at the elution peaks for NFD and DHNFD. Calibration curves for NFD (1–500 ng/mL) and DHNFD (0.3–50 ng/mL) exhibited linearity ($r^2 > 0.984$) and met standard criteria for inter- and intra-day accuracy, precision, and stability. Following an intravenous dose of NFD (0.2 mg/kg), no significant differences in the plasma concentrations of NFD and DHNFD were observed between the RGE-treated group (1.5 g/kg/day for 1 week) and the vehicle-treated group. However, after oral administration (1.0 mg/kg), the RGE-treated group exhibited increased plasma levels of NFD and decreased levels of DHNFD, indicating a distinct effect of RGE on oral, but not intravenous, NFD pharmacokinetics. While hepatic Cyp3a expression remained unchanged following RGE treatment, there was a reduction in Cyp3a levels in the enterocytes, suggesting that this downregulation in the gastrointestinal tract likely contributed to the altered pharmacokinetic profile observed with orally administered NFD. In conclusion, RGE administration affects the metabolism of NFD following its oral dosing, potentially through down regulation of intestinal Cyp3a protein levels, leading to reduced systemic DHNFD concentrations and increased NFD plasma exposure.

Keywords : red ginseng extracts (RGE); cytochrome P450 (Cyp)3a; pharmacokinetic; nifedipine (NFD); dehydronifedipine (DHNFD)

Introduction

Ginseng and its major pharmacological active component ginsenosides are reported to exert anticancer, antihypertensive, antidiabetic, anti-inflammatory, anti-oxidative, anti-aging, anti-allergic, neuroprotective, hepatoprotective, and immunologic effects.[1-5] In addition to their therapeutic effects, ginseng products are frequently administered as health supplements with therapeutic drugs such as anticancer drugs and anti-diabetes drugs, and for fatigue and physical performance among elderly patients.[1,2]

Due to the growing use of herbal supplementation - ease of taking herbal supplements with therapeutics drugs (i.e., does not require a prescription) and a trend of polypills with different modes of action for better therapeutic outcomes - there has been an increase in the rate of drug-drug interactions (DDIs) or herb-drug interactions (HDIs).[1] The most frequently-reported DDIs or HDIs include the modulation of herbal components on drug metabolizing enzymes and transporters as well as the causative pharmacokinetic alterations of co-administered therapeutic drugs as victim drugs.[1,2] HDIs of red ginseng extract (RGE) has been reported with anticoagulants such as aspirin and warfarin.[1-3,6-8] A reduction in cytochrome P450 2C (Cyp2c) activity has been implicated in the herb-drug interaction between warfarin and RGE in rats.[9]

Antihypertensive medications are among the most commonly prescribed pharmaceuticals taken alongside herbal supplements.[10] In a previous study, we examined the interactions between RGE and several antihypertensive drugs—specifically fimasartan, amlodipine, and hydrochlorothiazide.[11] Our findings indicated that repeated administration of RGE decreased intestinal permeability of amlodipine and delayed time to peak concentration (T_{max}), while no sig-

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nificant effects were observed on plasma concentration of amlodipine. Plasma exposure of fimasartan tended to increase but did not reach to statistical significance.[11] Amlodipine is highly absorbed and undergoes extensive hepatic metabolism via CYP3A, but has no significant intestinal or first-pass metabolism.[12] In case of fimasartan, only minimal amounts of fimasartan are metabolized by CYP3A.[13] CYP3A in human (or Cyp3a in rats) is known for its broad substrate specificity and is a critical enzyme in both intestinal and hepatic first-pass metabolism, as it is predominantly expressed not only in the liver but also in enterocytes.[14-16] To understand the effect of RGE on the pharmacokinetics of CYP3A substrate, the present study investigates the pharmacokinetics of nifedipine (NFD), which is a calcium channel blocker antihypertensive drug and undergoes significant first-pass metabolism by CYP3A (Cyp3a).[17,18] Nifedipine is one of drugs that have been suggested to undergo significant first-pass metabolism by CYP3A in the intestine. Therefore, this study aims to elucidate the herb-drug interaction between RGE and nifedipine (NFD) and its primary metabolite dehydronifedipine (DHNFD), through intravenous (IV) and oral (PO) administration in rats, to distinguish between hepatic and intestinal interactions.

Experimental

Chemicals and reagents

NFD, DHNFD, ^{13}C-caffeine (internal standard (IS)), and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) (Figure 1). Acetonitrile and water, used as solvents, were procured from Tedia (Fairfield, CT, USA). All chemicals and solvents were of HPLC and reagent-grade quality. The Korean RGE (Hwangpoonjung®, Lot No. 731902) was obtained from the Punggi Ginseng Cooperative Association (Punggi, Korea). Dried ginseng radix cultivated for 6 years were steamed with water at 85°C for 8 h, repeated by 4 cycles, and then extracted and evaporated under reduced pressure at 65°C. The extract were autoclaved at 121°C for 15 min and concentrated using freeze dryer under vacuum condition at -80°C for 72 h. The Korean RGE contained 13.2 mg of marker ginsenosides (GRb1 + GRg1 + GRg3), and 34.7 mg total ginsenosides per gram extract.[9]

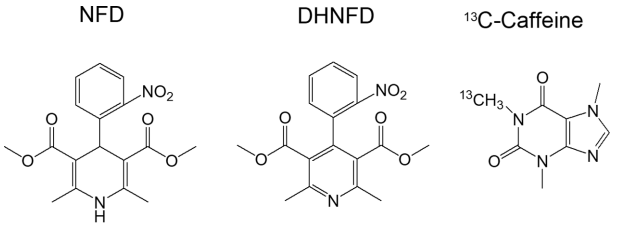

Figure 1. Structures of nifedipine (NFD), dehydronifedipine (DHNFD), and ^{13}C-caffeine (IS).

Preparation of calibration standards and quality control (QC) samples

Working solutions of NFD and DHNFD were prepared through serial dilutions of their stock solutions (2 mg/mL for NFD and 1 mg/mL for DHNFD) in acetonitrile and subsequently mixed to produce calibration standards and QC samples. The ^{13}C-caffeine solution was prepared at a concentration of 50 ng/mL by diluting its stock solution with acetonitrile. Both stock and working solutions were maintained at –80°C throughout the analysis process.

For calibration standards and QC samples, a 50 μL aliquot of the working solution was evaporated and reconstituted in 50 μL of blank rat plasma. The final concentrations of the calibration standards were 1, 2, 5, 20, 50, 100, 200, and 500 ng/mL for NFD and 0.3, 0.5, 1, 2, 5, 10, 20, and 50 ng/mL for DHNFD. QC samples were prepared with concentrations of 3, 35, and 400 ng/mL for NFD, and 0.9, 3, and 40 ng/mL for DHNFD.

Sample preparation

To prepare the calibration standards and QC samples, 50 μL of each sample was mixed with 200 μL of ^{13}C-caffeine solution (50 ng/mL in acetonitrile). The resulting mixture was vortexed vigorously for 10 min and subsequently centrifuged at 16,000 × g for 5 min. The supernatant was transferred to an autosampler vial, and a 5 μL aliquot was injected into the LC-MS/MS system. The sample preparation process for double blank and zero blank was carried out by adding 200 μL of acetonitrile solvent and internal standard solution to 50 μL of rat blank plasma, respectively, and the subsequent process was the same as the calibration standard and QC sample preparation process.

Instrument conditions

The quantification of NFD and DHNFD in rat plasma samples was performed using an Agilent 6430 triple quadrupole LC-MS/MS system coupled with an Agilent Infinity 1260 HPLC (Agilent Technologies, Wilmington, DE, USA). Chromatographic separation was achieved with a Synergi polar RP column (150 × 2.0 mm, 4 μm particle size; Phenomenex, Torrance, CA, USA). The mobile phase consisted of water and acetonitrile in a 23:77 (*v/v*) ratio with 0.1% formic acid, at a flow rate of 0.2 mL/min. The mass spectrometer operated in positive ion mode using multiple reaction monitoring (MRM). For NFD, the *m/z* transition was 347.1 → 315.1 with a fragmentor voltage (FV) of 85 V and a collision energy (CE) of 1 eV. For DHNFD, the *m/z* transition was 345.1 → 284.1, with a FV of 150 V and a CE of 25 eV. For the IS, ^{13}C-caffeine, the *m/z* transition was 197.9 → 139.8, with a FV of 120 V and a CE of 20 eV.

Method validation

The selectivity of the method was determined by comparing the chromatograms of blank plasma samples from six different rats with those of corresponding rat plasma sam-

ples spiked with lower limit of quantification (LLOQ) samples (1 ng/mL of NFD, 0.3 ng/mL of DHNFD) and the IS. A calibration curve for NFD (1–500 ng/mL) and DHNFD (0.3–40 ng/mL) was generated by plotting the ratio of the peak areas of NFD, DHNFD, and IS against their respective concentrations. The data were fitted using least squares linear regression with a weighting factor of 1/concentration^2 to assess the linearity of the calibration curve.

To evaluate inter-day precision and accuracy, two sets of QC samples (LLOQ, low, middle, and high QC) were analyzed over five independent days. Intra-day precision and accuracy were determined by analyzing six sets of QC samples (LLOQ, low, middle, and high QC) on the same day. Precision was expressed as the coefficient of variation (CV, %), while accuracy was reported as the percentage of the measured QC concentration relative to the nominal QC concentration.

Extraction recovery and matrix effects were assessed using QC samples at three different concentrations (3, 35, and 400 ng/mL for NFD; 0.9, 3, and 40 ng/mL for DHNFD) and IS (50 ng/mL). Extraction recovery was calculated by comparing the peak areas of pre-extraction spiked samples with those of post-extraction blank plasma spiked with QC samples. The matrix effect was determined by dividing the peak areas of post-extraction blank plasma spiked with QC samples by those from neat solutions of the corresponding concentrations. The extraction recovery of IS was determined using the same procedure, with an IS concentration of 50 ng/mL.

The stability of NFD and DHNFD in rat plasma was evaluated using QC samples at two concentrations (3 and 400 ng/mL for NFD; 0.9 and 40 ng/mL for DHNFD) under three conditions. Bench-top stability was assessed by incubating QC samples at 25°C for 6 h. Freeze-thaw stability was evaluated after three freeze-thaw cycles, with each cycle consisting of storage at –80°C for over 12 h followed by thawing at 25°C for 6 h. Autosampler stability was assessed by storing extracted QC samples in the autosampler at 6°C for 24 h. Concentrations of all QC samples subjected to stability tests were determined using freshly prepared calibration curves.

For the evaluation of dilution integrity, aliquots (50 μL) of NFD and DHNFD working solution (1500 ng/mL and 150 ng/mL in acetonitrile) were evaporated and the residues were reconstituted with 50 μL of blank rat plasma, followed by 5-fold dilutions with blank rat plasma to yield a diluted QC sample of the final concentration of 300 ng/mL for NFD and 30 ng/mL for DHNFD. Five sets of diluted QC samples were analyzed using freshly prepared calibration curve, and the accuracy and precision were calculated.

Pharmacokinetic study

All animal procedures were approved by the Animal Care and Use Committee of Kyungpook National University (Approval No. KNU-2019-0005). Male Sprague-Dawley rats (7 weeks old, 225–270 g) were obtained from Samtako (Osan, Korea). The rats were acclimated for one week in the animal facility of Kyungpook National University, with ad libitum access to food and water. Prior to the pharmacokinetic experiments, the rats were fasted for 12 h. RGE (1.5 g/kg/day, 2 mL/kg suspended in water) or water (2 mL/kg, vehicle treatment) was administered orally at 9 a.m. for 7 consecutive days via oral gavage. Femoral arteries and veins were cannulated using PE50 polyethylene tubing (Jungdo, Seoul, Korea) under oxygenated isoflurane anesthesia (1–3%) and the animals were allowed to recover. For IV administration, NFD solution (0.2 mg/kg in 1 mL saline containing 10% DMSO) was injected into the femoral vein, and blood samples (150 μL) were collected from the femoral artery using heparinized tubes at 0.083, 0.25, 0.5, 0.75, 1, 2, 4, 8, and 24 h. For PO administration, NFD solution (1.0 mg/kg in 2 mL saline containing 10% DMSO) was administered via oral gavage, and blood samples (150 μL) were collected from the femoral artery using heparinized tubes at 0.083, 0.25, 0.5, 1, 1.5, 2, 4, 8, and 24 h. Blood samples were centrifuged at 16,000 × g for 1 min to obtain plasma, which was stored at –80°C until further analysis.

Western blot analysis of Cyp3a

Enterocytes and liver tissues (approximately 100 mg) were homogenized in 1.0 mL CETi lysis buffer containing protease and phosphatase inhibitors (Translab, Daejeon, Korea) using a Wheaton Dounce tissue grinder. Protein samples (30 μg) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis on a 4–15% gradient gel. Western blotting for Cyp3a was performed following previously established protocols.[3] Primary antibodies against Cyp3a (dilution 1:1000, Santa Cruz Biotechnology, Dallas, TX, USA) and β-actin (dilution 1:1000, Santa Cruz Biotechnology) as a loading control were used. Protein bands were visualized using a Luminata Forte enhanced chemiluminescence system (Millipore, Burlington, MA, USA), and band intensity was quantitated using ImageQuant LAS 4000 Mini (GE Healthcare, Chicago, IL, USA).

Data Analysis

Pharmacokinetic parameters were determined using non-compartmental analysis with WinNonlin (version 5.1; Pharsight, Mountain View, CA, USA) applying student's t-test with a value of $p < 0.05$ deemed to indicate a statistically significant difference between the control and RGE-treated groups.

Results and Discussion

Optimization of MS conditions

To optimize electrospray ionization (ESI) conditions for NFD, DHNFD, and ^{13}C-caffeine (IS), each compound was directly injected into the mass spectrometer ionization

source. ESI yielded predominantly protonated ions ($[M+H]^+$) for NFD, DHNFD, and IS in the full scan spectra. The MRM transitions for NFD and DHNFD were selected based on the precursor ions ($[M+H]^+$ at *m/z* 347.1 and 345.1, respectively) and the most abundant product ions (*m/z* 315.1 and 284.1, respectively), as previously reported.[19] For ^{13}C-caffeine, both the precursor and product ions were consistent with values from published data.[20]

In this study, we used a protein precipitation method to shorten the sample preparation time, in which the matrix effect has been an important issue. Among the compounds tested for IS, ^{13}C-caffeine showed the consistent response and the least CV value in matrix effect in rat plasma. Moreover, the retention time of ^{13}C-caffeine was shorter than that of NFD and DHNFD, and as a result, total run time was 3.5 min in analysis, which might save the time in application for a large samples analysis. Therefore, ^{13}C-caffeine was chosen as an IS in this study.

Analytical method validation

Figure 3 presents the typical chromatograms for double blank, zero blank, LLOQ sample (1 ng/mL for NFD, 0.3 ng/mL for DHNFD), and plasma samples collected 1 h post-NFD PO administration. The retention times for NFD,

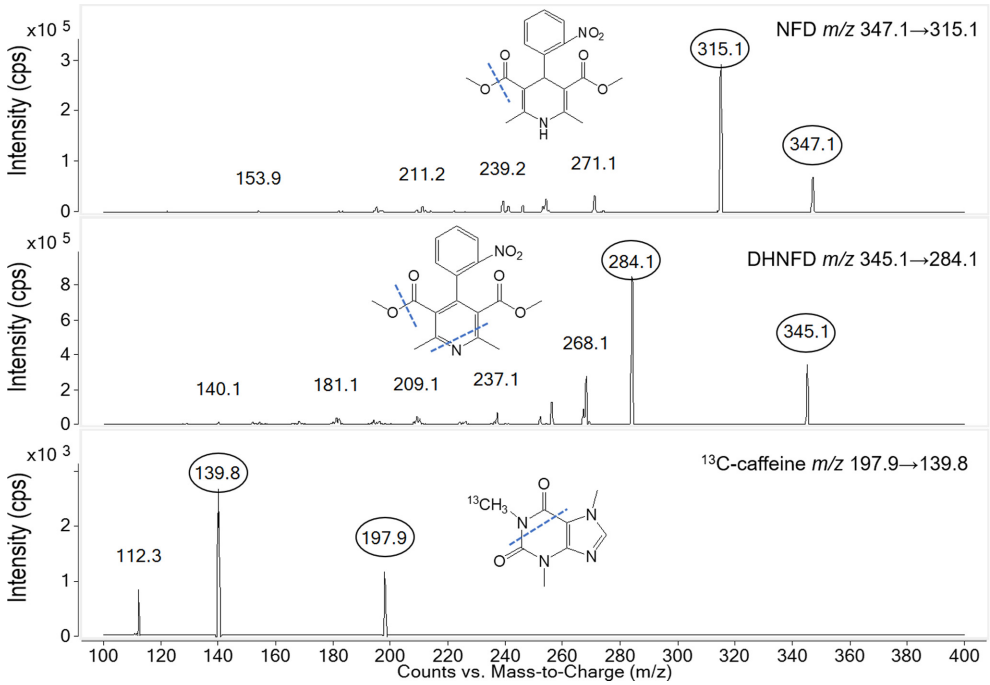

Figure 2. Product ion spectra of nifedipine (NFD), dehydronifedipine (DHNFD), and ^{13}C-caffeine.

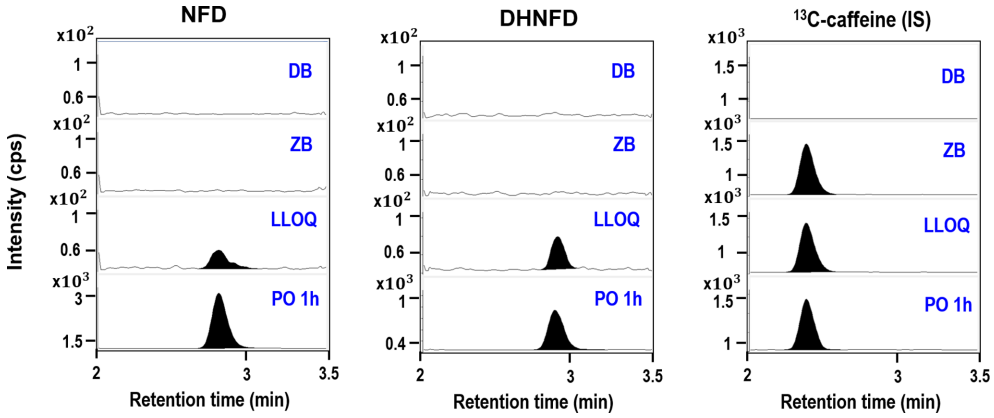

Figure 3. Representative MRM chromatograms of nifedipine (NFD), dehydronifedipine (DHNFD), and ^{13}C-caffeine (IS) in rat plasma: (A) double blank sample, (B) zero blank sample, (C) LLOQ sample, and (D) plasma sample at 1 h following PO administration of NFD.

DHNFD, and IS were 2.8, 2.9, and 2.4 min, respectively. In LLOQ samples, the signal-to-noise (S/N) ratio for NFD and DHNFD exceeded 10, with no significant matrix interference at their retention times (Figure 3).

The calibration curve was linear over the concentration range of 1–500 ng/mL for NFD and 0.3–50 ng/mL for DHNFD, with coefficients of determination (r^2) of 0.984 and 0.993, respectively (Table 1). Accuracy and precision for the calibration standards fell into the standard criteria (less than 15%).

Table 2 summarizes the inter- and intra-day precision and accuracy of NFD and DHNFD based on four levels of QC samples. Inter-day and intra-day precision for NFD ranged from 4.36% to 9.51%, and accuracy ranged from 91.67% to 101.1%. For DHNFD, inter-day and intra-day precision ranged from 2.61% to 6.79%, and accuracy ranged from 96.46% to 108.74%.

Table 3 presents the extraction recoveries and matrix effects for NFD and DHNFD. The extraction recoveries of NFD ranged from 90.12% to 90.59%, with a CV of 6.46% to 10.8% across three QC levels. For DHNFD, the extraction recoveries ranged from 84.50% to 91.06%, with a CV of 8.48% to 11.4%. These results demonstrate that the protein precipitation method employed in this study effectively and reproducibly extracts both NFD and DHNFD from rat plasma. The matrix effects for NFD ranged from 28.61% to 31.57%, with a CV of 3.84% to 9.94%. DHNFD exhibited matrix effects ranging from 44.54% to 46.36%, with a CV of 6.37% to 11.3%.

Table 4 summarizes the results of the stability experiments. The accuracy of NFD concentrations ranged from 93.84% to 100.1%, with precision spanning from 2.11% to 5.42% under three stability conditions. DHNFD concentrations showed an accuracy range of 91.68% to 103.4%, with precision values from 2.22% to 4.19% under the same con-

Table 1. Back-calculated concentrations for nifedipine (NFD) and dehydronifedipine (DHNFD) in calibration curve.

Norminal concentration (ng/mL)	Back-calculated concentrations (ng/mL)	Accuracy (%)	Precision (%)
	NFD		
1	0.97 ± 0.03	97.06	2.69
2	2.09 ± 0.13	104.6	5.99
5	5.20 ± 0.29	104.1	5.56
20	19.76 ± 1.76	98.81	8.92
50	47.71 ± 4.24	95.41	8.88
100	97.12 ± 7.02	97.12	7.23
200	190.4 ± 15.74	95.19	8.26
500	523.5 ± 14.30	104.7	2.73
Slope	0.015 ± 0.002		11.8
	DHNFD		
0.3	0.32 ± 0.02	106.78	5.02
0.5	0.48 ± 0.05	95.84	9.42
1	0.88 ± 0.05	88.17	5.11
2	1.89 ± 0.15	95.59	8.14
5	4.90 ± 0.41	97.99	8.39
10	10.31 ± 0.98	103.07	9.50
20	20.78 ± 0.63	103.92	3.04
50	55.41 ± 2.95	110.81	5.32
Slope	1.303 ± 0.165		12.7

Data represented as mean ± SD (n = 6).

Table 2. Inter- and intra-day precision and accuracy of nifedipine (NFD) and dehydronifedipine (DHNFD) in rat plasma.

Criteria	Nominal concentration (ng/mL)	Measured concentration (ng/mL)	Precision (%)	Accuracy (%)
		NFD		
Inter-day (n = 5)	1	1.00 ± 0.09	8.67	99.97
	3	2.97 ± 0.18	6.05	98.98
	35	32.85 ± 1.80	5.47	93.86
	400	389.3 ± 16.98	4.36	96.54
Intra-day (n = 6)	1	1.01 ± 0.09	9.14	101.1
	3	2.97 ± 0.23	7.66	99.09
	35	31.78 ± 1.65	5.19	91.67
	400	389.4 ± 37.02	9.51	97.34
		DHNFD		
Inter-day (n = 5)	0.3	0.32 ± 0.01	4.05	105.5
	0.9	0.88 ± 0.06	6.79	97.56
	3	3.04 ± 0.17	5.58	101.2
	40	41.69 ± 2.27	5.44	103.8
Intra-day (n = 6)	0.3	0.33 ± 0.01	4.26	108.7
	0.9	0.87 ± 0.02	2.61	96.46
	3	2.89 ± 0.13	4.49	97.78
	40	41.69 ± 1.55	3.72	104.2

Data represented as mean ± SD (n = 5 or 6).

Table 3. Extraction recoveries and matrix effects for nifedipine (NFD), dehydronifedipine (DHNFD), and IS.

Analyte	Nominal concentration (ng/mL)	Extraction recovery (%)	CV (%)	Matrix effects (%)	CV (%)
NFD	3	90.59 ± 8.21	9.06	28.61 ± 2.57	8.97
	35	90.49 ± 5.84	6.46	31.57 ± 3.14	9.94
	400	90.12 ± 9.75	10.8	29.62 ± 1.14	3.84
DHNFD	0.9	84.50 ± 9.63	11.4	46.36 ± 5.26	11.3
	3	84.65 ± 7.18	8.48	45.13 ± 3.75	8.30
	40	91.06 ± 9.87	10.8	44.54 ± 2.84	6.37
IS	50	81.10 ± 4.37	5.39	11.02 ± 0.45	4.06

Data represented as mean ± SD (n = 6).

Table 4. Stability of nifedipine (NFD) and dehydronifedipine (DHNFD) in rat plasma.

Analyte	Nominal concentration (ng/mL)	Measured concentration (ng/mL)	Accuracy (%)	Precision (%)
		Bench-top stability (at 25°C for 6 h)		
	3	3.00 ± 0.13	100.1	4.25
	400	396.9 ± 13.6	99.24	3.43
		Autosampler stability (at 6°C for 24 h)		
NFD	3	2.86 ± 0.10	95.23	3.65
	400	375.4 ± 7.92	93.84	2.11
		Freeze-thaw stability (3 cycles, at –80°C for 12 h and at 25°C for 6 h as 1 cycle)		
	3	2.91 ± 0.07	97.01	2.45
	400	378.1 ± 20.5	94.51	5.42
		Bench-top stability (at 25°C for 6 h)		
	0.9	0.87 ± 0.03	96.22	3.75
	40	39.4 ± 1.26	98.58	3.19
		Autosampler stability (at 6°C for 24 h)		
DHNFD	0.9	0.87 ± 0.02	96.52	2.22
	40	39.11 ± 1.64	97.77	4.19
		Freeze-thaw stability (3 cycles, at –80°C for 12 h and at 25°C for 6 h as 1 cycle)		
	0.9	0.83 ± 0.03	91.68	3.78
	40	41.37 ± 1.63	103.4	3.94

Data represented the means ± SD (n = 3).

Table 5. Dilution integrity of nifedipine (NFD) and dehydronifedipine (DHNFD)

Analyte	Nominal concentration (ng/mL)	dilution	Measured concentration (ng/mL)	Accuracy (%)	Precision (%)
NFD	1500	5	277.46 ± 7.43	92.49	2.68
DHNFD	150	5	31.71 ± 1.63	105.70	5.14

Data represented as mean ± SD (n = 5).

ditions. These findings indicate that NFD and DHNFD are stable in rat plasma for up to 6 h at 25°C (bench-top stability), stable for 24 h in the autosampler after sample treatment, and remain stable over three freeze-thaw cycles. Collectively, these results confirm that the analytical method for NFD and DHNFD is validated and suitable for pharmacokinetic studies.

The dilution integrity of NFD and DHNFD was assessed by calculating the accuracy and precision from five samples at 1500 ng/mL and 300 ng/mL. The accuracy and precision of the diluted QC samples were represented in Table 5. The accuracy and precision for NFD in diluted QC samples were 92.49% and 2.68%, and those for DHNFD were 105.70% and 5.14%, respectively.

Pharmacokinetic study

We investigated the pharmacokinetic profile of the CYP3A substrate NFD and its metabolite, DHNFD, following IV (0.2 mg/kg) and PO (1.0 mg/kg) administration of NFD to rats from both control and RGE-treated groups (1.5 g/kg, PO for one week). Analysis of plasma concentrations for NFD and DHNFD following IV administration revealed no significant differences between the control and RGE-treated groups (Figure 4A). Consequently, pharmacokinetic parameters such as area under the curve (AUC), half-life ($T_{1/2}$), and metabolic ratio (MR) of DHNFD remained unchanged by RGE treatment (Table 6). In contrast, following PO administration of NFD, rats in the RGE-treated group displayed increased plasma concentrations of NFD and decreased levels of DHNFD (Figure 4B). This resulted in elevated AUC and $T_{1/2}$ values for NFD, accompanied by reduced AUC and MR for DHNFD in the RGE-treated group compared with controls (Table 6).

Consequently, oral bioavailability (BA) of NFD was increased from 37.40% to 70.25% by RGE multi-dose treatment. These findings suggest that RGE multi-dose treatment reduced the metabolic activity of NFD in the case of PO but not for IV administration.

Such route-dependent pharmacokinetic alterations have been previously observed in scenarios where intestinal metabolism significantly influences the pharmacokinetics of substrate drugs.[11,21] For instance, DDIs between rifampi-

cin and orally administered drugs are more pronounced than with IV drugs since rifampin induced intestinal CYP3A and P-glycoprotein expression theregy rifampin affect the pharmacokientics of orally administered substrate drugs of CYP3A and/or P-glycoprotein.[22] NFD undergoes metabolism into the inactive metabolite DHNFD within the gastrointestinal tract and liver via CYP3A in humans (Cyp3a in rats), with an additional role played by the Cyp2c subclass in rats.[22,23] On average, CYP3A and CYP2C constitute the major intestinal CYP enzymes, accounting for approximately 80% and 15%, respectively, of total immunoquantified CYP enzymes in humans.[24]

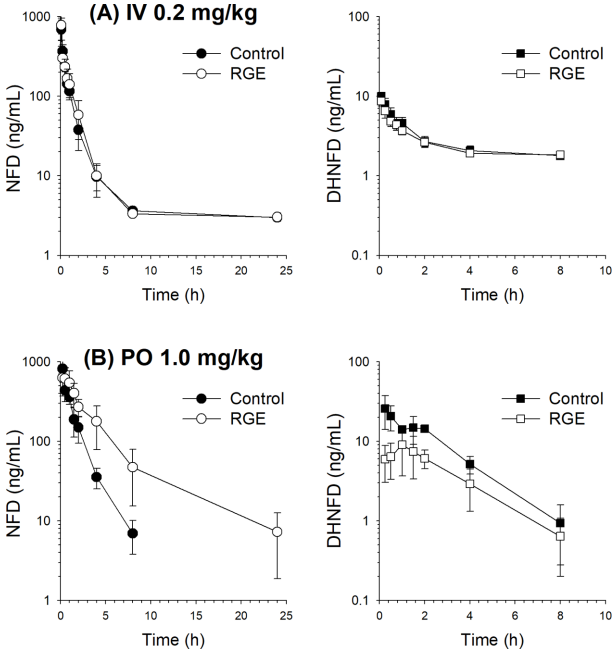

Figure 4. Plasma concentration-time profiles of NFD and DHNFD following IV (0.2 mg/kg) and PO (1.0 mg/kg) administration in control and RGE groups (1.5 mg/kg, PO for one week). Data are presented as means ± SD (n = 4).

Given the route-dependent pharmacokinetic interactions observed between NFD and RGE treatment, the study further explored whether these differences could be related to the expression of Cyp3a. Western blot analyses on Cyp3a protein expression were conducted on liver and intestinal tissues collected from control and RGE-treated rats. As depicted in Figure 5, while liver Cyp3a protein levels were not significantly affected, the intestinal Cyp3a protein levels were significantly reduced in the RGE multi-dose group. These results suggested that reduced Cyp3a expression in the intestine could inhibit NFD metabolism and increase plasma concentrations of orally administered NFD. However, intravenously administered NFD bypassed the intestinal tract thereby pharmacokinetics of NFD was not changed.

In this study, we used Hwangpoonjung® as RGE, which contains 34.7 mg of total ginsenosides per gram extract. Ginsenoside content may vary depend on the preparation method.[4] Therefore, we should note that the effect of RGE could be different depend on the various types of RGE. DDIs between NFD and RGE using more RGE from various sources need to be further investigated to correlate the

Table 6. Pharmacokinetic parameters of NFD and DHNFD following IV and PO administration in rats.

Parameters	Control		RGE	
	NFD	DHNFD	NFD	DHNFD
ROA	IV (0.2 mg/kg NFD)			
C_{max} (ng/mL)	-	9.97±0.64	-	8.98±1.06
T_{max} (h)	-	0.08±0.00	-	0.08±0.00
AUC_{last} (ng·h/mL)	512.2±51.73	50.93±3.30	546.6±134.2	30.94±17.55
AUC_{inf} (ng·h/mL)	527.6±51.07	88.47±8.78	557.6±133.3	46.33±19.45
$T_{1/2}$ (h)	3.59±0.17	14.88±4.53	2.45±1.48	5.33±0.90
MR (%)		9.26±0.56		5.39±3.01
ROA	PO (1.0 mg/kg NFD)			
C_{max} (ng/mL)	682.0±47.38	30.44±7.85	672.1±232.49	12.09±4.48*
T_{max} (h)	0.25±0.00	0.50±0.00	0.88±0.83	2.25±1.26
AUC_{last} (ng·h/mL)	918.2±62.56	53.94±16.04	1949.7±369.14*	34.95±9.33*
AUC_{inf} (ng·h/mL)	922.5±66.20	55.70±16.31	1958.8±363.22*	35.88±8.79*
$T_{1/2}$ (h)	1.58±0.38	3.25±0.89	2.50±0.57*	3.91±2.09
MR (%)		5.84±1.62		1.77±0.52*
BA (%)	37.40		70.25	

ROA, Route of administration; C_{max}, maximum plasma concentration; T_{max}, time to reach C_{max}; AUC, area under the curve; $T_{1/2}$, half-life; MR, metabolic ratio, calculated by dividing AUC_{DHNFD} by AUC_{NFD} × 100. BA, bioavailability which was calculated by dividing dose normalized $AUC_{NFD,PO}$ by dose normalized $AUC_{NFD,IV}$ × 100. Data represent the means ± SD (n = 4). * p<0.05

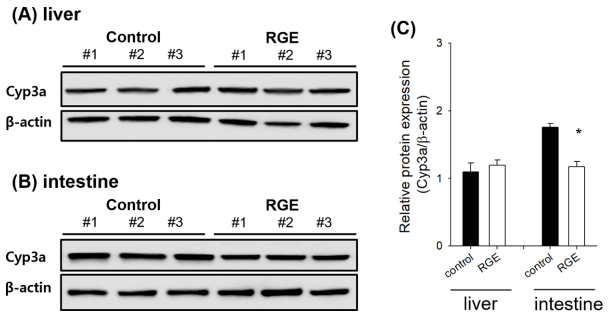

Figure 5. Cyp3a protein expression in (A) liver and (B) intestine of control and RGE-treated rats. β-actin was used as a loading control. (C) Quantitative analysis of relative Cyp3a protein expression, showing mean ± SD (n = 3). *p < 0.05 compared with the control group.

ginsenoside content with DDIs.

In summary, the pharmacokinetic analysis revealed no significant difference in plasma levels of NFD and DHNFD following IV administration of NFD. However, following PO administration, the RGE-treated group demonstrated significantly reduced plasma levels of DHNFD and metabolic ratio, suggesting that RGE treatment reduced the intestinal metabolism of NFD, as evidenced by decreased AUC and MR of its metabolite.

Conclusions

A sensitive and validated LC-MS/MS method was developed for quantifying NFD and DHNFD in rat plasma. This analytical approach successfully elucidated the pharmacokinetic interaction between RGE and NFD, a Cyp3a substrate antihypertensive drug, in rats. Repeated RGE administration (1.5 g/kg, for one week) influenced the pharmacokinetic profile of orally administered NFD but not IV NFD, which may be attributed to the reduced Cyp3a protein levels in the intestine, with no observable effect on hepatic Cyp3a protein levels.

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Conflict of interest

All authors declare that they have no conflict of interest.

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