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Characterization of the Interaction between White Ginseng Extract and Selegiline Using Triple Quadrupole-Mass Spectrometry

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Abstract : Korean ginseng (*Panax ginseng* Meyer) is a traditional herb used across the world to treat various diseases. Although, red ginseng is this herb's most famous product and has demonstrated diverse pharmacological activities, white ginseng (WG) is another ginseng product that is made fresh and individually regulated in Eastern Asia. Red and white ginseng show different characteristics due to distinct processing steps despite originating from the same plant, and the drug interactions induced by WG have not been well documented. Selegiline is a selective monoamine oxidase (MAO) inhibitor used as an antidyskinetic and antiparkinsonian agent. Here we developed a quantification method for selegiline in mouse plasma using a C8 stationary phase in triple quadrupole-mass spectrometry (LC-MS/MS) with multiple reaction monitoring (MRM). The validated LC-MS/MS method was successfully applied to determine the potential interaction with WG extract (0.1 g/kg/day) pre-administered for 4 weeks. The $AUC_{0\text{-}240\,min}$ of selegiline was altered due to a decrease in the absorption of selegiline with repeated administration of WG extract.

Keywords : White ginseng extract, LC-MS/MS, selegiline, herb-drug interaction

Introduction

Korean ginseng (*Panax ginseng* Meyer) is a traditional herb used to treat various diseases in Far East Asia due to its ability to normalize body functions and strengthen systems that have been compromised by stress.[1] Among the diverse bioactive constituents in ginseng extracts, ginsenosides are the major pharmacologically active ingredients of ginseng, with antiallergic, antioxidant, immune-stimulatory, anti-inflammation and anti-cancer activities.[2,3] Generally, the products made from fresh ginseng can be classified as white ginseng (WG) or red ginseng (RG) according to processing conditions, which are individually regulated in the Korean, Japanese, and Chinese Pharmacopoeias.[1,3] Korean red ginseng (KRG) extract is the most famous ginseng product, with diverse pharmacological activities due to its high ginsenoside content as a result of heat and steam processing.[4] Many studies have investigated KRG-induced drug interactions,[5,6] demonstrating that these interactions are primarily related to the action of ginsenosides.[7,8]

Red and white ginseng show different characteristics as a result of unique processing steps despite originating from the same plant.[1] Specifically, the levels of ginsenosides and oligosaccharides differ between WG and RG, with a higher primary ginsenoside content (Rg1, Rb1, Re, Rc, Rb2 and Rd, etc.) and a 40% higher sucrose level in WG compared with RG.[9] In our recent study, we also determined that the levels of Rb2, Rc, Re and Rg1 are higher in WG extract than RG extract.[4] While the drug interactions of RG have been extensively studied, the research on drug interactions with WG has not been well documented. Because the ginsenoside composition as the major active ingredient differs between the two groups, an evaluation of drug interactions with WG is required to ensure safe administration.

Here we evaluated WG-induced drug interactions using selegiline as a model compound in mouse plasma after oral administration of WG extract (0.1 g/kg/day) for four weeks. Selegiline [*l*-(−)-deprenyl; (−)-*R*-*N*,α-dimethyl-*N*2-propynyl-phenethylamine hydrochloride) is a selective inhibitor of monoamine oxidase (MAO) type B and is metabolized to desmethylselegiline and methamphetamine by cytochrome P450 (CYP).[10,11] Although selegiline is widely used in medicine as a classic antidyskinetic and

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antiparkinsonian agent, this drug is likely to be abused, and thus a test is needed to quantify selegiline in the blood or urine. In recent years, liquid chromatography coupled with triple quadrupole-mass spectrometry (LC–MS/MS) using multiple reaction monitoring (MRM) modes has been widely applied to analyze a variety of small molecules in biological matrices. In the present study, we developed a quantification method for selegiline in mouse plasma using a C8 stationary phase with LC-MS/MS following administration of white ginseng extract (WGE) for 4 weeks.

Materials and Methods

Preparation of white ginseng extract

The white ginseng products were prepared by the Punggi Ginseng Cooperative Association (Punggi, Korea) according to a previous study.[4] The WGE was prepared by refluxing with 500 mL of distilled water at 100°C for 3 h. After the extraction was completed, filtration was performed using a filter paper to obtain a filtrate, which was concentrated.

Animal experiments and blood collection

Male 5-week-old C54BL/6N mice were purchased from Orient Co. (Seongnam, Korea) and randomly housed at 3 mice per cage. Before the experiments, the mice were allowed one week of acclimation in a controlled environment (relative humidity: 60%, temperature: maintained at 25°C) under a 12-h/12-h light/dark cycle, with free access to standard rodent chow and tap water. Mice were fed a normal diet (ND) (n = 3) or ND + WGE (0.1 g/kg/day) (n = 3) for 4 weeks. All animal procedures were conducted in compliance with the guidelines of the Guide for the Care and Use of Laboratory Animals of the National Instituted of Health, and this study was approved by the Institutional Review Board of Kyungpook National University (KNU-2017-0089-1).

After fasting for 12 h, the mice (25 ± 2 g) were randomly divided into two groups (n = 3) receiving saline for control group or an oral administration of 20 mg/kg selegiline dissolved in saline for treated group. Approximately 20 μL of blood was obtained from the tail vein and collected in heparinized tubes at 0, 5, 10, 15, 30, 60, 120, and 240 min after oral selegiline administration. The samples were obtained by centrifuging blood sampled at 4,000 g for 15 min at 4°C, and the supernatants were separated and stored at -80°C until analysis.

Plasma samples (10 μL) were mixed with 90 μL 100% acetonitrile (ACN) in 0.1% formic acid containing 25 μM reserpine (internal standard, IS). The supernatant was vortexed and centrifuged (13,000 rpm, 10 min, 4°C), and a 10 μL sample was then injected into a C8 column for the LC-MS/MS analysis.

LC-MS/MS system

The LC system consisted of a LC-20AD liquid chromatograph, SIL-20A auto sampler, CTO-20A column oven and CVM-20A communication bus module (Shimadzu, Kyoto, Japan). A Triart C8 column (3 μm, 50 mm × 4.6 mm, YMC Co. Ltd., Kyoto, Japan) and a guard C18 column (2 mm, 2.1 mm i.d., Phenomenex, USA) were used for LC separation. A gradient program was employed with the mobile phase combining solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in ACN) at a flow rate of 500 μL/min. The gradient was as follows: 5-5% B (0-1.0 min), 5-95% B (1.0-4.0 min), 95-95% B (4.0-7.0 min), 95-5% B (7.0-8.0 min), 5-5% B (8.0-10.0 min). During the analysis, the column oven was maintained at 40°C. Samples were analyzed using an LC system coupled to a TSQ triple stage quadrupole mass spectrometer (Thermo Scientific Inc., MA, USA) with an electrospray ionization (ESI) source. The mass spectrometer was operated in the positive ESI mode with nitrogen as the aux gas pressure, Sheath gas pressure and vaporizer temperature were set at optimum values of 10, 40 psi and 300°C, respectively. The ESI spray voltage was adjusted to 3,000 V, and the capillary temperature was set at 350°C. For all the low-energy collision dissociations using the multiple reaction monitoring (MRM) scan, the transitions recorded were from m/z precursor protonated molecule → m/z product ion as follows: m/z 188.1 → 91.1 for selegiline and m/z 609.2 → 194.9 for the IS, at a collision energy of 15 eV for selegiline and 30 eV for the IS. Data were obtained using Xcalibur software (Version 3.0.63)

Linearity and calibration curve

Stock solutions of selegiline were prepared in methanol at a concentration of 2 mg/mL. Ten microliters of each stock solution were added to 90 μL of drug-free mouse plasma. The final concentrations of selegiline in the calibration standards were 3, 5, 10, 30, 50, 100, 300 and 500 ng/mL. Aliquots (10 μL) of standard plasma samples were then processed as described above. Calibration curves were constructed by plotting the peak-area ratios of selegiline to reserpine (IS) versus the concentrations of selegiline in mouse plasma.

Accuracy and precision

The precision and accuracy of the methodology were evaluated by analyzing five replicates of mouse plasma spiked with known concentrations of selegiline (3, 30 and 300 ng/mL). Intra-day accuracy and precision for selegiline quantification were determined by repeating the assay on the same day at each concentration. Inter-day accuracy and precision were evaluated on five consecutive days at each concentration.

Pharmacokinetic parameters

A non-compartmental model was used to evaluate the Pharmacokinetics (PK) parameters of selegiline using WinNonlin software (Version 2.1, Scientific Consulting, KY, USA). The parameters included the area under the plasma concentration-time curve for 240 min ($AUC_{0\text{-}240\ min}$), time to reach maximum plasma concentration (T_{max}), maximum plasma concentration (C_{max}), and half-life ($T_{1/2}$).

Statistics

The results were expressed as the mean ± S.E. and the statistical differences between two groups were determined by Paired Sample *t*-test. The significant value at $P<0.05$ was represented as asterisks (*).

Results and Discussion

To determine the WGE-induced drug interaction in mice, we monitored the plasma concentration of selegiline as a marker compound in blood by LC-MS/MS after four weeks of oral WGE 0.1 g/kg/day administration. The concentration of selegiline in blood was determined by MRM scan mode in the positive ionization mode. The product ions of selegiline and IS were produced by collision-induced dissociation, and the *m/z* transition of the highest produced peaks selected for MRM analysis were determined and optimized for each compound (Figure 1). The MRM transitions selected for selegiline and IS were *m/z* 188.1 → 91.1 and 609.2 → 194.9, respectively. We initially used the most popular reverse phase column (C18) for the separation of selegiline in chromatography, but as shown in Figure 2A, the leaching was too rapid, presumably due to the hydrophilic nature of selegiline. Figure 2B shows that selegiline was successfully separated using a C8 column, as the latter has the advantage of being more hydrophilic than C18.

As a result, we obtained final MRM chromatograms for selegiline and IS (Figure 3). No endogenous interference source was observed in blank plasma (Figure 3A). The

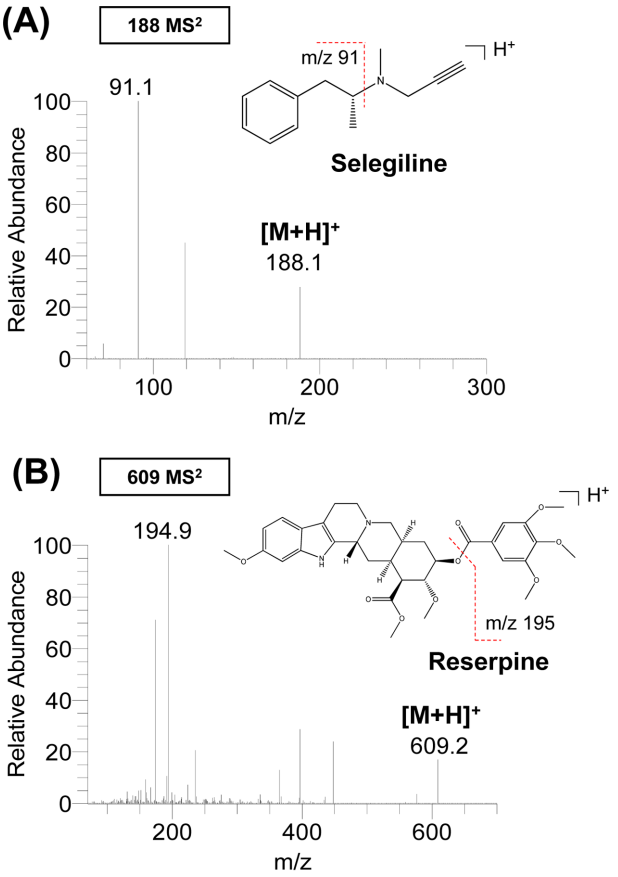

Figure 1. Product ion mass spectra of selegiline (A) and reserpine as the internal standard (B).

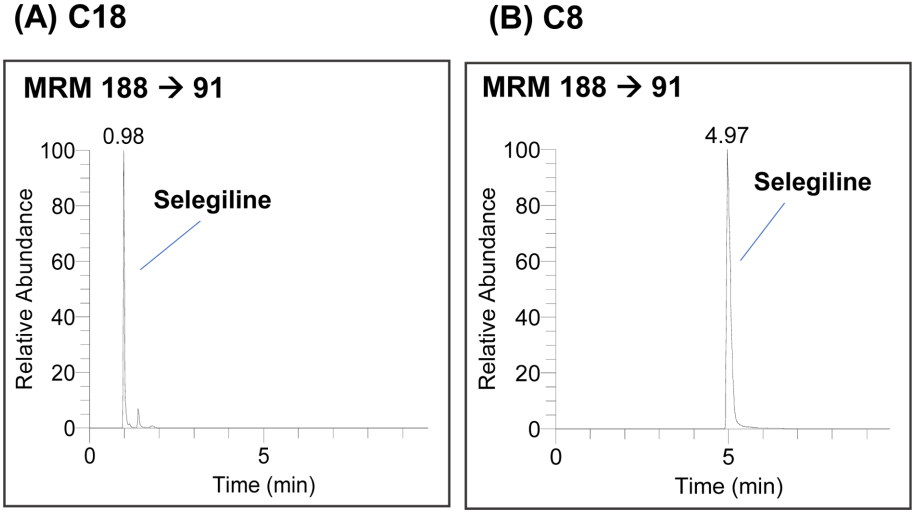

Figure 2. Representative MRM chromatograms of selegiline in reverse stationary phase using C18 (A) and C8 (B).

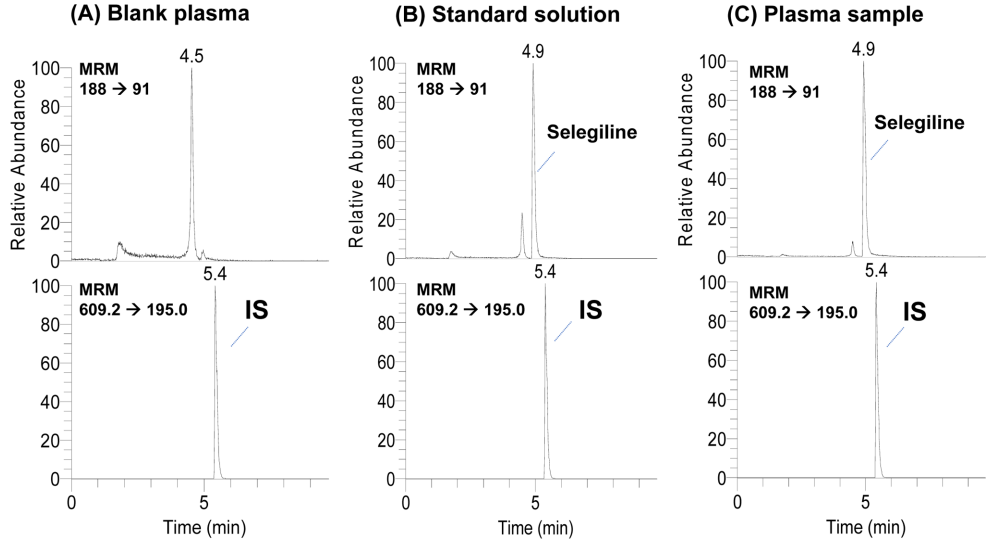

Figure 3. Representative MRM chromatograms of selegiline and IS (reserpine) in mouse blank plasma (A), blank plasma spiked with 50 ng/mL of selegiline (B) and a mouse plasma sample obtained 5 min after the administration of 20 mg/kg selegiline (C).

Table 1. Intra- and inter-day RSD and accuracy for the determination of selegiline in mouse plasma.

Theoretical concentration (ng/mL)	Intra-day			Inter-day		
	Observed Concentration (ng/mL)	RSD (%)	Accuracy (%)	Observed Concentration (ng/mL)	RSD (%)	Accuracy (%)
3	3.2 ± 0.02	1.5	93.5	3.2 ± 0.09	6.5	92.7
30	34.5 ± 0.2	1.7	87.0	34.4 ± 0.3	1.9	87.3
300	340.3 ± 3.3	2.2	88.1	342.2 ± 1.7	1.1	87.6

Values represent the means ± standard errors of five replicates.

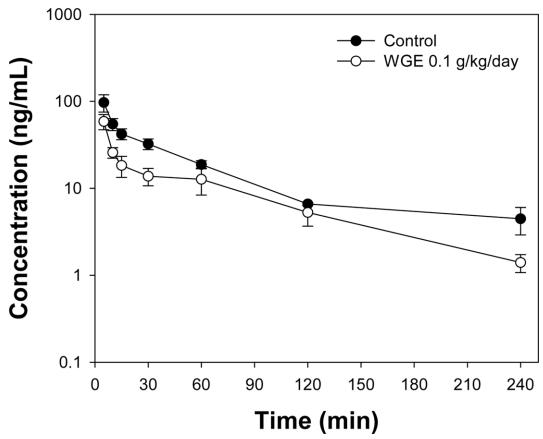

Figure 4. Mean plasma concentration-time curve of selegiline with or without pre-administration of WGE (*p.o.*, 0.1 g/kg/day) for four weeks in male ICR mice. Values represent the means ± standard errors for three animals.

selegiline and IS eluted at 4.9 and 5.4 min, respectively (Figure 3B and 3C). Based on our developed LC-MS/MS method, we set the calibration curve in the range 3-500 ng/mL (r^2 = 0.999) for selegiline, which showed linearity. The limit of detection (LOD) and lower limit of quantitation (LLOQ) of selegiline were 1 and 3 ng/mL, respectively. To validate the quantification method for selegiline, the precision and accuracy were evaluated, as shown in Table 1. The precision (relative standard deviation (RSD), %) ranged from 1.1 to 6.5, and the accuracy ranged from 87.0 to 93.5%, showing that the proposed method was suitable for the determination of selegiline in blood by LC-MS/MS. The validated LC-MS/MS method was successfully applied to determine the potential interaction with pre-administered WGE (0.1 g/kg). Figure 4 shows the mean plasma concentration-time curves of selegiline after oral administration at a dose of 20 mg/kg. The main PK parameters for selegiline in the groups with or without WGE administration are summarized in Table 2. All PK parameters were determined by non-compartment model analysis.

The C_{max} of the control and WGE-administration groups were found to be 96.9 ± 21.8 ng/mL and 58.7 ± 11.7 ng/mL, respectively, demonstrating a decreased value in the WGE-administration group. The $AUC_{0\text{-}240\ min}$ of the WGE group was also significantly decreased compared to

Table 2. Pharmacokinetic parameters of selegiline in the control and WGE-administered group (0.1 g/kg/day, *p.o.*) after 4 weeks.

Parameters	Group	
	Control	WGE (0.1 g/kg/day)
C_{max} (ng/mL)	96.9 ± 21.8	58.7 ± 11.7
T_{max} (min)	5.0 ± 0.0	5.0 ± 0.0
$AUC_{0\text{-}240\ min}$ (μg·min/mL)	4.1 ± 0.15	2.2 ± 0.41*
Half-life (min)	63.5 ± 11.1	60.9 ± 9.9
MRT (min)	56.1 ± 13.0	56.7 ± 7.7

Values represent the means ± standard errors of three replicates. The asterisks indicate values that significantly differed from the vehicle control at $P < 0.05$ (*).

controls, with the $AUC_{0\text{-}240\ min}$ of controls and the WGE-treated group of 4.1 ± 0.15 and 2.2 ± 0.41 μg·min/mL, respectively. Other parameters including T_{max}, half-life and mean residence time (MRT) did not show significant differences between control and WGE-treated animals. These data indicate that the PK parameters of selegiline were altered due to the decreased absorption of selegiline following repeated white ginseng administration.

P. ginseng is widely used as an adaptogen throughout the world, and ginsenosides are the major active constituents of *P. ginseng*.[12] A previous study showed that various ginseng components can influence CYP, glucuronidation, and drug transport activity, leading to potential ginseng-induced drug interactions.[12] According to the results of the present study, a decrease in the $AUC_{0\text{-}240\ min}$ of selegiline reflects the decreased absorption rate caused by chronic administration of white ginseng extract. However, studies on the correlation between ginseng-derived components and transporters involved in the absorption of low-molecular-weight substances are limited. In this study, although we could not identify the regulatory elements or mechanisms, we successfully developed a method to quantify selegiline in blood and evaluated its interaction with white ginseng extract.

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