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Short communication

Pharmacokinetic study of ginsenoside Re with pure ginsenoside Re and ginseng berry extracts in mouse using ultra performance liquid chromatography/mass spectrometric method

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ABSTRACT

Ginsenoside Re is the major ginsenoside in ginseng berry(GB) extract and its pharmacokinetics were studied following the intravenous and oral administration of pure Re or ginseng berry extract in mouse with doses of 10 and 50 mg/kg using ultra performance liquid chromatography mass spectrometric (UPLC/MS) method which can simultaneously determine ginsenoside Re, Rg1 and Rh1 in mouse serum. The serum samples were pretreated by protein precipitation and chromatographic separation was performed on AQUITY UPLC BEH C_{18} column using gradient elution with the mobile phase of 5 mM ammonium formate and acetonitrile. Analytes and digoxin (I.S.) were analyzed and identified using an electrospray negative ionization mass spectrometry in the selected ion monitoring mode with the linear concentration range of 5.0–5000 ng/mL and lower limits of detection (LLOD) under 2.5 ng/mL. Ginsenoside Re was rapidly cleared from the body with a short half-life (0.2 ± 0.03 h for male and 0.5 ± 0.08 h for female mice after i.v.) and oral absorption was generally poor (*F*% 0.19–0.28). Notably, GB extract showed a superior oral absorption of ginsenoside Re (*F*% 0.33–0.75) at equivalent ginsenoside Re dose to pure ginsenoside Re, indicating that GB extract might be a good form for ginsenoside Re intake.

1. Introduction

While the root of *Panax Ginseng* C.A. Meyer, ginseng, is the most popular herbal medicine in the world [1], recent attentions are given into ginseng berry (GB) due to its strong therapeutic efficacies against cancer, diabetes and ischemic heart diseases [2–4]. The GB is known to have a distinctive ginsenoside profile substantially different from that of the ginseng root [5]. Interestingly, among other ginsenosides, GB extract contains high level of ginsenoside Re amounting almost seven times that of ginseng root, indicating that GB extract can be a superior form to ginseng root extract for ingesting a large amount of ginsenoside Re. Currently, GB extract is being evaluated in preclinical and clinical trials for its efficacy over ginseng root extract.

Ginsenoside Re was regarded to be an active component of GB extract for the anti-diabetic and anti-ischemic effect [6,7]. Despite these well-known pharmacological effects, little is known about the pharmacokinetic profiles of ginsenoside Re. Only recently, the pharmacokinetic behavior of ginsenoside Re has been investigated using ginsenoside mixture [8] or traditional Chinese prescription drug using ginseng roots, Shenmai injection [9], however, the true pharmacokinetic behavior of ginsenoside Re with pure ginsenoside Re remained unexplored to our best knowledge. In addition, it is recently known that interactions from co-existing flavonoids or catechins could substantially affect the pharmacokinetic profiles of natural compounds [10,11], indicating the need for an independent assessment of pharmacokinetic profiles of ginsenoside Re to know the accurate systemic behavior of ginsenoside Re after consumption of pure ginsenoside Re or GB extract.

Recently, the bioanalytical methods for ginsenoside Re were developed using high performance liquid chromatography with ultraviolet detection (HPLC-UV)[12], fluorescence detection (HPLC-FLD)[13], evaporative light-scattering detection (HPLC-ELSD)[14], liquid chromatography mass spectrometry (LC–MS)[15,16] and liquid chromatography–tandem mass spectrometry (LC–MS/MS)[17]. This analytical method, however, has some drawbacks, such as long retention times to improve better separation for analysis of various gensenosides and excessive consumption of mobile phase.

Ultra performance liquid chromatography (UPLC) utilizes small silica particle column ranging 1.7 μm, which makes possible to perform efficient separations in a short analysis time [18]. It has

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many advantages in the analysis of multiple biological samples such as high speed, high peak capacity, high resolution and good sensitivity[19]. In this study, a rapid UPLC–MS quantification method was established, enabling the simultaneous determination of three ginsenosides in 6.5 min total run-time for the quantitation of ginsenoside Re and the potential metabolites, ginsenoside Rg1 and ginsenoside Rh1 in mouse serum. Using this sensitive, selective, and simple UPLC/MS method, the intravenous and oral pharmacokinetic profiles of ginsenoside Re were investigated with a pure ginsenoside Re in mouse to assess the absorption of ginsenoside Re. Additionally, the oral absorption of ginsenoside Re was measured after administration of GB extracts, which contain a high content of ginsenoside Re to explore its use as a new health food.

2. Experimental

2.1. Chemicals and reagents

Ginsenoside Re, Rg1, Rh1 were purchased from Wako Pure Chemical (Osaka, Japan) and other ginsenosides (Rb1, Rb2, Rc, Rd, Rg2) were from LKT Labs (St. Paul, MN, USA). HPLC grade acetonitrile and methanol were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Digoxin (I.S.), methylcellulose and ammonium formate were obtained from Sigma (St. Louis, MO, USA) and formic acid was purchased from Fluka (Burchs, Switzerland). The water used were ultra-pure deionized water (18.2 MΩ cm) produced from Millipore Milli-Q Gradient system (Millipore, Bedford, MA). All other reagents used were of the highest grade available.

2.2. Preparation of ginseng berry extract(GB)

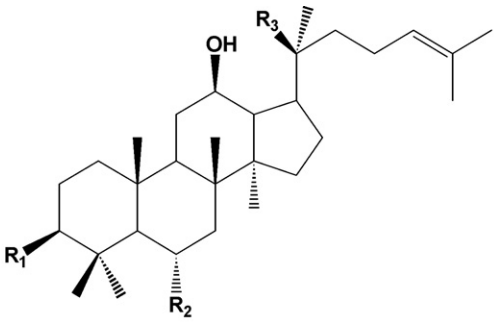
After fresh *P. Ginseng* berry was washed with water, the seeds were removed and the remainder (pulp and rinds) was collected. After extraction in 70% ethanol under reflux condition, extract was filtered and evaporated. Finally, the extract solution was lyophilized to obtain a powder state of GB extracts.

2.3. Assaying of ginsenoside Re in GB extract

The content of ginsenoside Re in GB extract was determined using HPLC-UV method. About 500 mg of extracts were dissolved with 100 mL of 50% methanol in water and were ultra-sonicated for 10 min. Without further cleaning, sample was filtered and resultant filtrates were directly injected to HPLC. Chromatographic separations were achieved using a Mightysil (Kanto, Japan) C18 column (250 mm × 4.60 mm, 5 μm), and UV detection was at 203 nm. The mobile phase consisted of a mixture of acetonitrile (A) and water (B). The initial composition was 20% A and 80% B and gradient elution was as follows; 0–10 min, 20% A; 10–40 min, 32% A; 40–48 min, 42% A; 48–50 min, 100% A; 50–60 min, 100% A; 60–62 min, 20% A; 62–70 min, 20% A. The flow rate was 1 mL/min and injection volume was 10 μL. The content of ginsenoside Re in GB extract was estimated to be 11.06% and other ginsenosides were as follows: Rb1, 0.77%, Rb2, 1.90%, Rc, 2.11%, Rd, 1.65%, Rg1, 1.66% and Rg2, 0.84%.

2.4. Liquid chromatographic and mass spectrometric condition

The chromatographic separation was carried out using ACQUITY UPLC system (Waters Co., Milford, MA). The column was ACQUITY UPLC BEH C_{18} column (1.7 μm, 2.1 mm × 50 mm). The column temperature and autosampler tray temperature were maintained at 40 and 10 °C, respectively. The mobile phase consisted of 5 mM ammonium formate (pH 3.3 with formic acid, solvent A) and acetonitrile (solvent B). Gradient elution was as follows: isocratic elution with 15% B for 0.5 min, followed by a 2 min gradient to 30% B, 3.5 min to 40% B, 4 min to 95% B, then isocratic elution with 95% B in 5 min, then returned to 15% B in 6.5 min. The flow rate was 0.45 mL/min and injection volume was 5 μL. LC–MS analysis was performed using Waters Micromass Quattro Premier XE triple quadrupole mass spectrometer. The mass spectrometer was operated in the negative ESI mode with following operation conditions. Capillary voltage, 4.0 kV; ion source temperature, 120 °C; desolvation temperature, 400 °C; desolvation gas flow rate, 850 L/h; cone gas flow rate, 50 L/h. The optimum cone voltages for ginsenoside Re, Rg1, Rh1 and digoxin (I.S.) were set to different voltages according to *m/z*. (Fig. 1) The selected ion monitoring (SIR) was set at *m/z* 991.5 for ginsenoside Re, *m/z* 845.4 for ginsenoside Rg1, *m/z* 683.2 for ginsenoside Rh1, *m/z* 825.3 for digoxin (I.S.). The data were acquired and processed with MassLynx Version 4.1 (Waters Co.).



Compound	R1	R2	R3	m/z	Cone voltage(V)
Re	-OH	-O-Glc-Rha	-O-Glc	991.5	21.0
Rg1	-OH	-O-Glc	-O-Glc	845.4	30.0
Rh1	-OH	-O-Glc	-OH	683.2	25.0
digoxin	-	-	-	825.3	30.0

Fig. 1. Chemical structures, *m/z* and optimized cone voltage of ginsenoside Re, Rg1, Rh1 and digoxin (I.S.).

2.5. Preparation of standard solution and quality control samples

Standard stock solutions of ginsenoside Re, Rg1, Rh1 and digoxin (I.S.) were prepared in methanol at 1 mg/mL and stored at 4 °C. Working standard solutions were serially diluted with methanol to obtain concentrations for calibration curve standards. The working solution for internal standard was diluted with methanol to get a final concentration of 500 ng/mL. Calibration standards of ginsenoside Re, Rg1, Rh1 (5.0, 10.0, 20.0, 50.0, 100, 500, 1000 and 5000 ng/mL, respectively) were prepared by spiking appropriate amount of the working standard solutions to pooled blank serum from 10 naive animals. QC samples were spiked at 10.0, 250, and 750 ng/mL, respectively. The standard spiked serum samples were aliquoted (100 μL) into polypropylene tubes and stored −20 °C until analysis.

2.6. Sample preparation

To 100 μL aliquots of biological samples, 10 μL of 500 ng/mL digoxin (as internal standard) was added. After protein was precipitated with 500 μL of methanol in 1.5 mL polypropylene tube by vortexing for 5 min, samples were centrifuged at 14,000 rpm, 4 °C for 10 min. The upper organic layer was transferred into another

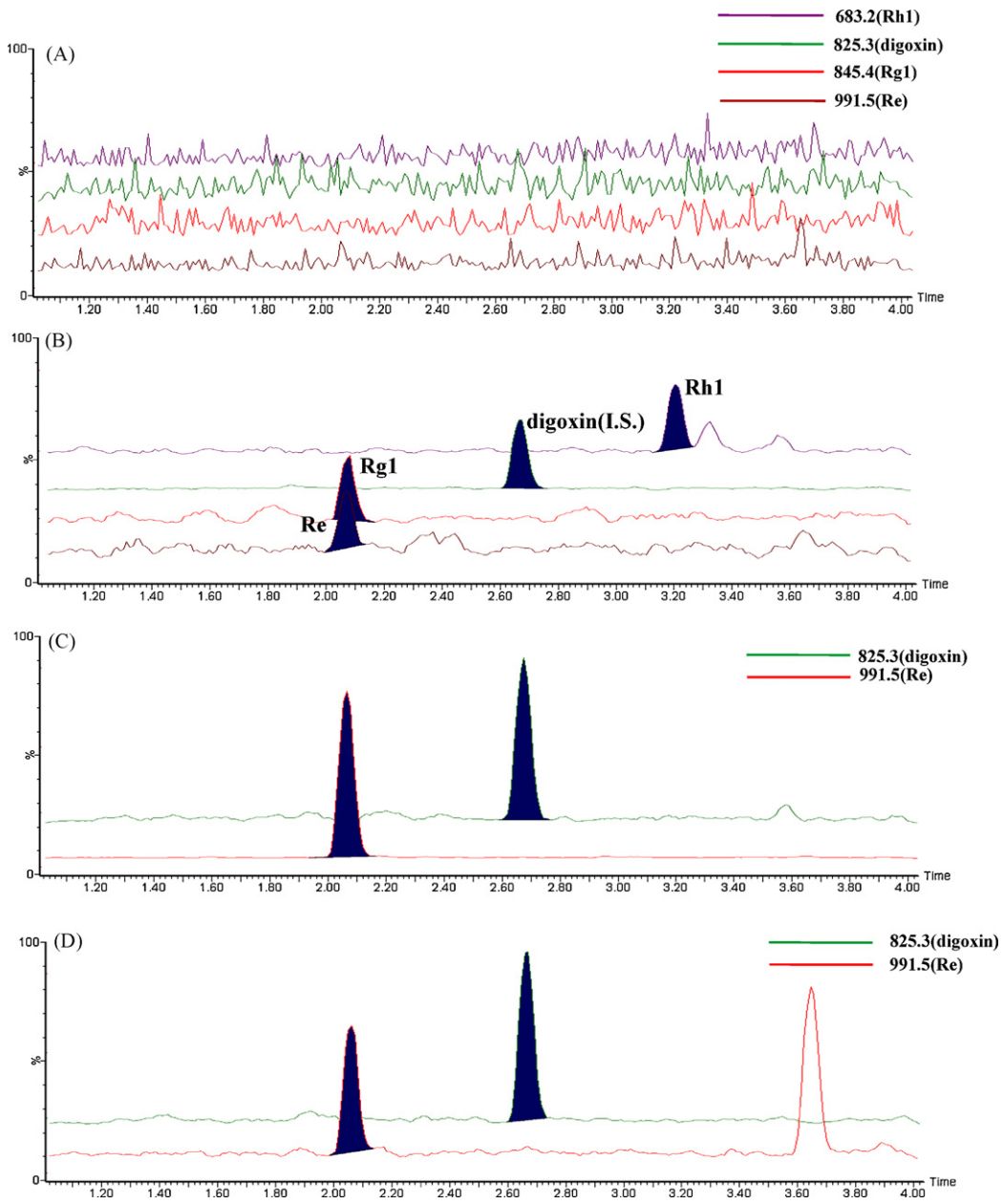


Fig. 2. Representative SIR UPLC/MS chromatograms. (A) blank serum sample, (B) blank serum sample spiked with ginsenoside Re, Rg1, Rh1 and I.S. at the lower limit of quantification (C) serum sample from a mouse, 0.5 h after intravenous administration of ginsenoside Re at a dose of 1 mg/kg (D) serum sample at 0.5 h after oral administration of GB extract at a dose of 50 mg/kg.

tube and evaporated to the dryness using speedvac (EZ-2 Plus, Genevac, Swiss) at 50 °C. The residues were reconstituted with 100 μL of methanol and vortexed for 5 min, followed by centrifuging at 14,000 rpm, 4 °C for 5 min. The 5 μL of sample solutions was injected into a UPLC/MS system.

2.7. Method validation

The method was validated by linearity, the lower limit of detection (LLOD), the lower limit of quantification (LLOQ), intra- and inter-day accuracy, precision, extraction recovery and stability. The LLOQ and LLOD were determined as the lowest concentration point of standard curve with a signal to noise, 10 or 3, respectively. To evaluate linearity, five sets of mouse serum calibration curves were prepared and assayed. Intra- and inter-day accuracy and precision were assayed by determining of QC samples using triplicates of mouse samples at three concentration levels (10.0, 250 and 750 ng/mL, respectively) for ginsenoside Re, Rg1, Rh1 on 4 validation days. The stability of analytes in mouse serum was investigated by analyzing QC samples stored for 24 h at ambient temperatures and after three freeze (−20 °C)–thaw (room temperature) cycles on 3 consecutive days.

2.8. Pharmacokinetic study

Seven weeks old male albino ICR mice, 20–25 g in weight, were obtained from Orient-Bio (Gyeonggi-do, Korea). All the mice were fed a standard experimental diet (PurinaKorea, Gyeonggi-do, Korea) *ad libitum*. A 12/12 h light/dark cycle was provided and the room temperature was maintained at approximately 23 °C. All aspects and protocols of the animal experiments and husbandry were carried out in compliance of, and approved by the

institutional animal care and use committee of AmorePacific R&D center.

Oral dose of pure ginsenoside Re was dispersed in 1% methylcellulose and Tween80 0.5% to a final concentration of 1 and 5 mg/mL. GB extract was dispersed in DW to contain ginsenoside Re to a final concentration of 1 and 5 mg/mL. After 6 h of fasting, mice received a single oral intubation of ginsenoside Re or GB extract. Intravenous dose of Re was dissolved in 10% DMSO to a final concentration of 0.2 mg/mL and administered into tail vein. Blood samples were collected from 16–20 animals per group at each time point (0, 10, 20, 30 min, 1, 2, 4, 8 and 24 h for oral and 5, 10, 20, 30, 60, 120, 240 and 480 min, respectively, for i.v.) immediately after dosing. For a mouse, only twice blood sampling were done at the retro-orbital plexus using a plain capillary tube (Chase Scientific, USA) and centrifuged at 3000 rpm for 10 min. The serum samples were stored at −70 °C until bioanalysis.

2.9. Statistics

The serum concentration vs. time data were analysed by a non-compartmental method using the nonlinear least squares regression program WinNonlin (Scientific Consulting Inc., Cary, NC, USA). All data were expressed as the mean ± standard deviation (SD) and statistical difference between groups was analyzed by Student's *t*-test.

3. Results and discussion

3.1. Method development

Ginsenoside Re, Rg1, Rh1 and digoxin (I.S.) were separated on UPLC BEH C_{18} column using a linear gradient of acetonitrile and ammonium formate (5 mM, pH 3.3). Using this UPLC/MS method, a method to determine three ginsenosides was developed for mouse serum sample, simultaneously. A gradient elution program was employed with 15% organic phase as the initial concentration of gradient program for retention time and peak shape for ginsenoside Re and Rg1. With the gradient program, the analysis of ginsenoside Re, Rg1, Rh1 and digoxin (I.S.) could be completed within 6.5 min. The retention times were about 2.07 min for ginsenosie Re, 2.08 min for Rg1, 3.21 min for Rh1 and 2.68 min for digoxin (I.S.), respectively (Fig. 2B).

The MS responses of ginsenoside Re, Rg1, Rh1 and digoxin (I.S.) to ESI were evaluated by measuring the full scan mass spectra in both positive and negative ionization modes. To obtain the maximum sensitivity, we investigated the effects of pH with various mobile phases on the ionization efficiency of analytes. The use of 10 mM ammonium acetate as mobile phase showed the maximum sensitivity of ginsenoside Re, Rg1, Rh1 and digoxin in positive mode. For all compounds, $[M+Na]^+$ was the most abundant adduct ion, but when target analytes in serum sample were monitored by $[M+Na]^+$ ion adduct, reproducibility was not satisfactory. In the negative mode, using of 5 mM ammonium formate adjusted to pH 3.3 as mobile phase, it can be observed that predominant formated adduct ions of ginsenoside Re, Rg1, and Rh1 were formed at *m/z* 991.5, 845.4 and 683.2, respectively. A higher signal of ginsenoside Re, Rg1, Rh1and I.S. were obtained when monitoring the adduct ions $[M+CO_2H]^-$, instead of $[M-H]^-$ in serum sample. The formated adduct ions of these analytes were selected as SIR monitoring ion to obtain the selectivity and sensitivity for the determination of ginsenosides. Digoxin was selected as internal standard because of its similarity of chemical structure, retention time, ionization and extraction efficiency at formated adducts ions of *m/z* 825.3.

Various sample treatment procedures were evaluated, including solid-phase extraction (SPE), protein precipitation and liquid–liquid extraction. Considering of recovery for all analytes, simple protein precipitation procedure was developed with methanol which yielded an over 80% extraction recovery for Ginsenoside Re, Rg1 and Rh1.

Table 1
Linear range, LLOQ, LLOD for ginsenoside Re, Rg1, Rh1.

Analytes	Linear range (ng/mL)	R^2	LLOQ ng/mL)	LLOD (ng/mL)
Ginsenoside Re	5.0–5000	0.9982	5.0	1.0
Ginsenoside Rg1	5.0–5000	0.9993	5.0	2.5
Ginsenoside Rh1	5.0–5000	0.9988	5.0	2.5

3.2. Method validation

The specificity of the method was demonstrated by comparing SIR chromatograms of Re, Rg1, Rh1 and the I.S. for a blank serum sample with a spiked serum sample. All the analytes and internal standard could be detected on their own selected ion chromatograms without any significant interference (Fig. 2A and B).

Ginsenoside Re, Rg1 and Rh1 showed a good linearity with a correlation coefficient (R^2) value greater than 0.998 as listed in Table 1. The lower limit of quantitation (LLOQ) and lower limits of detection (LLOD) of these analytes using 100 μL serum were 5 and 1.0–2.5 ng/mL, respectively. Table 2 summarized the intra- and the inter-day precisions and accuracies of ginsenoside Re, Rg1 and Rh1 at three concentration levels. As shown in Table 2, the intra- and inter-day accuracies of these analytes were within the range

Table 2
Accuracy and precision of ginsenoside Re, Rg1, Rh1 in ICR mice serum ($N=4$).

Spiked concentration (ng/mL)	Intra-day			Inter-day		
	Measured concentration (ng/mL)	Accuracy (%)	Precision RSD (%)	Measured concentration (ng/mL)	Accuracy (%)	Precision RSD (%)
Ginsenoside Re						
10.0	9.8 ± 0.4	97.5	3.9	9.7 ± 0.3	97.1	3.0
250.0	254.8 ± 9.7	101.9	3.8	248.8 ± 11.6	99.5	4.7
750.0	757.9 ± 17.9	101.1	2.4	753.8 ± 19.0	100.5	2.5
Ginsenoside Rg1						
10.0	9.6 ± 0.7	95.6	7.5	10.1 ± 0.7	101.0	7.0
250.0	247.4 ± 7.0	98.9	2.8	257.7 ± 12.8	103.0	5.0
750.0	752.2 ± 18.2	100.3	2.4	748.8 ± 12.4	99.8	1.7
Ginsenoside Rh1						
10.0	9.9 ± 0.7	99.3	7.0	9.5 ± 0.3	95.3	2.6
250.0	246.4 ± 9.9	98.6	4.0	250.4 ± 14.5	100.1	5.8
750.0	748.6 ± 11.8	99.8	1.6	748.2 ± 17.0	99.8	2.3

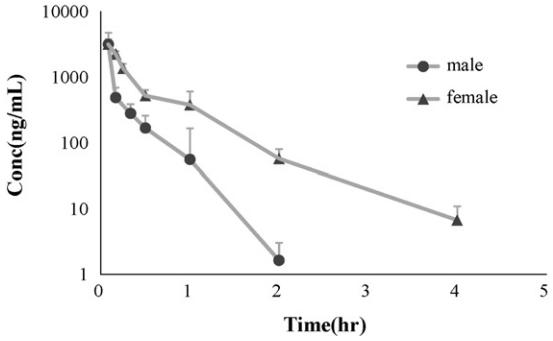


Fig. 3. Serum concentration–time curves of ginsenoside Re after intravenous administration of pure ginsenoside Re at a dose of 1 mg/kg. Values are mean ± SD (N = 4–5).

of 95.6–101.9% and 95.3–103.0%, respectively. The intra- and inter-day precisions were less than 7.5% for the respective QC samples.

The extraction recoveries for ginsenoside Re, Rg1, Rh1 were more than 80% and mean recovery were from 80.1% to 86.3%. The stability of ginsenoside Re, Rg1 and Rh1 in serum was also investigated. No significant changes of concentrations of analytes were detected after three freeze–thaw cycles and the storage period of 24 h at ambient temperature. The concentrations of analytes were still within 10% deviation of the initial values (data not shown).

3.3. *Pharmacokinetic studies of ginsenoside Re in mouse after intravenous and oral administration of pure ginsenoside Re and GB extract*

The developed and validated method was applied to the pharmacokinetic evaluation of ginsenoside Re in mice following intravenous and oral administration of ginsenoside Re or GB extract. To evaluate the pharmacokinetic character of intravenously administered a single dose of 1 mg/kg ginsenoside Re, serum samples were obtained after i.v. dosing in ICR mice. Ginsenoside Re was successfully determined, but no Rg1 and Rh1 above LLOD (2.5 ng/mL) were detected in serum after oral administrations of ginsenoside Re and oral administration of GB extract. Typical chromatogram of Re at the time of 0.5 h after i.v. administration is shown in Fig. 2C. The serum concentration–time profile of ginsenoside Re in male and female mice after i.v. administration is illustrated in Fig. 3. The pharmacokinetic parameters for i.v. administration are shown in Table 3. The systemic clearance was 1.7 ± 0.7 and 0.7 ± 0.11 L/h/kg for male and female, respectively. The volume of distribution in the terminal phase was 0.3 ± 0.2 and 0.2 ± 0.07 L/kg for male and female, respectively. From the profile, we could know that ginsenoside Re is rapidly eliminated from serum.

Fig. 4 shows the serum concentration–time profiles of ginsenoside Re after oral dose of pure ginsenoside Re or GB extract. As shown in Fig. 4A, after 10 mg/kg oral administration, ginsenoside Re was absorbed with C_{max} of 29.0 ± 25.4 ng/mL and AUC_{0-t} of 17.7 ± 4.5 ng h/mL with pure ginsenoside Re. C_{max}

Table 3
Pharmacokinetic parameters of ginsenoside Re after intravenous(1 mg/kg) administration to ICR mice.

i.v. (1 mg/kg)		
Parameter	Male	Female
$AUC_{(0-t)}$ (ng/h/mL)	638.8 ± 197.0	1437.6 ± 271.2
$AUC_{0-\infty}$ (ng/h/mL)	639.3 ± 196.8	1442.0 ± 271.0
$t_{1/2}$ (h)	0.2 ± 0.03	0.5 ± 0.08
MRT (h)	0.2 ± 0.07	0.5 ± 0.08
Vd (L/kg)	0.3 ± 0.2	0.2 ± 0.07
CL (L/h/kg)	1.7 ± 0.7	0.7 ± 0.11

Data are expressed as mean ± SD (N = 4–5).

Table 4
Pharmacokinetic parameters of ginsenoside Re and GB extract after oral(10, 50 mg/kg) administration to ICR mice.

Oral (10 mg/kg)			Oral (50 mg/kg)	
Parameter	Ginsenoside Re	GB extract	Ginsenoside Re	GB extract
T_{max} (h)	0.4 ± 0.2	0.3 ± 0.13	0.7 ± 0.7	0.5 ± 0.3
C_{max} (ng/mL)	29.0 ± 25.4	21.3 ± 10.1	35.0 ± 4.3	124.1 ± 127.9*
$AUC_{(0-t)}$ (ng/h/mL)	17.7 ± 4.5	21.3 ± 19.9	61.5 ± 37.0	238.3 ± 64.2**
MRT (h)	0.76 ± 0.20	1.06 ± 0.56	2.0 ± 1.2	4.0 ± 2.0
F%	0.28	0.33	0.19	0.75

Data are expressed as mean ± SD (N = 7). Difference from corresponding ginsenoside Re group, *P < 0.1, **P < 0.05

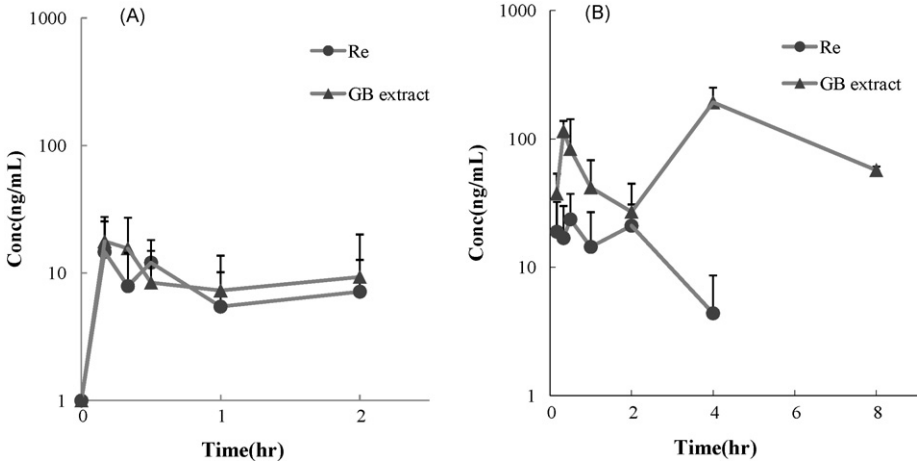


Fig. 4. (A) Serum concentration-time curves of ginsenoside Re after oral administration of pure ginsenoside Re and GB extract at a dose of 10 mg/kg (B) Serum concentration-time curves of ginsenoside Re after oral administration of pure ginsenoside Re and GB extract at a dose of 50 mg/kg. Values are mean ± SD (N = 7).

and AUC_{0-t} after oral administrations for GB extract 90.5 mg/kg (~10 mg/kg for Re) was 21.3 ± 10.1 ng/mL and 21.3 ± 19.9 ng/h/mL, respectively (Table 4). After the oral dose of 50 mg/kg, the mean maximum serum concentration of ginsenoside Re of pure Re was 35.0 ± 4.3 ng/mL and AUC_{0-t} was 61.5 ± 37.0 ng/h/mL. Interestingly, C_{max} and AUC_{0-t} after oral dose of 452.5 mg/kg (50 mg/kg for Re) of GB extract were 124.1 ± 127.9 ng/mL and 238.3 ± 64.2 ng/h/mL, significantly higher than those obtained after 50 mg/kg pure Re administration. As shown in Fig. 4B and Table 4, T_{max} was shorter and C_{max} was higher in GB extract administered group and it could be inferred that the dissolution of ginsenoside Re might be facilitated by co-existing component of GB extract when compared with pure Re preparation. Indeed, dissolution of pure ginsenoside Re and GB extract suspension in artificial intestinal fluid indicated higher solubility of ginsenoside Re in GB extract (recovery %, 51.9 ± 1.9 for pure ginsenoside Re vs. 98.9 ± 2.0 for GB extract) and resultant superior absorption, especially at high doses.

4. Conclusions

In the present study, a rapid and sensitive UPLC–ESI–MS method for the determination of ginsenoside Re, Rg1 and Rh1 in mouse serum was developed and validated. A simple protein precipitation procedure was used for sample clean-up procedure which yielded a good recovery, ranging over 80%. This method was proved to be selective, precise, accurate and reliable for the simultaneous determination of ginsenoside Re, Rg1 and Rh1 within 6.5 min of single chromatographic run. Furthermore, our method has been successfully applied to the pharmacokinetic study of ginsenoside Re in mouse, which showed very short half-life of 0.2 ± 0.03 h and low oral bioavailability ranging from 0.19% to 0.28% for pure ginsenoside Re. Most importantly, we proved that oral ingestion of GB extract can exhibit a significantly higher absorption of ginsenoside Re (0.33–0.75%), substantiating that whole herbal extract might be advantageous than isolated natural ingredients for the herbal medicine or alternative medicine.

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