



Research article

Effects of processing method on the pharmacokinetics and tissue distribution of orally administered ginseng



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ABSTRACT

Background: The use of different methods for the processing of ginseng can result in alterations in its medicinal properties and efficacy. White ginseng (WG), frozen ginseng (FG), and red ginseng (RG) are produced using different methods. WG, FG, and RG possess different pharmacological properties.

Methods: WG, FG, and RG extracts and pure ginsenosides were administered to rats to study the pharmacokinetics and tissue distribution characteristics of the following ginsenosides—Rg1, Re, Rb1, and Rd. The concentrations of the ginsenosides in the plasma and tissues were determined using UPLC-MS/MS. **Results:** The rate and extent of absorption of Rg1, Re, Rb1, and Rd appeared to be affected by the different methods used in processing the ginseng samples. The areas under the plasma drug concentration-time curves (AUCs) of Rg1, Re, Rb1, and Rd were significantly higher than those of the pure ginsenosides. In addition, the AUCs of Rg1, Re, Rb1, and Rd were different for WG, FG, and RG. The amounts of Rg1, Re, Rd, and Rb1 were significantly ($p < 0.05$) higher in the tissues than those of the pure ginsenosides. The amounts of Re, Rb1, and Rd from the RG extract were significantly higher than those from the WG and FG extracts in the heart, lungs, and kidneys of the rats.

Conclusion: Our results show that the use of different methods to process ginseng might affect the pharmacokinetics and oral bioavailability of ginseng as well as the tissue concentrations of Rg1, Re, Rd, and Rb1.

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1. Introduction

Panax ginseng is a traditional herbal medicine, which has been used in China for more than 2,000 years. The medicinal properties and efficacy of ginseng vary depending on the method used for processing it. This is because the different processing methods result in the production of different chemical compounds [1–3]. Ginseng is commercially available in the herbal market as a fresh, white, red, frozen, or other processed product. Fresh ginseng is prepared by simply washing ginseng, whereas white ginseng (WG) is usually prepared by sun-drying ginseng. However, red ginseng (RG) is prepared by steaming fresh ginseng at 90–100°C for a reasonable time, followed by drying until the moisture content is less than 15%. Frozen ginseng (FG) is produced by freeze-drying ginseng [4,5]. FG is also called “active *Panax ginseng*” because the active ingredients in the fresh ginseng are retained after the freeze-drying process. RG is reported to have better pharmacological properties than WG. This is

because during the steam treatment of ginseng, changes that occur in the chemical constituents enhance the biological activities of the plant [6–8]. Many new ginsenosides, such as Rh1(R), Rg3(s), and Rg5, have been detected in RG but not in WG and FG. Ginsenosides are the major active compounds in ginseng. They play very important roles as bioactive compounds and their anticancer, antiviral, and antioxidant activities have been studied [9,10]. There have been many reports on the pharmacokinetics and tissue distribution characteristics of ginsenosides using HPLC-diode array detection (DAD), evaporative light scattering detection, UV and fluorescence detections, micellar electrokinetic chromatography, LC-MS, HPLC-MS/MS [11], and UPLC-MS/MS [12]. However, the aforementioned studies paid more attention to pure ginsenosides and neglected interactions among ginsenosides and other components of ginseng. The effects of an herbal medicine are usually attributed to the synergistic effects of two or more herbs in the product because the constituent compounds usually interact with each other. The

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pharmacokinetics and tissue distribution characteristics of the ginsenosides in WG, FG, and RG are unclear; however, the differences in the pharmacological activities of the three ginseng products may be attributed to these characteristics. Currently, there are no reports on the effects of different ginseng processing methods on the pharmacokinetics, bioavailabilities, and tissue distribution characteristics of ginsenosides. Because of the complex nature of the chemicals in ginseng, one or several representative ginsenosides may be chosen as markers for investigating the pharmacokinetics of ginseng [13,14,21]. This allows for interactions among components to be clarified based on the selected compounds. The most abundant saponins in ginseng are ginsenosides Rg1, Re, Rb1, and Rd. The effects of the four compounds on the pharmacological activities of ginseng have been well demonstrated [15–17]. Therefore, in the present study, ginsenosides Rg1, Re, Rb1, and Rd were chosen as the marker compounds to study the effects of different processing methods on the pharmacological activities of ginseng.

The aim of this study was to explore whether different ginseng processing methods affect the pharmacokinetics, bioavailabilities, and tissue distribution characteristics of ginsenosides Rg1, Re, Rb1, and Rd. It is expected that the results of this study would be useful in improving the clinical applications of processed ginseng products.

2. Materials and methods

2.1. Materials

WG, RG, and FG were purchased from Tongrentang Traditional Pharmacy and Clinic (Changchun, China). To obtain RG, fresh ginseng roots were washed and then steamed in a closed chamber at 98°C for 3 h. After steaming, the steamed ginseng was dried in a chamber (65°C) until the moisture content was < 12%. To obtain WG, the fresh ginseng was dried by sun-drying until the moisture content was < 12%. To obtain FG, the fresh ginseng was dried by freeze-drying under –80°C until the moisture content was < 12% [4,5]. Rg1, Rb1, Re, Rd, and digoxin standards (Fig. 1) were obtained from Yuanye Biological Technology Co., Ltd. (Shanghai, China). Acetonitrile and methanol were obtained from Fisher Scientific

(Pittsburgh, PA, USA). Water was purified by redistillation and filtered before use with a 0.22- μ m membrane filter.

2.2. Preparation of WG, RG, and FG extracts and determination of Rg1, Rb1, Re, and Rd contents in the extracts

About 200 g of each ginseng sample was separately decocted in 70% ethanol (500 mL) overnight. The solution obtained was filtered and concentrated to 20 mL under reduced pressure at 60°C. The amounts of Rg1, Rb1, Re, and Rd in WG (6.8 mg/mL, 33.5 mg/mL, 12.5 mg/mL, and 3.5 mg/mL, respectively), RG (2.5 mg/mL, 8.6 mg/mL, 4.5 mg/mL, and 4.2 mg/mL, respectively), and FG (17.9 mg/mL, 45.5 mg/mL, 11.5 mg/mL, and 10.2 mg/mL, respectively) were determined by UPLC-DAD. The extracts were kept at 4°C for further studies.

2.3. Animals

Male Sprague Dawley rats (240–260 g) were provided by Yisi Laboratory Animal Technology Co. Ltd. (Changchun, China). The rats were maintained under a 12/12 h light/dark cycle at a temperature of 22–25°C and a relative humidity of 50–60%. The animals were allowed free access to food and water but were fasted 12 h prior to the experiment with free access to water. The animal experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals, and the study protocol was reviewed.

2.4. Instrumentation and analytical conditions

Chromatographic separation was performed using a UPLC-MS/MS system (Xevo TQ; Waters, 34 Maple Street, Milford, MA 01757, US) with an analytical column (ACQUITY UPLC BEH; C18, 1.7 μ m, 2.1 \times 50 mm; Waters) at 35°C. Water (A) and acetonitrile (B) were used as the mobile phase, which was run at a flow rate of 0.5 mL/min. The automatic sampler was set at a temperature of 4°C. A linear gradient elution was carried out as follows: 18% B at 0–2.0 min; 20%→40% B at 2.0–4.5 min; and 40%→58% B at 4.5–7.5 min. The mass spectrometer was operated in positive ionization mode using

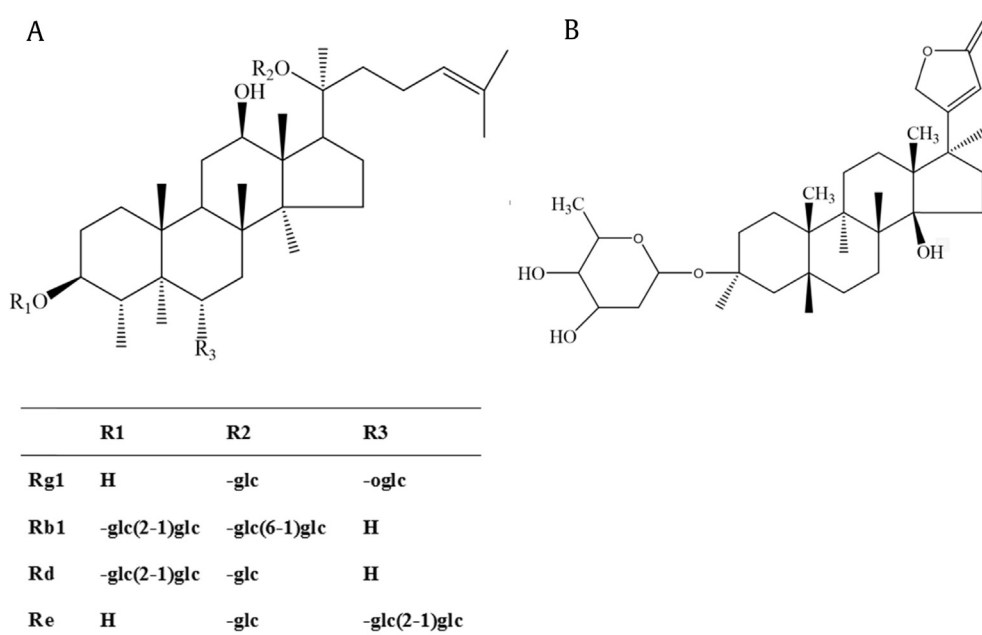


Fig. 1. Chemical structures. (A) Rg1, Rb1, Re, and Rd. (B) Internal standard digoxin.

multiple reaction monitoring (MRM) to assess the four ginsenosides and digoxin (internal standard, IS) as follows: m/z 823.35 \rightarrow 643.29 for Rg1, m/z 1,131.38 \rightarrow 365.03 for Rb1, m/z 969.39 \rightarrow 789.3 for both Re and Rd and m/z 803.27 \rightarrow 283.09 for digoxin (IS).

2.5. Preparation of plasma and tissue samples

The rats were randomly divided into 13 groups (5 rats per group). A mixture of pure Rg1, Rb1, Re, and Rd dissolved in water and decoctions of WG, RG, and FG were orally administered to the rats. Rg1, Rb1, Re, and Rd were administered at doses of 25 mg/kg, 86 mg/kg, 45 mg/kg, and 42 mg/kg, respectively. Blood samples (0.5 mL) were collected by retro-orbital bleeding into heparinized 1.5-mL tubes at 0.083 h, 0.25 h, 0.5 h, 1 h, 1.5 h, 2 h, 4 h, 6 h, 8 h, 12 h, 24 h, and 36 h after oral drug administration. Immediately after collection, the blood samples were centrifuged at 18,000 g for 10 min to obtain plasma. A 0.15-mL aliquot of plasma was then withdrawn and diluted with 0.3 mL of acetonitrile and 20 μ L of the IS (6 μ g/mL). The mixture was centrifuged at 18,000 g for 10 min at 4°C after 30 min. The supernatant was evaporated to dryness under a stream of N₂ at 50°C and 0.1 mL of methanol was used to dissolve the residue obtained. The samples were then stored at -20°C until analysis. The plasma concentrations of Rg1, Rb1, Re, and Rd were determined, and each was expressed as mean \pm standard deviation (SD). The mean plasma drug concentration-time curve for each ginsenoside was then plotted. All of the data were processed by non-compartmental analysis using the WinNonlin software (version 6.1; Pharsight Inc., Mountain View, CA, USA).

For the study on the brain concentration of the ginsenosides, the rats were randomly divided into 12 groups as was done in Section 2.5. The tissue samples were collected at 30 min after the administration of WG, FG, RG, and pure compounds to the rats. Next, four rats were sacrificed, and samples of their tissues were carefully collected and washed with sodium phosphate buffer (pH 7.4) to remove blood. Each tissue (0.5 g) was homogenized in normal saline (1 mL), after which 1 mL of acetonitrile and 20 μ L of the IS (6 μ g/mL) were added. The mixture was centrifuged at 18,000 g for 10 min at 4°C. A 1-mL aliquot of the supernatant was evaporated to dryness under a stream of N₂ at 50°C. The residue was dissolved in 0.1 mL of methanol, and the solution obtained was stored at -20°C until analysis.

2.6. Preparation of quality control and calibration standards

Stock standard solutions of Rg1, Rb1, Re, and Rd were prepared by dissolving the reference compound in methanol to obtain a final concentration of 2.0 μ g/mL for Rg1, 10 μ g/mL for Rb1, 2.0 μ g/mL for Rd, and 2 μ g/mL for Re. Each stock solution was then diluted with methanol to obtain five working solutions over the following concentration ranges: 0.1–2 μ g/mL for Rg1, 0.1–1 μ g/mL for Re, 2–10 μ g/mL for Rb1, and 0.1–2 μ g/mL for Rd. The IS solution was digoxin dissolved in methanol at a concentration of 1 μ g/mL. All the solutions were stored at 4°C. Low, medium, and high quality control (QC) samples were similarly prepared.

2.7. Method validation

The UPLC-MS/MS method was validated for assaying the ginsenosides in rat plasma and tissue samples by determining the linearity, precision, accuracy, recovery, stability, and lower limit of quantification (LLOQ) of the method. Linearity was studied in rat plasma over the following concentration ranges: 0.1–2 μ g/mL for Rg1, 0.1–1 μ g/mL for Re, 2–10 μ g/mL for Rb1, and 0.1–2 μ g/mL for Rd. By contrast, linearity of the method in the rat tissues was studied over the following concentration ranges: 0.01–0.2 μ g/mL

for Rg1, 0.01–0.1 μ g/mL for Re, 0.2–1 μ g/mL for Rb1, and 0.01–0.2 μ g/mL for Rd. The LLOQ was evaluated as the lowest amount of analyte at which the signal-to-noise ratio was 10. The intra- and interday precision and accuracy of the method were determined as the relative standard deviation of the concentrations of the QC samples. The absolute extraction recoveries of the analytes from the rat plasma and tissues were also studied. These were estimated by comparing the analyte concentrations obtained from spiked extracts and the respective analyte concentrations obtained from the standard solutions. The stability of each ginsenoside during storage was evaluated by determining the concentrations of five replicates of each QC plasma and tissue sample stored at -80°C for 1 mo. In addition, the effects of freezing and thawing on analyte stability were evaluated by determining the concentrations of QC plasma and brain samples after subjecting the samples to three freeze (-80°C)-thaw (room temperature) cycles. Lastly, the stability of each sample after its preparation was evaluated by determining the concentrations of extracted QC samples stored in the automatic sampler at 15°C for 24 h.

2.8. Application of the method in a pharmacokinetic study

The UPLC-MS/MS method was used to determine the concentrations of Rg1, Re, Rb1, and Rd in rat plasma and tissues after oral administration of WG, FG, RG, and pure ginsenoside compounds to rats. The WinNonlin software (Phoenix, version 6.1; Pharsight Inc., Mountain View, CA, USA) was used to calculate the following pharmacokinetic parameters: C_{max} (peak plasma concentration), T_{max} (time at C_{max}), $AUC_{0-36 h}$ (AUC from time 0 h to 36 h), $AUC_{0-\infty}$ (AUC from time 0 h to infinity), mean residence time (MRT), clearance rate (CL/F), and $t_{1/2}$ (elimination half-life).

3. Results

3.1. Method validation

The interference of analyte elution by the components of plasma was assessed by inspecting the chromatograms of blank plasma samples. Representative chromatograms of blank plasma, plasma spiked with the standard compounds and the IS, and plasma after oral administration of the WG, FG, and RG extracts and pure ginsenoside compounds to the rats are presented in Fig. 2. The retention times of Rg1, Re, Rb1, Rd, and the IS were approximately 1.88 min, 1.89 min, 3.03 min, 3.56 min, and 2.56 min, respectively. Observation of the MRM chromatograms showed that the developed UPLC-MS/MS method is highly sensitive and specific for the compounds studied. The chromatograms for Rg1, Re, Rb1, and Rd in rat plasma and tissues showed low baseline noise and no interfering peaks (Fig. 2). In addition, the method showed good linearity over the concentration ranges studied as the correlation coefficients (R^2) obtained were > 0.99 for all the rat plasma and tissue samples (Table 1).

For the plasma samples, the intra- and interday precisions were found to be $< 8.9\%$, accuracy was 91.3–105.3%, and the extraction recovery was $> 80.2\%$ (Tables 2 and 3). The LLOQs were found to be 1.0 ng/mL, 1.0 ng/mL, 5.0 ng/mL, and 2.5 ng/mL for Rg1, Re, Rb1, and Rd, respectively (Table 1). For the tissue samples, the intra- and interday precisions were found to be $< 9.8\%$, accuracy was 90.6–105.2%, and the extraction recovery was $> 75.9\%$ (Tables 2 and 3). The LLOQs were obtained as 1.5 ng/g, 0.5 ng/g, 2.5 ng/g, and 1.0 ng/g for Rg1, Re, Rb1, and Rd, respectively (Table 1). The stability results are shown in Table 4. The results obtained showed that Rg1, Re, Rb1, and Rd were stable in rat plasma and the various tissue homogenates during sample extraction and storage.

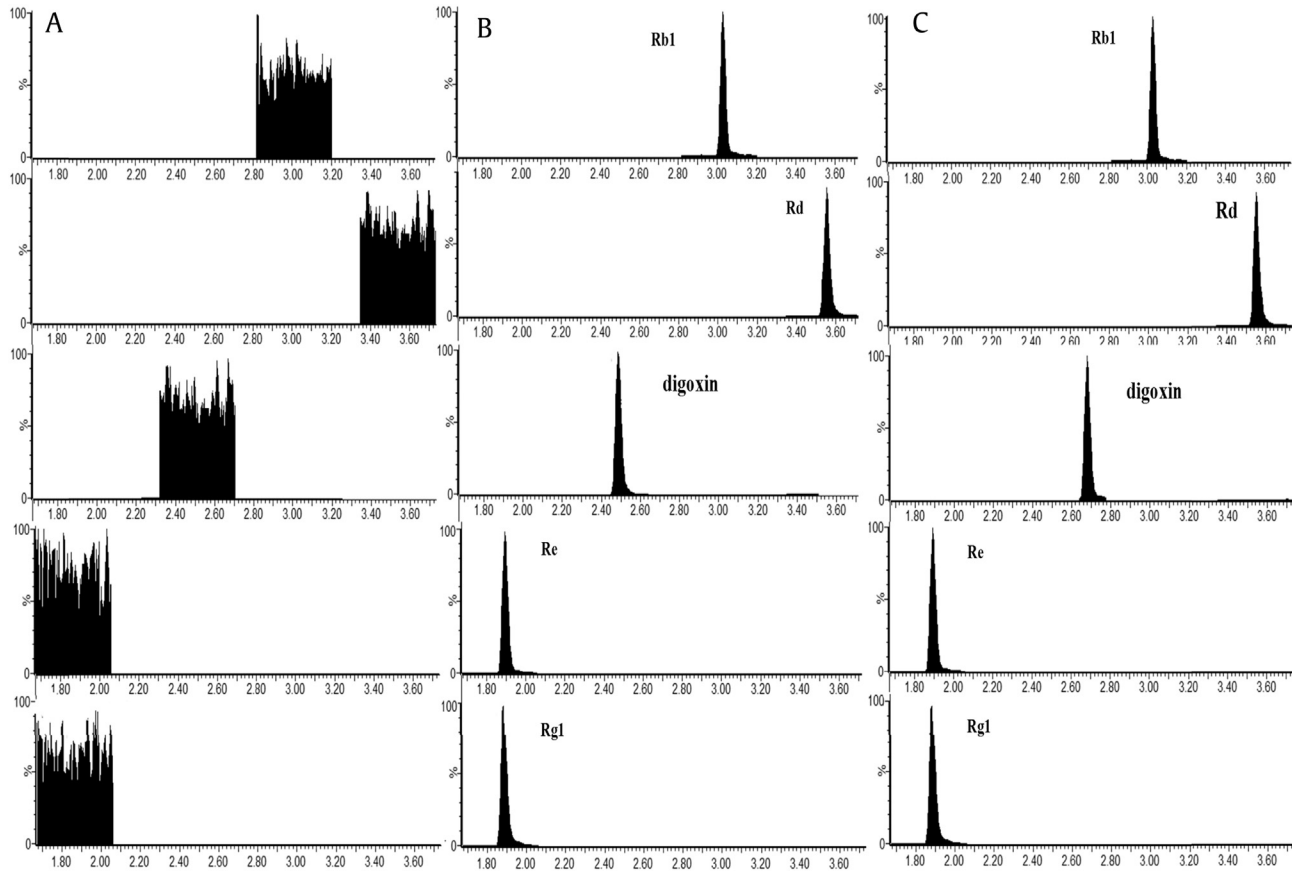


Fig. 2. Typical multiple reaction monitoring chromatograms. (A) Blank plasma. (B) Blank plasma spiked with standard compounds Rg1, Rb1, Re, and Rd and digoxin (internal standard). (C) Plasma sample 15 min following oral administration of white ginseng.

Table 1
Regression data and LLOQ of the analytes determined ($n = 6$)

| Composition | | Linear regression equation | R^2 | Linear range ($\mu\text{g/mL}$) | LLOQ (ng/mL) |
|-------------|-----|----------------------------|-------|-----------------------------------|--------------|
| Plasma | Rg1 | $Y = 0.312x + 0.05$ | 0.998 | 0.1–2.0 | 1.0 |
| | Re | $Y = 0.207x + 0.22$ | 0.999 | 2.0–10.0 | 1.0 |
| | Rb1 | $Y = 0.199x + 0.06$ | 0.995 | 0.1–1.0 | 5.0 |
| | Rd | $Y = 0.114x + 0.12$ | 0.999 | 0.1–2.0 | 2.5 |
| Tissue | Rg1 | $Y = 0.023x + 0.111$ | 0.997 | 0.01–0.2 | 1.5 |
| | Re | $Y = 0.014x - 0.121$ | 0.995 | 0.2–1.0 | 0.5 |
| | Rb1 | $Y = 0.004x + 0.022$ | 0.997 | 0.01–0.1 | 2.5 |
| | Rd | $Y = 0.013x - 0.054$ | 0.996 | 0.01–0.2 | 1.0 |

LLOQ, lower limit of quantification

3.2. Determination of Rg1, Re, Rd, and Rb1 in plasma

The mean plasma drug concentration-time profiles for Rg1, Re, Rd, and Rb1 after orally administering WG, RG, and FG extracts and free ginsenosides to the rats are illustrated in Fig. 3. The pharmacokinetic parameters obtained from the profiles are presented in Table 5. The plasma concentration-time curves for Rg1, Re, and Rd show atypical bimodal phenomena (Figs. 3A, 3B, and 3D). The first and second peaks occurred at about 15–30 min and 8–12 h, respectively, for each analyte. However, only a single peak was observed at about 8–10 h for Rb1 in Fig. 3C. The data obtained from the pharmacokinetic study show that the different processing methods affected the absorption rates and extents of Rg1, Re, Rb1, and Rd from the extracts. The $AUC_{0-36\text{ h}}$ values obtained for the ginsenosides from the extracts were higher (Rg1: 1.5–4.6 times,

Table 2
Accuracy and precision of the analytes in blank plasma

| Compound | Spiked ($\mu\text{g/mL}$) | Intraday ($n = 5$) | | Interday ($n = 5$) | | | |
|----------|-----------------------------|----------------------|----------------|----------------------|----------------|-------|-----|
| | | Accuracy (%) | Precision RSD% | Accuracy (%) | Precision RSD% | | |
| Plasma | Rg1 | 0.5 | 101.0 | 7.2 | 102.3 | 3.6 | |
| | | 1.0 | 91.4 | 4.8 | 101.2 | 5.4 | |
| | | 2.0 | 97.2 | 8.4 | 95.6 | 4.8 | |
| | | 5.0 | 96.5 | 6.8 | 103.6 | 5.6 | |
| | Rb1 | 10 | 102.2 | 5.0 | 95.8 | 4.8 | |
| | | 0.1 | 103.6 | 5.9 | 91.3 | 4.7 | |
| | | 0.5 | 93.4 | 4.7 | 97.6 | 5.3 | |
| | | 1.0 | 95.6 | 7.8 | 96.3 | 2.1 | |
| | Rd | 0.1 | 93.3 | 4.6 | 98.5 | 2.8 | |
| | | 1.0 | 94.2 | 8.9 | 102.3 | 6.8 | |
| | | 2.0 | 98.3 | 5.8 | 105.3 | 6.5 | |
| | | 5.0 | 96.5 | 6.8 | 103.6 | 5.6 | |
| | Tissue | Rg1 | 0.01 | 94.2 | 6.5 | 105.2 | 7.5 |
| | | | 0.05 | 95.2 | 9.7 | 93.9 | 5.6 |
| 0.2 | | | 92.3 | 5.6 | 98.3 | 6.3 | |
| 0.2 | | | 105.2 | 6.9 | 104.3 | 3.2 | |
| Rb1 | | 0.5 | 103.2 | 5.9 | 106.3 | 4.5 | |
| | | 1.0 | 102.3 | 6.9 | 104.6 | 5.6 | |
| | | 0.01 | 90.6 | 7.8 | 98.3 | 6.3 | |
| | | 0.05 | 94.5 | 9.8 | 103.5 | 6.2 | |
| Re | | 0.2 | 96.5 | 2.6 | 105.4 | 2.5 | |
| | | 0.2 | 102.3 | 2.9 | 96.5 | 2.1 | |
| | | 0.5 | 103.6 | 3.6 | 98.4 | 1.9 | |
| | | 1.0 | 99.6 | 4.5 | 98.6 | 3.6 | |

RSD, relative standard deviation

Table 3
Extraction recovery of the analytes in rat plasma and various tissue homogenates ($n = 3$)

| Sample | Compound | Spiked ($\mu\text{g/mL}$) | Extraction recovery (%) | RSD (%) |
|--------|----------|-----------------------------|-------------------------|---------|
| Plasma | Rg1 | 0.5 | 84.5 | 8.6 |
| | | 1.0 | 81.2 | 6.4 |
| | | 2.0 | 87.2 | 8.8 |
| | Rb1 | 2.0 | 90.1 | 7.2 |
| | | 5.0 | 87.5 | 10.6 |
| | | 10 | 85.2 | 8.8 |
| | Re | 0.1 | 84.2 | 11.7 |
| | | 0.5 | 87.2 | 7.3 |
| | | 1.0 | 90.3 | 9.1 |
| | Rd | 0.1 | 88.2 | 6.8 |
| | | 1.0 | 87.5 | 9.8 |
| | | 2.0 | 80.2 | 7.5 |
| Tissue | Rg1 | 0.01 | 81.3 | 5.5 |
| | | 0.05 | 78.9 | 9.6 |
| | | 0.2 | 84.5 | 10.3 |
| | Rb1 | 0.2 | 87.3 | 11.2 |
| | | 0.5 | 79.5 | 12.5 |
| | | 1.0 | 82.8 | 9.6 |
| | Re | 0.01 | 86.3 | 8.3 |
| | | 0.05 | 75.9 | 8.2 |
| | | 0.2 | 89.6 | 7.5 |
| | Rd | 0.2 | 84.5 | 9.1 |
| | | 0.5 | 85.5 | 8.9 |
| | | 1.0 | 88.6 | 9.6 |

RSD, relative standard deviation

$p < 0.05$; Re: 1.1–5.4 times, $p < 0.05$; Rb1: 1.4–2.0 times, $p > 0.05$; and Rd, 1.1–1.5 times, $p < 0.05$) than those obtained for the respective pure ginsenosides (Table 5). In addition, the results showed that the AUCs for Rg1, Re, Rb1, and Rd from the WG, FG, and RG extracts were different from each other for each respective pair compared. The T_{max} values for Rg1, Re, Rb1, and Rd were lower in rats that were administered the RG extract than the respective values in rats that were administered the FG or WG extract or the pure ginsenosides. As shown in Table 5, the free Re, Rb1, and Rd

compounds were more rapidly eliminated than the respective ginsenosides from the RG, FG, and WG extracts. The results indicate that the RG, FG, and WG extracts may require multiple-dose administration to achieve the desired therapeutic efficacies of the extracts. The $t_{1/2}$ values for Re, Rb1, and Rd after administering the RG, FG, and WG extracts were two- to three-fold longer ($p < 0.05$) than those for the respective free compounds. Additionally, the MRT values obtained for the ginsenosides from the RG, FG, and WG extracts were higher ($p < 0.05$) than those obtained for the respective free compounds.

3.3. Tissue distribution characteristics of Rg1, Re, Rd, and Rb1

The distribution of Rg1, Re, Rd, and Rb1 in various tissues, including the heart, liver, spleen, lungs, kidneys, brain, muscle, and testes, at 30 min after oral administration of the RG, WG, and FG extracts and free ginsenosides to the rats are shown in Fig. 4. The results obtained indicated that all the analytes were widely distributed in the aforementioned tissues. As shown in Fig. 4, Rg1 was extensively distributed in the heart, lungs, and kidneys; Re was mainly distributed in the heart and lungs; Rb1 was mainly distributed in the heart; and Rd was mainly distributed in the kidneys. Rg1, Re, Rd, and Rb1 from the RG, WG, and FG extracts were more distributed in the tissues ($p < 0.05$) than the free ginsenosides. The levels of Re, Rb1, and Rd in the heart, lungs, and kidneys were significantly higher for the RG extract than those for the WG and FG extracts for the respective ginsenosides. The mean concentrations of the four ginsenosides in the brain were 10–15-fold lower than the corresponding ginsenosides in the plasma. This poor permeation of the ginsenosides into the brain may be because of the blood–brain barrier.

4. Discussion

Ginsenosides are the major active compounds in ginseng. Pharmacokinetic and tissue distribution studies of drugs are important aspects of the modernization of traditional Chinese

Table 4
Stability of the analytes under different conditions

| Sample | Compound | Spiked ($\mu\text{g/mL}$) | Accuracy (mean \pm SD,%) | | | | |
|--------|----------|-----------------------------|----------------------------|---------------------|-----------------------|----------------------------|-----------------|
| | | | Short-term stability | Long-term stability | Freeze–thaw stability | Post-preparative stability | |
| Plasma | Rg1 | 0.5 | 102.0 \pm 6.4 | 103.7 \pm 8.0 | 106.1 \pm 3.4 | 93.6 \pm 6.3 | |
| | | 1.0 | 93.2 \pm 7.0 | 103.1 \pm 9.4 | 107.0 \pm 8.2 | 105.1 \pm 6.2 | |
| | | 2.0 | 92.1 \pm 8.6 | 94.6 \pm 5.6 | 94.3 \pm 4.0 | 94.8 \pm 7.5 | |
| | Rb1 | 2.0 | 91.3 \pm 5.3 | 95.6 \pm 7.7 | 96.2 \pm 8.5 | 94.4 \pm 4.0 | |
| | | 5.0 | 91.5 \pm 6.8 | 106.6 \pm 6.8 | 103.3 \pm 6.0 | 102.0 \pm 7.5 | |
| | | 10 | 94.4 \pm 7.9 | 93.9 \pm 8.5 | 98.5 \pm 8.2 | 94.5 \pm 8.0 | |
| | Re | 0.1 | 102.5 \pm 9.0 | 96.6 \pm 4.1 | 93.3 \pm 6.2 | 94.7 \pm 7.5 | |
| | | 0.5 | 94.4 \pm 4.4 | 98.7 \pm 5.0 | 97.5 \pm 7.0 | 95.7 \pm 6.0 | |
| | | 1.0 | 97.6 \pm 3.5 | 92.2 \pm 7.4 | 99.0 \pm 7.4 | 92.1 \pm 6.5 | |
| | Rd | 0.1 | 92.3 \pm 4.3 | 93.6 \pm 8.5 | 92.5 \pm 7.7 | 108.7 \pm 7.8 | |
| | | 1.0 | 94.6 \pm 5.2 | 102.3 \pm 9.6 | 106.5 \pm 6.5 | 96.8 \pm 8.0 | |
| | | 2.0 | 105.3 \pm 6.0 | 102.5 \pm 2.3 | 104.3 \pm 8.6 | 96.3 \pm 8.5 | |
| | Tissue | Rg1 | 0.01 | 92.2 \pm 2.3 | 101.9 \pm 5.3 | 100.2 \pm 3.6 | 103.2 \pm 4.8 |
| | | | 0.05 | 102.5 \pm 3.1 | 94.7 \pm 7.4 | 93.5 \pm 9.1 | 95.6 \pm 7.5 |
| | | | 0.2 | 92.3 \pm 6.6 | 92.3 \pm 7.5 | 95.3 \pm 6.6 | 96.2 \pm 8.9 |
| Rb1 | | 0.2 | 102.4 \pm 5.1 | 104.5 \pm 8.6 | 108.5 \pm 6.0 | 102.2 \pm 4.5 | |
| | | 0.5 | 101.1 \pm 7.0 | 108.1 \pm 6.7 | 109.2 \pm 8.1 | 94.5 \pm 7.5 | |
| | | 1.0 | 105.0 \pm 5.1 | 101.0 \pm 8.7 | 103.7 \pm 7.0 | 95.0 \pm 8.9 | |
| Re | | 0.01 | 92.2 \pm 8.7 | 93.2 \pm 5.8 | 92.7 \pm 8.0 | 106.7 \pm 7.8 | |
| | | 0.05 | 96.6 \pm 2.8 | 105.5 \pm 9.4 | 103.5 \pm 5.5 | 104.6 \pm 5.6 | |
| | | 0.2 | 92.4 \pm 4.8 | 103.8 \pm 6.5 | 100.7 \pm 7.4 | 92.9 \pm 3.5 | |
| Rd | | 0.2 | 107.2 \pm 7.3 | 95.5 \pm 5.6 | 96.5 \pm 8.4 | 103.0 \pm 8.5 | |
| | | 0.5 | 106.4 \pm 8.5 | 92.0 \pm 9.4 | 95.7 \pm 9.5 | 102.9 \pm 7.7 | |
| | | 1.0 | 93.8 \pm 5.1 | 94.4 \pm 7.0 | 99.6 \pm 3.4 | 93.5 \pm 8.0 | |

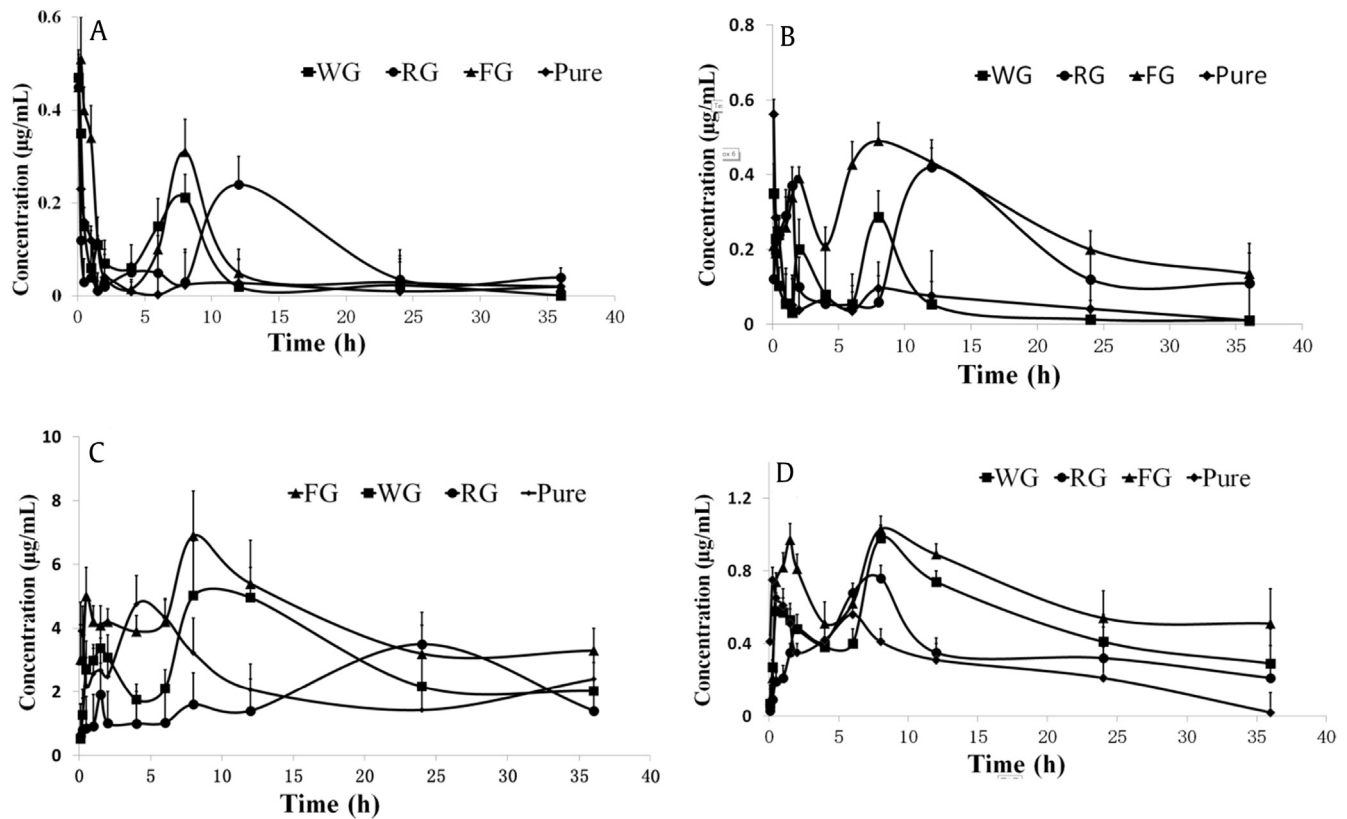


Fig. 3. Mean plasma concentration-time profiles of ginsenosides. (A) Rg1. (B) Re. (C) Rb1. (D) Rd following oral administration of different preparations to rats. FG, frozen ginseng; RG, red ginseng; WG, white ginseng.

Table 5

Pharmacokinetic parameters of ginsenosides Rg1, Re, Rb1, and Rd in rat plasma following oral administration of different preparations

| Compound | Ginseng | C _{max} (ng/mL) | T _{max} (h) | T _{1/2} (h) | AUC _{0–36 h} (g min/mL) | AUC _{0–∞} (g min/mL) | MRT (h) | CL/F (L/min/kg) |
|----------|---------|-----------------------------|-------------------------|-------------------------|-------------------------------------|----------------------------------|-------------|--------------------|
| Rg1 | WG | 0.5 ± 0.12 | 0.08 ± 0.03 | 32.2 ± 4.1* | 1.2 ± 0.7* | 1.8 ± 0.8* | 8.9 ± 2.9* | 32.2 ± 5.8* |
| | RG | 0.5 ± 0.11 | 12.8 ± 2.1 | 9.2 ± 1.5* | 3.7 ± 1.2* | 4.2 ± 1.3* | 12.6 ± 5.5* | 16.8 ± 5.5* |
| | FG | 0.5 ± 0.12 | 0.25 ± 0.06* | 18.5 ± 4.2 | 3.1 ± 1.9* | 3.8 ± 1.8* | 18.8 ± 7.1 | 19.1 ± 7.8 |
| | PG | 0.5 ± 0.14 | 0.08 ± 0.02 | 7.5 ± 4.2 | 0.84 ± 0.2 | 1.2 ± 0.3 | 10.9 ± 5.1 | 44.5 ± 9.8 |
| Re | WG | 0.3 ± 0.07* | 0.08 ± 0.02 | 6.5 ± 1.2* | 2.2 ± 0.4* | 2.9 ± 0.5* | 9.1 ± 3.2* | 21.6 ± 5.4 |
| | RG | 0.4 ± 0.11 | 12 ± 2.1* | 5.6 ± 1.6* | 6.4 ± 1.4* | 7.4 ± 1.3* | 15.7 ± 5.6* | 18.6 ± 5.5* |
| | FG | 0.5 ± 0.12 | 8.0 ± 1.2* | 14.6 ± 5.9 | 10.3 ± 2.8* | 18.3 ± 2.7* | 14.3 ± 8.9* | 3.7 ± 1.5* |
| | PG | 0.6 ± 0.16 | 0.08 ± 0.02 | 3.2 ± 2.9 | 1.9 ± 0.8 | 2.5 ± 0.9 | 12.6 ± 7.9 | 23.3 ± 6.5 |
| Rb1 | WG | 5.0 ± 1.51 | 8.0 ± 2.5 | 18.5 ± 3.8* | 108.5 ± 19.5* | 129.5 ± 23.5* | 15.6 ± 7.6* | 0.3 ± 0.08 |
| | RG | 3.5 ± 1.01* | 24 ± 4.1* | 48.2 ± 9.8 | 73.6 ± 10.1* | 93.6 ± 10.5* | 20.5 ± 8.9* | 0.2 ± 0.04* |
| | FG | 6.9 ± 1.71 | 8.0 ± 1.2 | 33.7 ± 6.1* | 150.5 ± 26.8* | 193.5 ± 28.9* | 12 ± 8.7* | 0.16 ± 0.02 |
| | PG | 6.9 ± 1.91 | 8.0 ± 2.2 | 13.7 ± 7.1 | 50.5 ± 12.8 | 68.5 ± 17.3 | 11.0 ± 6.7 | 0.16 ± 0.03 |
| Rd | WG | 0.9 ± 0.32 | 8.0 ± 2.9* | 17.7 ± 4.9* | 25.9 ± 8.8* | 29.9 ± 8.9* | 15.4 ± 9.7* | 1.1 ± 0.3* |
| | RG | 0.8 ± 0.42 | 8.0 ± 0.2 | 32.5 ± 6.2* | 13.3 ± 4.6* | 20.3 ± 4.7* | 18.1 ± 9.9* | 1.2 ± 0.2* |
| | FG | 0.5 ± 0.11* | 0.8 ± 0.13 | 17.6 ± 5.5 | 18.3 ± 6.2* | 26.3 ± 7.7* | 12.8 ± 7.6 | 1.1 ± 0.5 |
| | PG | 0.8 ± 0.15 | 0.25 ± 0.04 | 6.9 ± 2.5 | 9.7 ± 2.2 | 11.7 ± 3.1 | 11.9 ± 4.6 | 3.0 ± 1.1 |

Data are presented as mean ± SD (n = 5)

* $p < 0.05$ compared to the pure compound group

AUC, area under the plasma drug concentration-time curve; CL/F, clearance rate; FG, frozen ginseng; MRT, mean residence time; PG, pure ginsenosides compounds; RG, red ginseng; WG, white ginseng

herbal medicine [18–20]. Usually, one or more of the effective ingredients in the herbal medicine are used to study the pharmacokinetics and tissue distribution of the product [13,21]. In the present study, Rg1, Re, Rb1, and Rd were used as the target compounds to study the pharmacokinetics and tissue distribution of ginseng after oral administration. A simple and sensitive UPLC-MS/MS method was developed and validated to assay Rg1, Re, Rb1, and Rd in rat plasma and tissues. The AUCs of Rg1, Re, Rb1, and Rd from the WG, FG, and RG extracts were significantly higher

than those of the respective free ginsenosides ($p < 0.05$). In addition, Rg1, Re, Rd, and Rb1 from the extracts were more distributed in the tissues than the respective free ginsenosides group ($p < 0.05$). The reasons for the higher bioavailabilities of Rg1, Re, Rd, and Rb1 from the extracts might be high absorption of the ginsenosides from the gastrointestinal tract and a lower CL/F. Another reason was that there were significant differences in the $t_{1/2}$ values for Rg1, Re, Rb1, and Rd in plasma after oral administration of the different preparations to the rats. The results

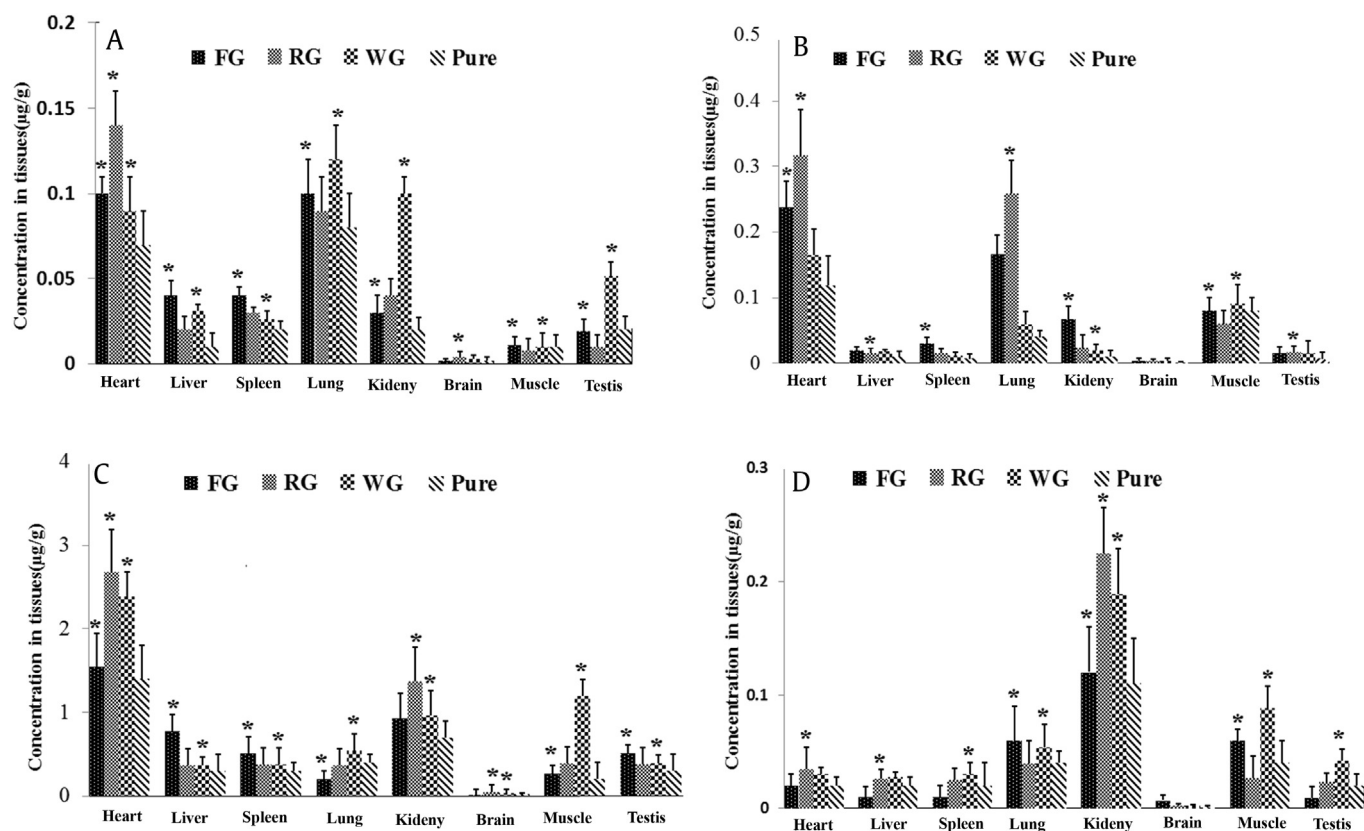


Fig. 4. Tissue distribution profile of Rg1, Rb1, Re and Rd after oral administration of different preparations in rats ($n = 5$). * $p < 0.05$ compared to the pure compound group. FG, frozen ginseng; RG, red ginseng; WG, white ginseng [15].

obtained also showed that the blood–brain barrier was poorly permeable to the ginsenosides as the concentrations of Rg1, Re, Rb1, and Rd in the brain tissues was much lower than those in the plasma. These results are in agreement with those obtained in a previous study [22]. The plasma/tissue concentrations of the ginsenosides from the extracts were higher than the respective free ginsenosides. WG, FG, and RG contain different chemical constituents, which could interact with Rg1, Re, Rb1, and Rd and lead to differences in the bioavailabilities and tissue concentrations of the three ginseng products. In addition, it appears that administration of the extracts resulted in higher plasma/tissue levels of the ginsenosides even though the extracts contained equivalent amounts of Rg1, Re, Rb1, and Rd as the free compounds did. The different bioavailabilities may have resulted in the absorption of ginsenosides from the intestine and the unabsorbed fraction is degraded by intestinal bacterial [23]. All glycosides in ginseng might be degraded by intestinal bacterial through the approximate mechanism as free ginsenosides. Thus, the competitive inhibition between free ginsenosides and other glycosides might reduce the degradation and increase the concentration of ginsenosides in intestine, which finally enhances the bioavailability of ginsenosides from the extracts. For example, a similar study showed that ginseng berry extract can exhibit a significantly higher absorption of ginsenoside Re (0.33–0.75%) than free ginsenoside Re in mouse [24] and statistically significant increases in pharmacokinetic parameters of paeoniflorin, including AUC and MRT, were obtained after oral administration of Cortex Moutan or Shuang-Dan decoction comparing with pure paeoniflorin, indicating that the enhancement of its bioavailability might be due to some ingredients in the Cortex Moutan extract. The authors indicated that the higher bioavailability of paeoniflorin from the

decoctions might be due to the effects of some other compounds in the decoctions [20,25].

The mechanisms accounting for the differences between the pharmacokinetic parameters obtained for Rg1, Re, Rb1, and Rd from the RG, FG, and WG extracts and those for the respective free ginsenosides are not clear. However, a possible explanation is the different interactions among the compounds in the WG, FG, and RG extracts. Possible interactions between ginsenosides and other compounds in WG, FG, and RG should be studied to further elucidate the reasons for the differences observed in the pharmacokinetics and tissue distribution characteristics of WG, FG, and RG for clinical applications.

Conflicts of interest

The authors have declared no conflicts of interest.

Acknowledgments

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