Comparative Pharmacokinetics of Ginsenoside Rg₃ and Ginsenoside Rh₁ after Oral Administration of Ginsenoside Rg₃ in Normal and Walker 256 Tumor-bearing Rats

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ABSTRACT

Background: Ginseng is Chinese traditional herbal medicine, and the ginsenoside Rg₃ is the main bioactive ingredient for the anti-tumor effect. However, there is no study on pharmacokinetics (PKs) of ginsenoside Rg₃ and its main metabolite after oral ginsenoside Rg₃ in tumor-bearing plasma. The aim of this study was to investigate the PK profiles of ginsenoside Rg₃ and ginsenoside Rh₁ after oral administration of pure ginsenoside Rg₃ were administered, and compare the difference of the PK profiles between normal and Walker 256 tumor-bearing rats. Materials and Methods: The concentrations of two ginsenosides in plasma were determined by using a simple and rapid high-performance liquid chromatography (HPLC) method to simultaneously determine the concentration of ginsenoside Rg₃ and ginsenoside Rh₁ in rat plasma after oral administration of ginsenoside Rg₃ to rats. Ginsenoside Rg₃ had shown better absorption than ginsenoside Rh₁, whether the oral administration of ginsenoside Rg₃, normal rats showed better absorption than tumor-bearing rats. Discussion and Conclusion: The PKs properties of the ginsenoside Rg₃ and ginsenoside Rh₁ differed between tumor-bearing rats and normal rats, including area under the plasma level/time curve and concentration maximum (P < 0.05). Key words: Ginsenoside Rg₃, ginsenoside Rh₁ high-performance liquid chromatography, pharmacokinetic

SUMMARY

• Ginsenoside Rh₁ was found in plasma after oral administration of ginsenoside Rg₃ to rats

INTRODUCTION

Ginseng is Chinese traditional herbal medicine; ginsenosides are the main bioactive ingredients. At present, there are many monomer components had been separated. The ginsenoside Rg₃ is the saponin from Ginseng, its structure belongs to protopanaxadiol type saponin in monomer, the molecular formula is C₆₃H₁₀₂O₁₅, and its relative molecular mass is 784. In various isolated saponins, the anti-tumor effect of Rg₃ was the most significant and had been widely used in clinical treatment. The ginsenoside Rg₃ is the main active ingredient in Shenyi capsule, the first anti-tumor Chinese medicine, in China. Up to now, there have been many reports about ginsenoside Rg₃ in vitro and in vivo pharmacokinetic (PK) studies. It was reported that ginsenoside Rg₃ may be prodrug and it can be hydroxylated ginsenoside Rh₁ and ginsenoside Rh₂ played an important role in anti-cancer action. However, there is no page about detecting ginsenoside Rh₁ in vivo after oral ginsenosides Rg₃, and also no study on PKs of ginsenoside Rg₃ in tumor-bearing plasma. Therefore, the purpose of this study was to develop a sensitive, simple, and accurate high-performance liquid chromatography (HPLC) method to simultaneously determine the concentration of ginsenoside Rg₃ and ginsenoside Rh₁ in normal and tumor-bearing rat plasma and to investigate, and compare the PK parameters of ginsenoside Rg₃ and ginsenoside Rh₁ after oral administration of ginsenoside Rg₃.

MATERIALS AND METHODS

Materials and chemicals

Pure ginsenoside Rg₃ was obtained from Prof. Fu Li (Dalian Fusheng Natural Drug Development Co., Ltd). The purities of ginsenoside Rg₃ was determined to be up to 98% by HPLC. Ginsenoside Rh₁ (>98%) and the internal standard (IS) ginsenoside Rb₁ (>98%) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The solvents (HPLC grade) used were reagent grade methanol, acetonitrile, and water. The isocratic HPLC was used to determine simultaneously the concentration of ginsenoside Rg₃ and ginsenoside Rh₁ in rat plasma after oral administration of ginsenoside Rg₃.

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for chromatographic analysis were purchased from Fisher Company Inc., USA. Deionized water was prepared in a Mill-Q academic water purification system (Millipore, Bedford, MA, USA). All the other reagents were of analytical grade and provided by Kernel Chemical Co., (Tianjin, China).

**Apparatus and chromatographic conditions**

The concentrations of two ginsenosides in plasma were assayed using reverse-phase HPLC (Agilent 1200 series) equipped with a variable wavelength ultraviolet (UV) detector and pump (Agilent model G1314A VWD). The separation was accomplished on a Welch Ultimate AQ-C$_{18}$ column (150 mm × 4.6 mm, 5 μm particle size). The mobile phase was composed of acetonitrile (A): Water (B) (0 → 5 min, 35:65; 5 → 10 min, 60:40; 10 → 20 min, 60:40; v/v) at a flow rate of 1.0 mL/min with gradient elution. The column temperature was 30°C. The detector was set at 203 nm. The injection volume was 20 μL. The chromatographic run time for each analysis was 35.0 min.

**Animals**

Male Wistar rats, weighing 200–250 g, were obtained from Liao Ning Chang Sheng Biotechnology Co., Ltd. (Benxi, China). Animal welfare and experimental procedures were strictly in accordance with the Guide for the Care and Use of Laboratory Animals (US National Research Council, 1996) and the related ethics regulations of Liaoning University of Traditional Chinese Medicine. Rats were housed in an air-conditioned animal quarter at a temperature of 22°C ± 2°C and a relative humidity of 50% ± 2%. All animals received food and water *ad libitum*. The animals were acclimatized to the facilities for 5 days and then fasted with free access to water for 24 h prior to each experiment.

**Animal model**

Walker 256 carcinosarcoma cells were purchased from Beijing AnBona Science and Technology Co., Ltd., (Beijing, China). Tumor cells for the establishment of the experimental animal model were obtained from ascitic fluid in Wistar rats, after two cycles of 7 days cell passage by intraperitoneal injection of 10$^7$ Walker 256 carcinoma cells. When cell harvesting dilute the tumor cells suspension to 10$^7$ cells/mL with sterile saline.

After 3 days of acclimatization in metabolic cages, rats were randomly divided into two groups: Model group ($n=6$, labeled M01−M06) and control group ($n=6$, labeled C01−C06). The rats in model group were subjected to three freeze/thaw cycles. The stock solutions were prepared by dissolving 2.73 mg of ginsenoside Rg$_3$, 5.52 mg of ginsenoside Rh$_2$, and 5.27 mg IS in 10 mL methanol, respectively. A series of mixture standard working solutions with concentrations of 5.46, 6.83, 13.7, 27.3, 54.6, 81.9, 109 μg/mL for ginsenoside Rg$_3$, and 0.552, 1.10, 2.21, 4.17, 5.70, 8.28, 11.4 μg/mL for ginsenoside Rh$_2$, were obtained by diluting the mixture of the stock standard solutions with methanol. The IS working solution was prepared by diluting the IS stock solution with methanol. All solutions were stored at 4°C.

**Preparation of stocks, calibration samples, and quality control samples**

The stock solutions were prepared by dissolving 2.73 mg of ginsenoside Rg$_3$, 5.52 mg of ginsenoside Rh$_2$, and 5.27 mg IS in 10 mL methanol, respectively. A series of mixture standard working solutions with concentrations of 5.46, 6.83, 13.7, 27.3, 54.6, 81.9, 109 μg/mL for ginsenoside Rg$_3$, and 0.552, 1.10, 2.21, 4.17, 5.70, 8.28, 11.4 μg/mL for ginsenoside Rh$_2$, were obtained by diluting the mixture of the stock standard solutions with methanol. The IS working solution was prepared by diluting the IS stock solution with methanol. All solutions were stored at 4°C.

**Sample preparation**

Plasma samples (200 μL) were spiked with 50 μL IS, and the mixtures were extracted with 1000 μL acetonitrile by vortex mixing for 3 min. After centrifugation at 4000 × g for 5 min, the solution was transferred to a polypropylene tube and dried under nitrogen gas at room temperature. The plasma residue was reconstituted in 50 μL of methanol, respectively. The injection volume was 20 μL for analysis.

**Method validation**

The method has been validated according to the Food and Drug Administration guidelines.

**Linearity and quantification**

The method was fully validated for its specificity, linearity, lower limits of detection (LLOD), lower limits of quantification (LLOQ), accuracy, and precision. The LLOD was determined during the evaluation of linear range of the calibration curve and is defined as the lowest concentration level resulting in a signal-to-noise ratio of 3:1. The LLOQ was determined as the lowest concentration of the analyte in rat plasma and tissue that could be quantified with an inter-assay relative standard deviation (%RSD) lower than 20% and with accuracy rates between 80% and 120%.

**Accuracy and precision**

The precision and accuracy of the method was evaluated by analyzing quality control (QC) samples with different concentrations. The intra-day variability was determined by assaying five replicates on the same day, and the inter-day variability was determined by assaying five replicates on three consecutive days. Precision was defined as the coefficient of variation expressed as a percentage. The accuracy of these samples was determined by comparing the calculated concentration obtained from the calibration curve with the known concentration.

**Extraction recovery**

Extraction recoveries from rat plasma were determined at three concentrations by comparing the peak areas extracted from rat plasma with those of the same quantities added to methanol.

**Stability**

Stability of ginsenoside Rg$_3$ and ginsenoside Rh$_2$ in rat plasma was assessed with QC samples ($n=3$) stored at −20°C for 30 days. Freeze-thaw stability of ginsenoside Rg$_3$ and ginsenoside Rh$_2$ in rat plasma was investigated with QC samples ($n=3$) subjected to three freeze/thaw cycles.

**Pharmacokinetic study**

The normal Wistar rats ($n=6$) and Walker 256 tumor-bearing rats ($n=6$) were assigned to receive a ginsenoside Rg$_3$ solution by oral administration at the dose of 50 mg/kg of ginsenoside Rg$_3$, respectively. Serial blood samples (0.4 mL) were obtained via the rats’ orbital vein at 1, 1.5, 2, 3, 4, 6, 8, and 12 h after administration and were collected into heparinized centrifuge tubes. The blood samples were immediately centrifuged at 667 × g for 10 min at room temperature. The plasma samples were analyzed by the previously described methods.

**Statistical analysis**

The content of ginsenoside Rg$_3$ and ginsenoside Rh$_2$ in plasma at different times were evaluated by means of linear regression analysis. All data were calculated using Microsoft Excel 2003 (Microsoft). The relevant PK parameters were calculated using the computer program DAS 2.0 (Chinese Society of Mathematical Pharmacology, Beijing, China) from the Chinese Pharmaceutical Association.

**RESULTS AND DISCUSSION**

High-performance liquid chromatography assay

The selectivity of the method was evaluated by analyzing blank plasma samples prior to administration. The chromatograms of the plasma and
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The method presented here was successfully used to quantify the ginsenoside Rg3 and ginsenoside Rh2 in rat plasma after oral administration of ginsenoside Rg3. The concentration-time profiles of the ginsenoside Rg3 and ginsenoside Rh2 are shown in Figure 2. According to the F-test and the Akaike's information criterion, a two-compartment PK model fitted best the plasma data of the ginsenoside Rg3 and ginsenoside Rh2. The calculated PK parameters are listed in Table 1.

The noncompartmental model was applied to the PK evaluation of ginsenoside Rh2 against the original compound ginsenoside Rg3. Ginsenoside Rh2 exhibited a rapid and poor absorption phase followed by a sharp but lasting disappearance of ginsenoside Rh2. The concentration peak values of ginsenoside Rg3 were much higher than ginsenoside Rh2, indicating that ginsenoside Rg3 should also be a major compound in vivo. The data suggested that ginsenoside Rg3 was a major compound for pharmacological effects because there were significant differences in the area under the plasma level/time curve (AUC[0→t]) between ginsenoside Rg3 and ginsenoside Rh2.

| Parameter            | Unit  | Normal rats | Tumor-bearing rats |
|----------------------|-------|-------------|--------------------|
| AUC[0→12h]           | mg/L×h| 219±81.4    | 11.5±3.72          |
| AUC[0→∞]             | mg/L×h| 326±36.1    | 14.9±4.33          |
| CLz/F                | L/h/kg| 67.3±25.4   | 134±527            |
| T                    | max   | 2.3±0.58    | 1.7±0.26           |
| T1/2                 | h     | 4.2±1.33    | 3.2±0.10           |
| Cmax                 | mg/L  | 81.6±24.6   | 6.1±1.34           |

SD: Standard deviation; AUC: Area under the plasma level/time curve; T1/2: Terminal half-life; Cmax: Concentration maximum; CLz/F: Clearance

**Figure 1**: Chromatograms of rat plasma samples: (a) Blank plasma; (b) blank plasma spiked with internal standard, ginsenoside Rg3, and ginsenoside Rh2; (c) plasma sample obtained 2 h after oral administration of ginsenoside Rg3 at a dose of 50 mg/kg to rat; (internal standard, t0.5 = 7.064 min; ginsenoside Rg3, t0.5 = 13.250 min; ginsenoside Rh2, t0.5 = 18.822 min)

**Figure 2**: The mean plasma concentration-time curves of ginsenoside Rg3 and ginsenoside Rh2 after oral administration of ginsenoside Rg3 to normal rat and tumor-bearing rats at different doses. (a) ginsenoside Rg3 and (b) ginsenoside Rh2
After oral administration of Rg$_3$, the AUC values, terminal half-life, and concentration maximum of ginsenosides Rg$_3$ and ginsenoside Rh$_2$ in normal rats were higher than those in the tumor-bearing rats. The result showed that the absorption of Rg$_3$ in tumor-bearing was lower than in normal rats, but the clearance is higher than normal mice. It may be that the tumor changes rats’ body environment, which affect the absorption and metabolism of drug. Therefore, the dosage needs to be adjusted appropriately, according to the practical applications and achieve the desired therapeutic effect.

**CONCLUSION**

Our study is the first evaluation of the plasma PKs of ginsenoside Rg$_3$, as well as its metabolite, ginsenoside Rh$_2$. The ginsenoside Rg$_3$ and ginsenoside Rh$_2$ have been quantified by HPLC-UV. The validated method was simple, fast, reproducible, and suitable for the research of ginsenoside Rg$_3$ and ginsenoside Rh$_2$ in rat plasma with ginsenoside Rb$_1$ as the IS. The assay utilized an acetonitrile extraction method and a reversed-phase separation with sufficient selectivity and sensitivity. The evaluation of the PKs of ginsenoside Rg$_3$ and ginsenoside Rh$_2$ will help further the understanding of their pharmacological activity and clinical use. We need to take caution when extrapolating PK and exposure data from healthy animals to diseased animals in designing pharmacological studies.

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**Conflicts of interest**

There are no conflicts of interest.

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