

Comparative Pharmacokinetics of Ginsenoside Rg₃ and Ginsenoside Rh₂ after Oral Administration of Ginsenoside Rg₃ in Normal and Walker 256 Tumor-bearing Rats

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ABSTRACT

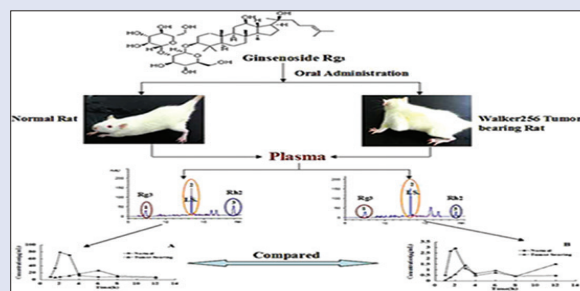
Background: Ginseng is Chinese traditional herbal medicine, and the ginsenoside Rg₃ is the main bioactive ingredient for the anti-tumor effect. However, there is no study on pharmacokinetics (PKs) of ginsenoside Rg₃ and its main metabolite after oral ginsenoside Rg₃ in tumor-bearing plasma. The aim of this study was to investigate the PK profiles of ginsenoside Rg₃ and ginsenoside Rh₂ 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. All the rats were divided randomly into two groups (Walker 256 tumor-bearing and normal groups). Each group received oral administration of 50 mg/kg ginsenoside Rg₃. **Results:** The results showed that ginsenoside Rh₂, possibly as a glycosylation hydrolysis product of ginsenoside Rg₃, were found in plasma after oral administration of ginsenoside Rg₃ to rats. Ginsenoside Rg₃ had shown better absorption than ginsenoside Rh₂, 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 Rh₂ 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 Rh₂, high-performance liquid chromatography, pharmacokinetic

SUMMARY

- Ginsenoside Rh₂ was found in plasma after oral administration of ginsenoside Rg₃ to rats

- HPLC could be used to determine simultaneously, the concentration of ginsenoside Rg₃ and ginsenoside Rh₂ in rat plasma after oral administration of ginsenoside Rg₃.
- Normal rats showed better absorption than tumor-bearing rats after oral administration of ginsenoside Rg₃.



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INTRODUCTION

Ginseng is Chinese traditional herbal medicine; ginsenosides are the main bioactive ingredients. At present, there are many monomer components had been separated.^[1] The ginsenoside Rg₃ is the saponin from Ginseng, its structure belongs to protopanaxadiol type saponin in monomer, the molecular formula is C₄₂H₇₂O₁₃, and its relative molecular mass is 784.^[2] In various isolated saponins, the anti-tumor effect of Rg₃ was the most significant and had been widely used in clinical treatment.^[3,4] 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.^[5-7] It was reported that ginsenoside Rg₃ may be prodrug,^[8-10] and it can be hydrolysed ginsenoside Rh₂ and ginsenoside Rh₂ played an important role in anti-cancer action.^[11-13] However, there is no page about detecting ginsenoside Rh₂ *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 Rh₂ in normal and tumor-bearing rat plasma and to investigate, and compare the PK parameters of ginsenoside Rg₃ and ginsenoside Rh₂ 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 Rh₂ (>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

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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 Kermel 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-C₁₈ 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 10⁷ Walker 256 carcinoma cells. When cell harvesting dilute the tumor cells suspension to 10⁷ 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 established by subcutaneous injection of a 200 μL suspension of Walker 256 carcinosarcoma cells (1 × 10⁷ cells/mL) into the right armpit while the control group was injected with an equal volume of sterile saline.

Preparation of stocks, calibration samples, and quality control samples

The stock solutions were prepared by dissolving 2.73 mg of ginsenoside Rg₃, 5.52 mg of ginsenoside Rh₂, 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 Rg₃ and 0.552, 1.10, 2.21, 4.17, 5.70, 8.28, 11.4 μg/mL for ginsenoside Rh₂, 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 validation has been performed 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 Rg₃ and ginsenoside Rh₂ in rat plasma was assessed with QC samples ($n = 3$) stored at –20°C for 30 days. Freeze-thaw stability of ginsenoside Rg₃ and ginsenoside Rh₂ 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 Rg₃ solution by oral administration at the dose of 50 mg/kg of ginsenoside Rg₃, 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 Rg₃ and ginsenoside Rh₂ 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

organs are shown in Figure 1. Ginsenoside Rb₁ (IS), ginsenoside Rg₃, and ginsenoside Rh₂ were well separated at 7.064, 13.250, and 18.822 min, respectively with no endogenous interference.

The linear calibration curves were obtained in the given concentration range of ginsenoside Rg₃ or ginsenoside Rh₂ in plasma samples, respectively. The standard curves were fitted to a first-degree polynomial, $Y = aX + b$, where Y was the peak area of ginsenoside Rg₃/IS or ginsenoside Rh₂/IS, a and b were constants, and X was the concentration ($\mu\text{g/mL}$) of ginsenoside Rg₃ or ginsenoside Rh₂. Calibration curves were found to be linear over the calibration range of 5.46–109 $\mu\text{g/mL}$ for ginsenoside Rg₃ and 0.552–11.4 $\mu\text{g/mL}$ for ginsenoside Rh₂ in rat plasma. All curves have correlation coefficients of >0.99 . The LLOQ of ginsenoside Rg₃ and ginsenoside Rh₂ were 5.46 and 0.552 $\mu\text{g/mL}$ for plasma with the RSD $<20\%$.

The RSD for the intra-day (repeatability) and inter-day precision ranged from 1.13% to 10.7%, for QC standards. The percentage of extraction recoveries of ginsenoside Rg₃ and ginsenoside Rh₂ for plasma were between 75.6% and 91.1%, respectively. These data indicated that the sample preparation method were satisfied and resulted in no appreciable matrix effect for ginsenoside Rg₃, ginsenoside Rh₂ and IS.

The stability tests were designed by taking into account the anticipated conditions that real samples may experience. The RSD of the stability studies were 5.75–12.8%, respectively.

Pharmacokinetics of ginsenoside Rg₃ and ginsenoside Rh₂ after oral administration of ginsenoside Rg₃ to rats

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 Rg₃ 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 were a major compound for pharmacological effects because there were significant differences in the area under the plasma level/time curve ($\text{AUC}_{[0-t]}$) between ginsenoside Rg₃ and ginsenoside Rh₂.

Table 1: Pharmacokinetic parameters for ginsenoside Rg₃ and ginsenoside Rh₂ in normal rats and tumor-bearing rats (mean \pm SD, $n=6$) after a single oral administration of ginsenoside Rg₃

Parameter	Unit	Normal rats		Tumor-bearing rats	
		Ginsenoside Rg ₃	Ginsenoside Rh ₂	Ginsenoside Rg ₃	Ginsenoside Rh ₂
AUC ₍₀₋₁₂₎	mg/L \times h	219 \pm 81.4	11.5 \pm 3.72	120 \pm 45.6	9.88 \pm 3.28
AUC _(0-∞)	mg/L \times h	326 \pm 36.1	14.9 \pm 4.33	137 \pm 51.7	10.0 \pm 3.06
CLz/F	L/h/kg	67.3 \pm 25.4	1344 \pm 527	165 \pm 38.9	2132 \pm 625
T	max	2.33 \pm 0.58	1.72 \pm 0.26	1.83 \pm 0.632	1.5 \pm 0.132
T _{1/2}	h	4.27 \pm 1.35	3.25 \pm 0.17	2.47 \pm 0.975	1.74 \pm 0.17
C _{max}	mg/L	81.6 \pm 24.6	6.17 \pm 1.34	36.4 \pm 11.3	3.81 \pm 0.989

SD: Standard deviation; AUC: Area under the plasma level/time curve;

T_{1/2}: Terminal half-life; C_{max}: Concentration maximum; CLz/F: Clearance

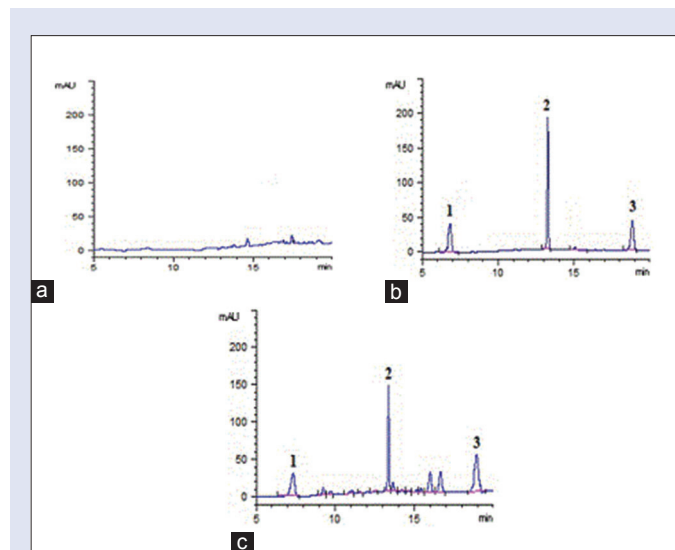


Figure 1: Chromatograms of rat plasma samples: (a) Blank plasma; (b) blank plasma spiked with internal standard, ginsenoside Rg₃ and ginsenoside Rh₂; (c) plasma sample obtained 2 h after oral administration of ginsenoside Rg₃ at a dose of 50 mg/kg to rat; (internal standard, $t_R = 7.064$ min; ginsenoside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

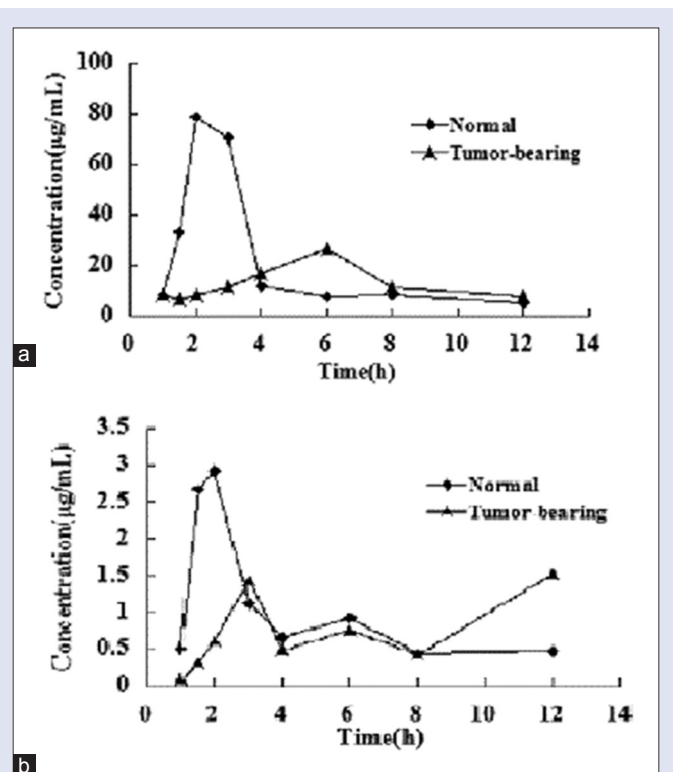


Figure 2: The mean plasma concentration-time curves of ginsenoside Rg₃ and ginsenoside Rh₂ after oral administration of ginsenoside Rg₃ to normal rat and tumor-bearing at different doses. (a) ginsenoside Rg₃ and (b) ginsenoside Rh₂

After oral administration of Rg₃, the AUC values, terminal half-life, and concentration maximum of ginsenosides Rg₃ and ginsenoside Rh₂ in normal rats were higher than those in the tumor-bearing rats. The result showed that the absorption of Rg₃ 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 Rg₃, as well as its metabolite, ginsenoside Rh₂. The ginsenoside Rg₃ and ginsenoside Rh₂ have been quantified by HPLC-UV. The validated method was simple, fast, reproducible, and suitable for the research of ginsenoside Rg₃ and ginsenoside Rh₂ in rat plasma with ginsenoside Rb₁ 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 Rg₃ and ginsenoside Rh₂ 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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