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Short communication

Determination of ginsenosides Rb_1, Rb_2, and Rb_3 in rat plasma by a rapid and sensitive liquid chromatography tandem mass spectrometry method: Application in a pharmacokinetic study

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ABSTRACT

A sensitive rapid resolution liquid chromatography–tandem mass spectrometry method was developed to determine the pharmacokinetics of ginsenoside Rb_1, Rb_2, and Rb_3 in rats, after oral administration (50 mg/kg) and intravenous administration (10 mg/kg) of Rb_1, Rb_2, and Rb_3, respectively. The plasma samples were extracted by saturated N-butanol with Rg_2 as internal standard. Chromatographic separation was performed on a Zorbax SB-C18 column (50 mm × 4.6 mm, 1.8 μm) with a mobile phase consisting of methanol and 1 mM ammonium formate (74:26, v/v). Multiple reaction monitoring mode was performed using the fragmentation transitions of m/z 1107.7 → m/z 178.9, m/z 1077.7 → m/z 148.6, and m/z 1077.7 → m/z 783.4 for Rb_1, Rb_2, and Rb_3, respectively. Calibration curves were recovered over a concentration range of 20–1000 ng/ml for Rb_1 and Rb_2, and 50–2500 ng/ml for Rb_3. The limits of detection were 3.0 ng/ml, 4.0 ng/ml, and 6.5 ng/ml. Both intra-day and inter-day variances were less than 15% and the accuracy was within 86–114% for the three ginsenosides. All three ginsenosides had poor oral bioavailability (0.78%, 0.08%, and 0.52% for Rb_1, Rb_2, and Rb_3, respectively). The value of Rb_1 is higher than that of Rb_2 or Rb_3, indicating that ginsenosides with hexose and hydroxyl groups (Rb_1) could present better pharmacokinetic behaviors than those with pentose groups in the same glycosylation site by oral administration.

1. Introduction

Ginsenosides have been regarded as the main active components responsible for the pharmacological activities of *Panax* herbs [1]. Ginsenoside Rb_1 is the main saponin with Rb_2 and Rb_3 as the other minor chief constituents in *Panax* extract. The three ginsenosides all are 20(S)-protopanaxadiol saponins (PPD) and have the same glycosylation site (C-3 site, C-20 site). The difference only lies in glycoside connected to the C-20 site. Rb_1 connects with two glucoses, Rb_2 connects with one glucose and one α-L-arabinopyranosyl, and Rb_3 connects with one glucose and one β-D-xylose (Fig. 1).

Until now, the pharmacokinetic characteristics of ginsenosides have been published by many literatures. Liu et al. found that Rb_1, Rb_2, Rc and Rd were all distributed rapidly in rat brain, muscle and nervous tissues after intravenous administration [2]. Song et al. reported a pharmacokinetic study of R_1, Rg_1 and Rb_1 in dogs after oral administration. The results showed the C_{max} for Rb_1 was 28.6 ng/ml [3]. Chen et al. reported that the $t_{1/2}$ of Rb_1 is much longer than Rg_1 and R_1 [4]. The bioavailability of Rb_1 has been reported to be 4.35% [5]. The study on the absorption and disposition of Ra_3, Rb_1, Rd, Re, Rg_1, R_1 showed that most ginsenosides were subject to rapid extensive biliary excretion, resulting in their short $t_{1/2}$ values [6].

Although the pharmacokinetic characteristic of Rb_1 is known, the information of Rb_2 and Rb_3 are rare. Actually, limited information has been published on pharmacokinetic behaviors of Rb_2 and Rb_3. Therefore, pharmacokinetic studies of Rb_2 and Rb_3 are necessary and could help clarify the relationship between pharmacokinetics and structural preference of ginsenosides. Since ginsenosides have poor UV radiation in maximum absorption of 203 nm [5]. The liquid chromatography mass spectrometry (LC–MS) [7–9] and liquid chromatography–tandem mass spectrometry (LC–MS/MS) [10–12] method are widely used for analyzing ginsenosides.

In this study we developed a simple, rapid, and sensitive LC–MS/MS method to determine plasma concentrations of Rb_1, Rb_2, and Rb_3 after oral and intravenous administration of the three

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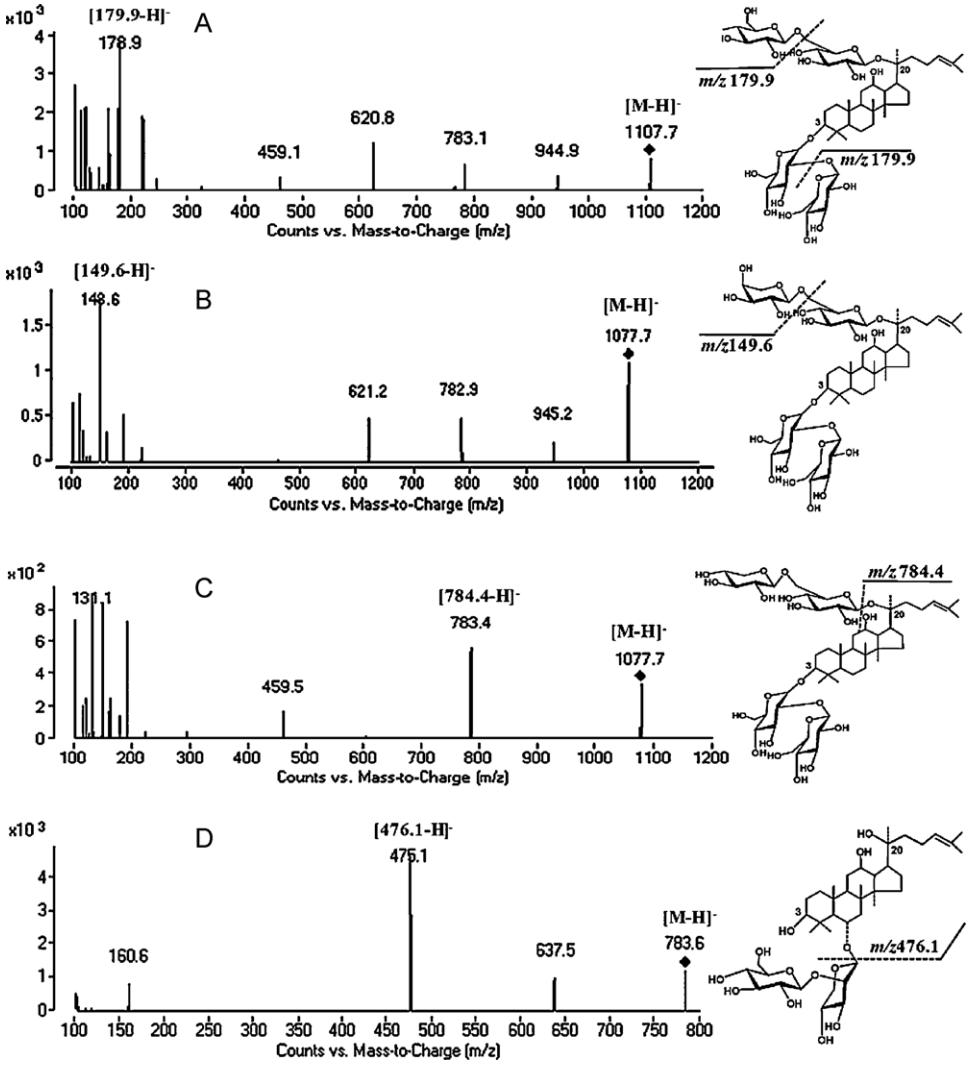

Fig. 1. Chemical structures and product ion mass spectra of Rb_1 (A), Rb_2 (B), Rb_3 (C) and IS (D).

ginsenosides and to systematically compare the similarities and differences in pharmacokinetic behaviors of the three ginsenosides.

2. Materials and methods

2.1. Chemicals and reagents

Ginsenoside Rb_1, Rb_2, Rb_3, and Rg_2 (internal standard, IS) (99.0%) were obtained from the College of Chemistry, Jilin University (Changchun, China). Ammonium formate was obtained from Agilent Technologies. All other reagents were of HPLC grade.

2.2. Instrumentation and analytical conditions

The Agilent 1200 rapid resolution HPLC system was interfaced with an Agilent 6410 triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI). The column was an Agilent Zorbax SB-C18 column (50 mm × 4.6 mm, 1.8 μm). The mobile phase was methanol and 1 mM ammonium formate (74:26, v/v, pH 6.0), the flow rate was 0.4 ml/min. Quantitation was performed in negative multiple reaction monitoring of m/z 1107.7 → m/z 178.9, m/z 1077.7 → m/z 148.6, m/z 1077.7 → m/z 783.4, and m/z 783.6 → m/z 475.1 for Rb_1, Rb_2, Rb_3, and IS, respectively. The values of fragmentation/collision energy were at 250 V/55 V, 210 V/67 V, 200 V/58 V for Rb_1, Rb_2, and Rb_3, respectively. Flow rate, 9 l/min; nebulizer pressure, 40 psi; gas temperature, 350 °C.

2.3. Preparation of calibration curve and plasma samples

Calibration curve were prepared by spiking blank rat plasma with the stock solution to the concentrations: 20, 50, 100, 500, 1000 ng/ml for Rb_1 and Rb_2; 50, 100, 500, 1000, and 2500 ng/ml for Rb_3. The sample (100 μl) was mixed with the IS solution (1 μg/ml, 10 μl) and extracted with 900 μl saturated N-butanol. After centrifugation at 13,000 × g for 10 min, the supernatant was evaporated to dryness under a stream of nitrogen. The residue was dissolved in 100 μl mobile phase and centrifuged for 10 min. 20 μl of the supernatant was injected into the LC–MS/MS.

2.4. Method validation

The matrix effect was evaluated by comparing the response of analyte spiked after extraction to that of an equivalent concentration of the standard solution. QC samples were prepared at three concentrations: 50, 100, and 1000 ng/ml for Rb_1 and Rb_2; 100, 500, 2500 ng/ml for Rb_3. The intra- and inter-day assays were evaluated of QC samples (n = 5) in rat plasma on three independent days. The extraction recovery was determined by comparing the response of

Table 1
Pharmacokinetic parameters of Rb_1, Rb_2, and Rb_3 after i.v. (10 mg/kg) and oral (50 mg/kg) administration in rats ($n = 6$).

Pharmacokinetic parameters	Rb_1		Rb_2		Rb_3	
	i.v.	p.o.	i.v.	p.o.	i.v.	p.o.
AUC_{0-t} (mg h/l)	1627.5 ± 138.9	63.5 ± 36.3	1642.7 ± 222.1	6.4 ± 1.9	1437.0 ± 108.6	37.4 ± 20.2
$AUC_{0-\infty}$ (mg h/l)	1859.1 ± 190.0	66.8 ± 34.0	2006.3 ± 283.0	9.7 ± 3.2	2229.9 ± 684.6	55.1 ± 29.4
$t_{1/2}$ (h)	12.5 ± 1.8	9.8 ± 6.9	15.4 ± 3.7	23.1 ± 3.7	24.9 ± 12.6	21.1 ± 9.8
MRT_{0-t} (h)	11.9 ± 0.7	9.7 ± 2.5	12.7 ± 0.9	14.4 ± 1.3	13.4 ± 0.5	13.9 ± 3.8
T_{max} (h)	0.083	2.0 ± 0.9	0.083	4.8 ± 3.5	0.083	1.5 ± 2.0
C_{max} (mg/l)	199.6 ± 12.4	6.1 ± 3.4	198.1 ± 27.9	0.4 ± 0.1	160.9 ± 31.6	3.3 ± 1.9

the analyte from the QC samples ($n = 5$) with the analyte spiked in post-extracted blank rat plasma at the same concentrations. QC samples ($n = 3$) were determined on the bench for 8 h at room temperature, three freeze–thaw cycles and processed-sample stability.

2.5. Pharmacokinetics study in rats

Sprague Dawley rats (200–220 g) were provided by Experimental Animal Center of Guangdong Province (Guangzhou, China). All studies were approved by the Ethics Committee of Southern Medical University. The rats were randomly assigned into six groups: three groups were intravenous administered of Rb_1, Rb_2, and Rb_3, respectively through tail vein (10 mg/kg), the three other groups were orally administered of Rb_1, Rb_2, and Rb_3, respectively (50 mg/kg). Blood samples were collected at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, and 36 h (i.v.) and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, and 36 h (p.o.). Blood samples were centrifuged immediately and stored at −20 °C until analysis.

3. Results and discussion

3.1. Method validation

The calibration curve was linear over the concentration range of 20–1000 ng/ml for Rb_1 and Rb_2, and 50–2500 ng/ml for Rb_3. The limits of detection (LOD) were 3.0 ng/ml, 4.0 ng/ml, and 6.5 ng/ml.

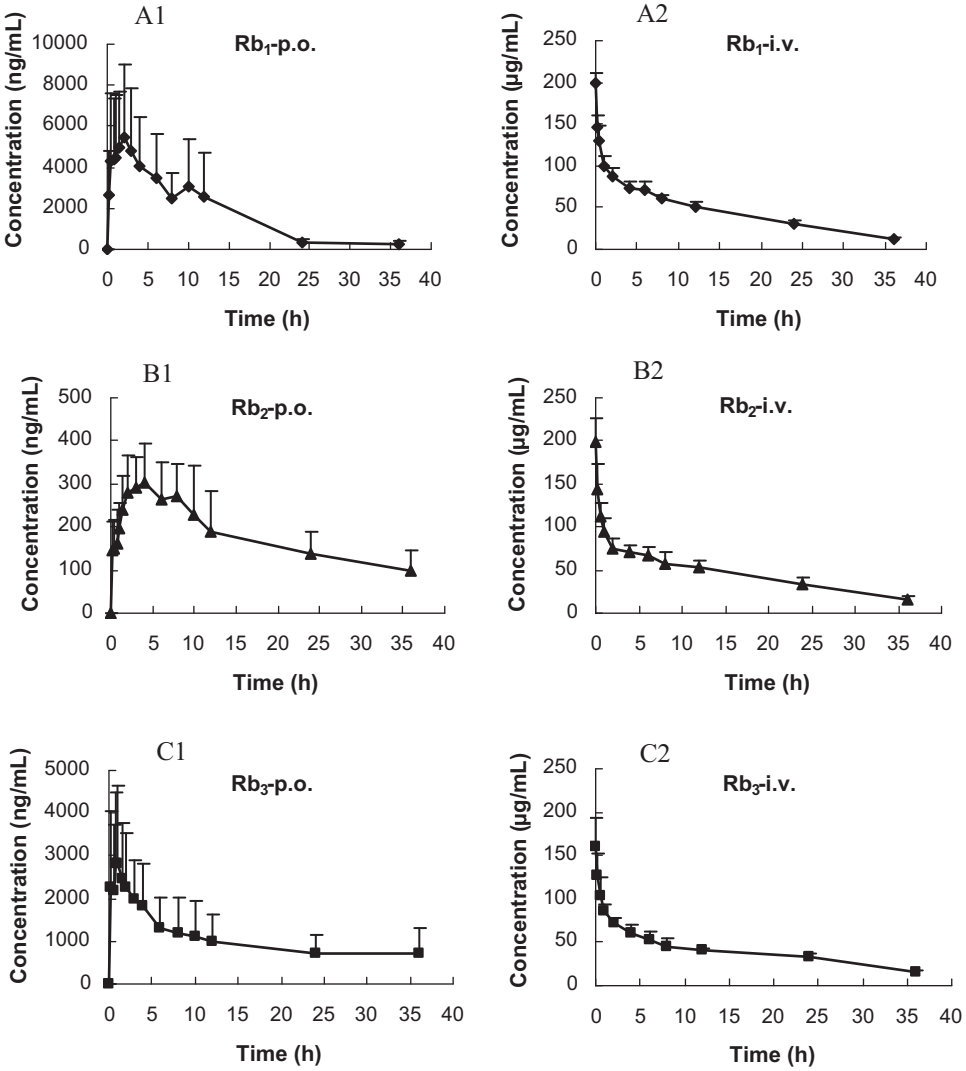

Fig. 2. Plasma concentration–time curve of Rb_1 (A), Rb_2 (B) and Rb_3 (C) after oral dose of 50 mg/kg and i.v. dose of 10 mg/kg ($n = 6$).

The results of matrix effect were in the range of 90–112% ($n = 5$). The intra- and inter-day accuracy were within 86–114% and the precision were within acceptable limits at three concentrations ($n = 5$). The extraction recovery was within 52–117%. All samples in stability evaluated displayed variability of less than 15% (R.S.D.).

3.2. Application to pharmacokinetics study

The method was successfully applied to pharmacokinetic study of Rb_1, Rb_2, and Rb_3 in rats. The pharmacokinetic parameters (Table 1) were calculated by the non-compartmental mode using DAS2.0 software. The plasma concentrations versus time profiles are presented in Fig. 2. The results of T_{max} indicated that the absorption of Rb_3 is the fastest and Rb_1 is the slowest. The $t_{1/2}$ after intravenous administration indicates that the three ginsenosides are eliminated slowly in vivo. Comparatively, the elimination of Rb_1 is the fastest, while Rb_3 is the slowest. The $AUC_{0-36\,h}$ value for Rb_1 following oral administration was about 10 times larger than that of Rb_2 and was nearly two times larger than that of Rb_3, suggesting that the absorption of Rb_1 in rats is much higher than Rb_2 and Rb_3. The bioavailability was 0.78% for Rb_1, 0.08% for Rb_2, and 0.52% for Rb_3, all the three ginsenosides are poor oral absorption. Since the pharmacokinetic characteristics of the three ginsenosides have been compared systematically, we presume that the pharmacokinetic behaviors of ginsenosides have structural preferences. In particular, ginsenosides with hexose and hydroxyl groups (Rb_1) could present better oral absorption than those with pentose groups in the same glycosylation site.

4. Conclusions

A simple, rapid, and sensitive LC–MS/MS method for determination of ginsenoside in rat plasma was developed. The assay was successfully applied to pharmacokinetic study of Rb_1, Rb_2, and Rb_3 in rats. All three ginsenosides had poor bioavailability. The pharmacokinetic behaviors of ginsenosides have structural preference.

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