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Short communication

Determination of ginsenoside Rc in rat plasma by LC–MS/MS and its application to a pharmacokinetic study

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ABSTRACT

Ginsenoside Rc (GRc) is a potential pharmacologically active ingredient isolated from ginseng (*Panax ginseng* C.A. Meyer, *Araliaceae*). A simple, rapid and sensitive method for determination of GRc in rat plasma was developed based on liquid chromatography–tandem mass spectrometry (LC–MS/MS). The analyte and internal standard (IS), ginsenoside Rb_1 (GRb_1), were extracted from plasma with *n*-butanol and chromatographied on a C_{18} column eluted with a mobile phase of methanol and water containing 0.1% formic acid. The detection was performed by positive ion electrospray ionization in selective reaction monitoring mode (SRM), monitoring the transitions m/z 1101.6 → 789.3 and m/z 1131.7 → 364.7 for GRc and IS, respectively. The assay was linear over the concentration range of 5–5000 ng/mL with a limit of quantitation (LOQ) of 5 ng/mL. The accuracy was between 86.7% and 114.9%, and the precision was less than 9.7%. This method was successfully applied to investigate the pharmacokinetic study of GRc in rats after intravenous (2 mg/kg) and oral (20 mg/kg) administration, and the result showed that the ginsenoside was poorly absorbed with an absolute bioavailability being approximately 0.17%.

1. Introduction

Ginseng, a well-known medicinal herb, has been consumed for preventive and therapeutic purposes for thousands of years in the oriental countries. Ginsenosides are the major active compounds in ginseng. Based on its chemical structure, ginsenosides are generally classified into two groups: protopanaxadiol (GRb_1, GRb_2, GRc and GRd) and propanaxatriol (GRg_1, GRg_2, GRe and GRf). GRc is an active ingredient found in ginseng. Pharmacological experiments demonstrated that GRc exhibited sedative [1–3], antiallergic [4], antalgic [5] and antitumor effects [6]. The results from the in vitro studies showed that GRc possessed the potent activity that enhanced both sperm motility and sperm progression [7]. In addition, it was reported that GRc could be used as a powerful natural antidiabetic agent owing to remarkably enhancing glucose uptake by inducing ROS generation [8].

GRc, anaerobically incubated with human fecal microflora, was metabolized to ginsenoside C-K (GC-K), the main metabolite, and protopanaxadiol [4]. Apart from this, Kong et al. reported that GRc could be metabolized to GC-K in in vitro models of the gastro-intestinal tract [9]. However, to our knowledge, the pharmacokinetic characteristics and absorption profile in the gastrointestinal tract of GRc are unknown so far, due to lack of a sensitive assay. Although Zhou et al. [10] developed an LC–MS method for determination of nine ginsenosides including GRc in rat plasma following intravenous administration of Yi-Qi-Fu-Mai injection, the oral pharmacokinetics and bioavailability were not covered. As a result, the objective of the present study was to develop a sensitive and reliable LC–MS/MS method for the quantification of GRc in rat plasma, moreover, the assay was successfully applied to investigate the pharmacokinetic behaviors and oral bioavailability of GRc for the first time.

2. Experimental

2.1. Chemicals and reagents

GRc (purity 98.0%) was obtained from the College of Chemistry, Jilin University (Changchun, China). GRb_1 (purity 92.9%) was purchased from the Chinese National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol (HPLC grade) was obtained from Fisher Scientific (Pittsburgh, USA), while other reagents were analytical grade and used without further purification. Milli-Q (Milford, MA, USA) water was used throughout the study.

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2.2. LC–MS/MS instrument and conditions

Sample analysis was carried out with a Surveyor HPLC system coupled to a Thermo Finnigan TSQ Quantum triple quadrupole mass spectrometer (Thermo Finnigan, USA) equipped with an ESI interface in positive ionization mode for the LC–MS/MS analysis. Quantification was obtained by using selective reaction monitoring (SRM) mode, monitoring the transitions m/z 1101.6 → 789.3 and m/z 1131.7 → 364.7 for GRc and GRb_1, respectively. The optimized instrument conditions were as follows: spray voltage of 4000 V, capillary temperature of 350 °C, nitrogen was used as the sheath gas and auxiliary gas at the pressures of 20 psi and 10 psi, respectively. The collision energy of 50 eV and 55 eV used for GRc and GRb_1. Chromatographic separation was performed on a Zorbax SB-C_{18} column (150 mm × 2.1 mm, 3.5 μm) with the mobile phase comprised of methanol and 0.1% formic acid (85:15, v/v) at a flow rate of 0.2 mL/min.

2.3. Extraction procedure

After thawing at room temperature, an aliquot of plasma sample (100 μL) was mixed with IS solution (1 μg/mL, 50 μL) and extracted with 500 μL *n*-butanol. After vortexing for 2 min, the sample was centrifuged at 6500 × *g* for 15 min, and the supernatant was evaporated to dryness with nitrogen. The residue was dissolved in 150 μL mobile phase and centrifuged for 5 min. 20 μL of the supernatant was injected into the LC–MS/MS.

2.4. Method validation

2.4.1. Linearity and sensitivity

Calibration curve samples were prepared by spiking blank rat plasma with the GRc standard solution (prepared in methanol) to the concentrations: 5, 15, 50, 150, 500, 1500 and 5000 ng/mL. The samples were dealt with Section 2.3 and injected into LC–MS/MS. The calibration curves of GRc were constructed by plotting the peak-area ratios of GRc/IS versus concentrations of GRc in plasma, and the linearity was assessed using linear regression analysis. The LOD was defined as the plasma concentration that produced a signal-to-noise ratio (S/N) at 3. The LOQ was determined as the lowest plasma concentration that produced a S/N ≥ 10.

2.4.2. Precision and accuracy

Precision and accuracy were evaluated through determining replicate QC samples (15, 150, and 4000 ng/mL) on three independent days. Precision was measured by inter- and intra-day RSD (%). The accuracy was evaluated by the deviation or bias (%) of the observed concentration from the actual concentration.

2.4.3. Extraction recovery and matrix effect

The extraction recovery of GRc was estimated by comparing the peak area ratio of GRc extracted from QC samples (15, 150, and 4000 ng/mL) with those obtained from direct injection of the analyte dissolved in the supernatant of the processed blank plasma. The matrix effect was evaluated by comparing the response of analyte spiked after extraction to that of an equivalent concentration of the standard solution.

2.4.4. Stability

Stability was examined in plasma samples under different conditions: (1) freeze–thaw stability through three freeze/thaw cycles; (2) post-preparative stability during storage in the autosampler at room temperature (about 20 °C) for 24 h; (3) long-term stability of GRc in rat plasma stored at −70 °C for 4 weeks. The stability was acceptable when 85–115% of the initial analytes were found.

2.5. Application to a pharmacokinetic study

Male Wistar rats, weighing (290 ± 10) g, were provided by Vital River Lab Animal Technology Co. Ltd. (Beijing, China) and housed with a 12 h light/12 h night cycle at ambient temperature (24–26 °C) and 60% relative humidity. Free access to food and water except for fasting 12 h before the experiment. 10 rats were randomly assigned into two groups, and then the rats were given GRc at a single oral dose (20 mg/kg) by gastric gavage and intravenous dose (2 mg/kg) through tail vein, respectively. Blood samples were collected at 0, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 32, 48 and 72 h, and the samples were centrifuged immediately and stored at −70 °C until analysis. All the animal experiment procedure was approved by the Animal Ethics Committee of Tianjin Tasly Institute.

3. Results and discussion

3.1. Method development

GRc and GRb_1 were investigated for the abundant precursor ions $[M+Na]^+$ at m/z 1101.6 and 1131.7, respectively. The quantification of analyte was performed using SRM mode for high selectivity and sensitivity of acquisition data. It is found that the fragmentation ion at m/z 789.3 and m/z 364.7 were the prominent product ions for GRc and GRb_1, respectively. Therefore, the transition of 1101.6 → 789.3 and 1131.7 → 364.7 for were selected for quantification of GRc and GRb_1.

During the optimization of chromatographic conditions, a number of different commercial column available and mobile phase were evaluated. To achieve symmetric peak shape of GRc and short chromatographic runtime, finally, the Zorbax SB-C18 column (150 mm × 2.1 mm, 3.5 μm) and the mobile phase consisted of methanol-water containing 0.1% formic acid were selected for analysis. Typical chromatograms of the blank plasma and plasma spiked with GRc and IS were presented in Fig. 1. There were no significant interfering peaks at the retention time of GRc and IS due to endogenous substances in blank rat plasma.

3.2. Assay validation

3.2.1. Linearity and sensitivity

The calibration curves for GRc were found to be linear over the concentration range of 5–5000 ng/mL ($r > 0.99$). Typical equations for the calibration curves were $y = (0.0013 \pm 0.0001)x - (0.0017 \pm 0.001)$. The LOD and LOQ of this method were 0.5 ng/mL and 5 ng/mL, respectively.

3.2.2. Precision and accuracy

The precision and accuracy data are shown in Table 1. The results indicate that the developed LC–MS/MS method had good reproducibility with precision less than 9.7% and excellent accuracy ranging from 86.7 to 114.9%. In addition, the precision and accuracy of LOQ were 9.1% and 106.4%.

3.2.3. Extraction recovery, matrix effect and stability

The extraction recoveries of GRc were 76.4 ± 5.2%, 70.2 ± 6.9%, and 72.4 ± 3.9% at concentrations of 15, 150, and 4000 ng/mL,

Table 1
Precision and accuracy data for GRc in rat plasma (three days, six replicates per day).

Spiked (ng/mL)	Measured (ng/mL)	Intra-day RSD (%)	Inter-day RSD (%)	RE (%)
15	14.4 ± 0.8	9.7	4.7	−4.1
150	153.6 ± 10.4	7.0	6.8	2.4
4000	4104.3 ± 340.5	2.9	8.8	2.6

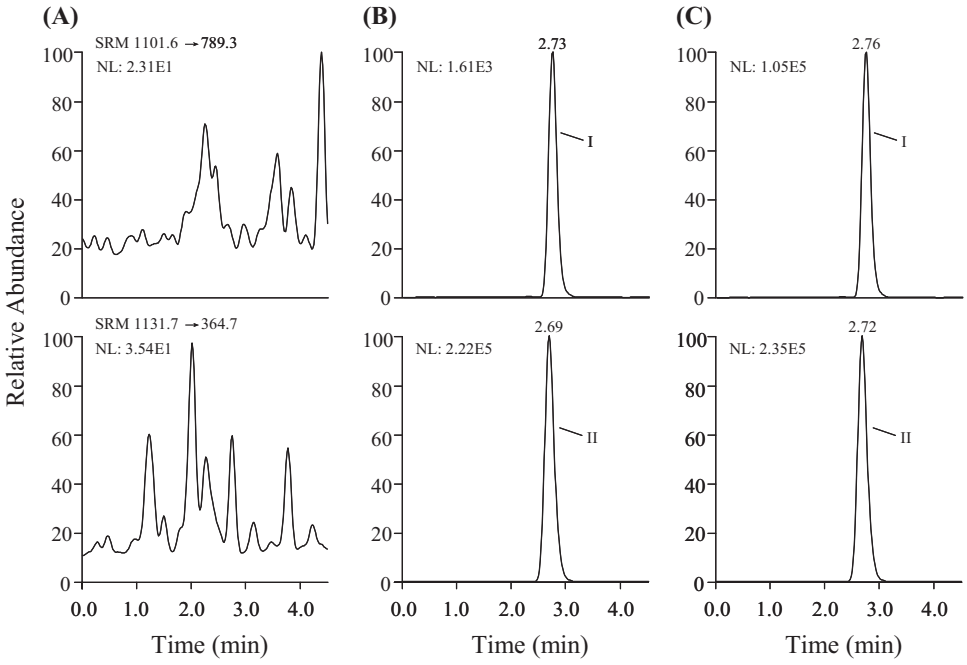

Fig. 1. Typical chromatograms of blank plasma (A), blank plasma spiked with GRc at concentration of 5.0 ng/mL and IS (B), and the plasma sample 12 h following oral administration of GRc at 20 mg/kg (C). Peak I, GRc; Peak II, GRb_1 (IS).

Table 2
Stability results of GRc in rat plasma ($n = 3$).

Spiked (ng/mL)	24 h (autosampler)		3 freeze/thaw cycles		4 weeks at −70 °C	
	Measured (ng/mL)	RSD (%)	Measured (ng/mL)	RSD (%)	Measured (ng/mL)	RSD (%)
15	16.5 ± 0.5	3.0	15.2 ± 1.0	6.7	15.4 ± 1.0	6.7
150	154.1 ± 7.9	5.1	156.7 ± 0.9	0.6	144.0 ± 5.6	3.9
4000	4166.3 ± 340.5	8.2	4142.0 ± 302.7	7.3	3903.3 ± 56.6	1.5

respectively. The results of matrix effect were in the range of 92.6–121.9%. As is reported in Table 2, GRc was stable in the autosampler at room temperature for 24 h, repeated three freeze/thaw cycles and frozen condition at −70 °C for 4 weeks.

3.3. Application to a pharmacokinetic study

The plasma concentrations versus time profiles of GRc after intravenous and oral administration were illustrated in Fig. 2. The pharmacokinetic parameters calculated using non-compartmental analysis with Topfit 2.0 software were given in Table 3. It was found that GRc was fast absorbed into the circulation system and reached its peak concentration at around 1 h after oral administration. The $t_{1/2}$ after intravenous and oral administration indicated that GRc was eliminated slowly in vivo. The oral bioavailability of GRc was quite low with a value being 0.17%, which value is similar with those of other ginsenosides, such as GRb_1 (0.78%), GRb_2 (0.08%) and GRb_3 (0.52%) [11]. The potential hydrolysis in the gastrointestinal tract [4] and poor permeability through the intestinal epithelial membrane might be responsible for the low bioavailability of these ginsenosides including GRc.

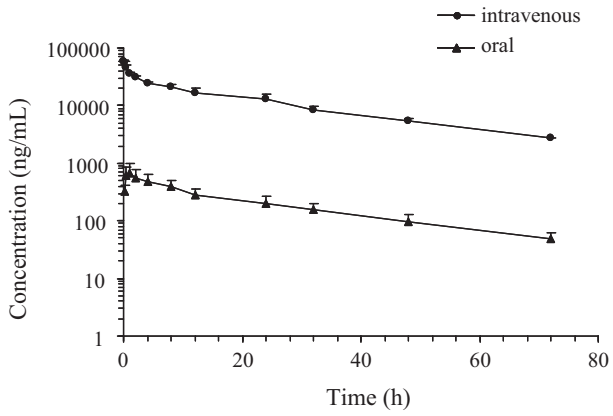

Fig. 2. Mean plasma concentration–time profiles of GRc in rats following intravenous (2 mg/kg) and oral (20 mg/kg) administration. All data were expressed as mean ± SD ($n = 5$).

Table 3
Main pharmacokinetic parameters of GRc in rats after intravenous and oral administration (mean ± SD, $n = 5$).

Parameters	Dose	
	i.v. (2 mg/kg)	p.o. (20 mg/kg)
C_{max} (ng/mL)	–	701.4 ± 292.4
T_{max} (h)	–	1.10 ± 0.55 h
$T_{1/2}$ (h)	22.0 ± 1.9	23.6 ± 0.6
AUC_{0-t} (ng h/mL)	779,808.3 ± 18,558.4	13,363.7 ± 3875.7
$AUC_{0-\infty}$ (ng h/mL)	862,997.1 ± 19,811.2	14,990.1 ± 4288.7
F		0.17%

$F = (AUC_{p.o.} \times Dose_{i.v.})/(AUC_{i.v.} \times Dose_{p.o.}) \times 100\%$.

4. Conclusion

A sensitive and reliable LC–MS/MS method has been established and validated for the determination GRc in rat plasma. This assay

showed excellent sensitivity, linearity, precision and accuracy and was successfully applied to evaluate the pharmacokinetics and oral bioavailability of GRc in rats for the first time.

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