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Short communication

Determination of ginsenoside Rg3 in human plasma and urine by high performance liquid chromatography–tandem mass spectrometry

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ABSTRACT

Here we report a method capable of quantifying ginsenoside Rg3 in human plasma and urine. The method was validated over linear range of 2.5–1000.0 ng mL^{-1} for plasma and 2.0–20.0 ng mL^{-1} for urine using ginsenoside Rg1 as I.S. Compounds were extracted with ethyl acetate and analyzed by HPLC/MS/MS (API-4000 system equipped with ESI^{-} interface and a C_{18} column). The inter- and intra-day precision and accuracy of QC samples were $\leq$8.5% relative error and were $\leq$14.4% relative standard deviation for plasma; were $\leq$5.6% and $\leq$13.3% for urine. The Rg3 was stable after 24 h at room temperature, 3 freeze/thaw cycles and 131 days at $-30\,^{\circ}$C. This method has been applied to pharmacokinetic study of ginsenoside Rg3 in human.

1. Introduction

Ginsenoside Rg3 (Fig. 1), extracted from Red Panax ginseng, is a tetracyclic triterpenoids saponins monomer. Pharmacological experiments showed that ginsenoside Rg3 significantly inhibited tumor growth and metastasis in mice and *in vitro* tumor cell invasion, as well as enhanced body immunity [1–8]. The anti-tumor drug-Rg3 Shenyi Capsule, which mainly consists of ginsenoside Rg3, has been approved by State Food and Drug Administration as a first-class new drug in China in 2003, and some commercial products containing ginsenoside Rg3 are applying for clinical trail.

Numerous methods have been introduced during recent years for the determination of many kinds of ginsenosides. They include gas chromatography coupled with mass spectrometry (GC/MS), high performance liquid chromatography (HPLC), HPLC coupled with mass spectrometry (HPLC/MS) and tandem mass spectrometry (HPLC/MS/MS) [9–25]. However, these methods appear to have either a complicated extraction procedure, low sensitivity, or a long chromatographic run-time, or perderivatization. Using the GC/MS method, 20(S)-protopanaxadiol and 20(S)-protopanaxatriol were measured as the genuine aglycones of ginsenosides in human urine [25]. HPLC methods with UV detection suffer from limited selectivity due to the presence of more than 20 structurally similar ginseng saponins and endogenous interferences from biological fluids.

Several methods have been established for evaluate pharmacokinetics profiles of ginseng and ginseng preparations in animals or human [16–18,23,24]. Li et al. [16] used an HPLC/UV coupled with solid-phase extraction method for determination of four active saponins (ginsenoside Rg1, Rb1, Rd and notoginsenoside R1) from Panax notoginseng in rat urine. Xu et al. [18] used another HPLC/UV method to evaluate the pharmacokinetics and bioavailability of ginsenoside Rb1 and Rg1 from Panax notoginseng in rats. Cai and Qian [17,24] established an HPLC/Q/TOF analytical method for the determination of ginsenoside Rg3 in rat plasma and its major metabolites in samples from *in vitro* and *in vivo* metabolism studies. Xie et al. [23] established an HPLC/MS method for the determination of ginsenoside Rg3 and its metabolites in rat plasma using solid-phase extraction for pharmacokinetic studies.

However, there are few reports on the determination of ginsenoside Rg3 in human for pharmacokinetic study [9,13]. Wang et al. reported some pharmacokinetic parameters of Rg3 after oral administration of the ginsenoside in human at 3.2 mg kg^{-1}. The human plasma was prepared with solid-phase extraction and the extract was analyzed by using HPLC/UV for Rg3 concentrations. Human pharmacokinetic parameters, including $C_{\max}$ of 15.67 ± 6.14 ng mL^{-1} and $t_{\max}$ of 0.66 ± 0.01 h were obtained. The lower limit of quantitation of this method was 2.5 ng mL^{-1}, and the run-time for each sample was 20 min [13].

Summing up the above, there is no specific and rapid HPLC/MS/MS method reported for determining ginsenoside Rg3 for evaluating the pharmacokinetics of it after administration in human until now. In order to provide a useful clinical tool for proper medication, we develop an HPLC/MS/MS method for quantitation of ginsenoside Rg3 in human plasma and urine, which is accurate, sensitive, specific, and with a high throughput. This method is applied to approximately 1000 samples from healthy male and female volunteers.

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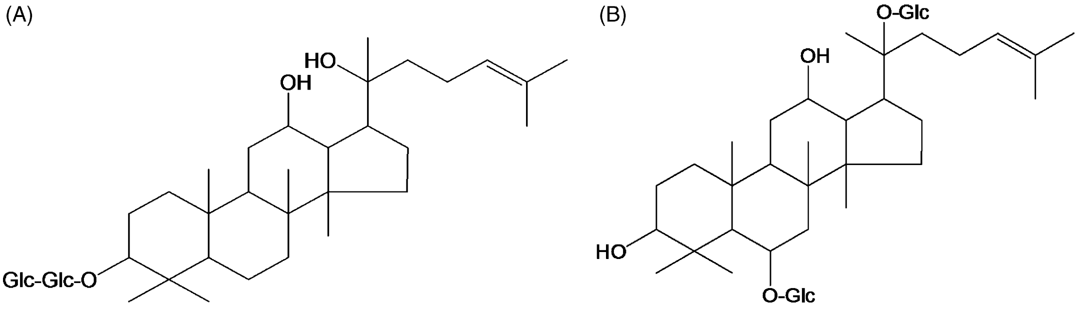

Fig. 1. Chemical structures of ginsenoside Rg3 and Rg1. (A) Ginsenoside Rg3 and (B) ginsenoside Rg1.

2. Experimental

2.1. Chemicals and reagents

Ginsenoside Rh3 and Rg1 (internal standard, Fig. 1) were obtained from Beijing Xinlinheng Company (China). Ammonium acetate was purchased from Beijing Chemical Reagents Company (China). HPLC grade methanol was purchased from Fisher (USA). All liquids used for experiments were filtered through 0.22 μm membrane filters from Millipore (USA). Heparinized blank human plasma and blank urine were obtained from volunteers at Peking Union Medical College Hospital, Beijing, China.

2.2. Standard curve and quality control sample preparation

Stock solutions of ginsenoside Rg3 were prepared from separate weightings for standards and quality control samples (QCs). The stock solutions and subsequent working solutions were prepared in methanol and stored at −30 °C during the validation.

Calibration standards were prepared by spiking an appropriate amount of concentrated stock solutions into blank control plasma and urine. The calibration ranges were 2.5–1000.0 ng mL^{-1} for plasma and 2.0–20.0 ng mL^{-1} for urine. Three levels of QC samples were prepared at 7.5 ng mL^{-1}, 80.0 ng mL^{-1} and 800.0 ng mL^{-1} for plasma and 3.0 ng mL^{-1}, 7.5 ng mL^{-1} and 15 ng mL^{-1} for urine. The QC concentrations were chosen near the low, medium and high calibration standard concentrations being prepared. QCs were stored at −30 °C with clinical sample to be analyzed.

2.3. Instrumentation

The HPLC system consisted of a Shimadzu DGU-20A pump (Japan), a Shimadzu DGU-20A autosampler (Japan) and a Waters SymmetryShield® RP18 column (4.6 mm × 100 mm, 3.5 μm). The HPLC system was operated isocratically at a flow rate of 1 mL min^{-1}. The mobile phase consisted of methanol:10 mM ammonium acetate (95:5, v/v).

The mass spectrometer was an AB API-4000 triple quadrupole mass spectrometer (Thornhill, Canada) equipped with a turboionspray (ESI) interface. The heated nebulizer was set at 500 °C and the needle voltage (IS) was set at −4500.00 V; the nebulizer gas (GS1), the auxiliary nitrogen gas (GS2), the curtain gas (CUR) and the collision gas (CAD) were set at 11 units, 12 units, 10 units and 10 units, respectively. Ion monitored in the multiple reaction monitoring (MRM) mode were m/z 783.5 (parent ion) to m/z 160.9 (daughter ion) for ginsenoside Rg3 and m/z 799.5 (parent ion) to m/z 637.4 (daughter ion) for ginsenoside Rg1 (I.S.). Nitrogen was used as the collision gas. The electron multiplier was set at 2000 V.

2.4. Extraction procedure

To 200 μL heparinized plasma (300 μL urine) samples, 50 μL I.S. in methanol (2.5 μg mL^{-1} for plasma samples and 250 ng mL^{-1} for urine samples) was added. After vortex-mixing briefly, 900 μL ethyl acetate was added. Then vortex-mixed for 1 min. The organic phase was then separated from the aqueous phase by centrifugation at 3500 × g for 3 min. The organic phase was transferred to a clean tube. After evaporation to dryness under nitrogen gas in 40 °C water bath, the residue was reconstituted in 300 μL of methanol:water (95:5 v/v), and 10 μL was injected onto the HPLC/MS/MS system.

2.5. Assessment of validation

The specificity, precision/accuracy of the assay and the stabilities of ginsenoside Rg3 in human plasma and urine were determined using three levels of quality control samples.

2.5.1. Specificity and selectivity

Blank human plasma and urine from six different lots were prepared and tested for endogenous interference. One blank matrix sample spiked with I.S. only and one blank matrix sample spiked with ginsenoside Rg3 alone at 1000 ng mL^{-1} without I.S. were analyzed in order to assess potential interferences that may affect either the analyte or the I.S.

Ginsenoside Rh2 (loss of one glucose from ginsenoside Rg3), the potential metabolite of ginsenoside Rg3 in human which was reported in rats study was analyzed to assessing the interferences for ginsenoside Rg3 and I.S.

2.5.2. Precision and accuracy

Five replicates of each quality control samples were analyzed on three batches and the intra- and inter-day means, relative standard deviation (RSD%) and relative error (RE%) were calculated.

2.5.3. Stability testing

The stability of ginsenoside Rg3 stock solution was calculated by comparing the peak area of stock solution after stored for a period of time with that of stock solution freshly prepared at the same concentration (freshly weighing) under the same determination condition. To mimic the possible freezing and thawing conditions of samples, quality control samples were subjected to multiple cycles of freezing and thawing and then analyzed. The values of quality control samples after three freeze/thaw cycles were comparable to the theoretical concentration. The autosampler stability was evaluated by comparing the concentration of quality control samples after being stored in autosampler for a period with the theoretical concentration of quality control samples. Short-term stability test was done by comparing the data from quality control samples thawed and stored on the bench to nominal value. Long-term storage stability test was done by comparing the data from quality control samples stored in biological matrix in freezer conditions