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Short communication

Relative bioavailability of gastrodin and parishin from extract and powder of Gastrodiae rhizoma in rat

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ABSTRACT

A rapid, sensitive and reliable UHPLC-ESI-MS/MS method was developed for simultaneous determination of gastrodin and parishin in rat plasma. The LLOQ of the two analytes were 1.00×10^{-1} and 8.30×10^{-5} μg/mL, respectively. The intra-day and inter-day precision were all less than 10% of the relative standard deviation (RSD), whilst the accuracy were all within ±15% of the relative error (RE). The proposed method was successfully applied for pharmacokinetics study on the two analytes in rats after oral administration of Gastrodiae rhizoma (GR) extract and powder at low, medium and high dosages. Blood samples were collected from the suborbital vein at predetermined time points and were precipitated using methanol. Chromatographic separations were carried out on a Kinetex XB-C18 column (2.1 mm × 150 mm, 1.7 μm) with a gradient mobile phase of acetonitrile-water with 0.1% formic acid as a modifier. The pharmacokinetic parameters of the two analytes in rats were obtained and the relative bioavailability of gastrodin and parishin in two formulations were calculated. The results indicated that higher bioavailability was obtained when low dosage of GR powder was used, whereas, higher bioavailability values were obtained when medium and high dosages of GR extract were used.

1. Introduction

Gastrodiae rhizoma (GR), *Tianma* in Chinese, is the dried rhizomes of *Gastrodia elata* Blume. (Orchidaceae) and is officially listed in Chinese Pharmacopeia (CP) (Edition 2010) [1] as one of the commonly used Chinese medicines for the treatment of febrile convulsion, epileptoid convulsion, tetanus, headache or dizziness and paralysis or numbness of the limbs. Modern pharmacological studies indicated that aqueous extract of GR had anxiolytic-like effects [2] and relatively good favorable effect on prevention and/or treatment of ovariectomy (OVX)-induced osteoporosis [3]. Fifty percent alcohol extract of GR was testified to have a protective effect against neuronal damage in KA-treated rats by reducing nNOS, microglia activation and apoptosis [4] and have anticonvulsive and free radical scavenging activities [5]. Ether fraction of methanol extract of GR was also investigated and the anticonvulsive effect and putative neuroprotective effect against excitotoxicity were reported [6].

Phytochemical studies on GR have revealed the presence of gastrol, phenolic compounds [7], polysaccharides [8], amino acids, nucleosides [9], adenosines [10], vanillyl alcohol [11], gastrodin [10,12,13] and parishins [13,14]. Gastrodin is one of the earliest compounds found in GR and is specified as a marker for quality assessment on the herb in CP (edition 2010). The compound was reported to decrease immune reactivities of gamma-aminobutyric acid shunt enzymes in the hippocampus of seizure sensitive gerbils [15], to play protective action against liver injury induced by CCl_4 in mice [16] and to cure vascular headache with effective rate at 91%. The compound is available in oral formulations and has been reported to be used intravenously and intramuscularly in clinical trials. Parishin, a main highly polar compound from aqueous extract of GR, significantly enhanced the ADCC reaction [17]. The two analytes have been assayed for quality assessment on GR and its related formulas in different analytical approaches [18,19].

GR extract has been manufactured by many Chinese pharmaceutical or biotechnological companies used clinically as therapeutic medicine. However, due to the valuableness of the herb, more and more pharmaceutical companies are inclined to use GR powder. Generally, the herb was smashed into super fine powder, mixed with the extract of other herbs and then manufactured to different formulations with excipients in commercial products on market. In that way, are there any differences on bioactivities

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between GR extract and GR powder? The issue arouses our great interest.

Several studies on pharmacokinetics of gastrodin were reported [20,21], however, no report on pharmacokinetics of parishin has been published so far. In the present study, a reproducible, rapid, sensitive and selective UHPLC-ESI-MS/MS method for simultaneous determination of gastrodin and parishin in rat plasma was developed for the first time, which was then applied to pharmacokinetics of the two analytes after oral administration of GR extract and GR powder. Moreover, on the basis of the pharmacokinetic parameters of the two analytes, bioavailability values of the two formulations were calculated, which will provide useful information for clinical treatments. To the best of our knowledge, this is the first study on bioavailability comparison between two different medication forms of GR, based on the pharmacokinetics of the two analytes.

2. Experimental

2.1. Material and reagents

GR was purchased from Tong-Ren-Tang Pharmacy (Beijing, China) in Guiyang city. Half was ground to fine powder (60 mesh) using a common grinder with a knife blade for extraction. Another half was smashed to superfine powder (less than 10–25 μm in diameter) using micronizer in Guizhou Holy Pharmaceutical Co. Ltd. for intragastric administration directly. They were stored at 4 °C before use.

Reference compounds of gastrodin (No. 110807-2002-5) and bergenin (Internal Standard, IS, No. 1532-200202) were purchased from the National Institute for the Control of Biological and Pharmaceutical Drugs (Beijing, China). Parishin (No. 12031) was obtained from Guizhou Dida Technology Co. Ltd (Guiyang, Guizhou, China). Their structures are shown in Fig. 1.

MS-grade methanol and acetonitrile were purchased from Tedia Co. Inc. (Fairfield, OH, U.S.). Formic acid was from ROE Scientific, Inc. (DE, U.S.). Super purified water from Robust Food & Beverage Co. Ltd. was used for all preparations. All other reagents used in the present study were of analytical grades.

2.2. Experimental animals

Adult, male Sprague-Dawley (SD) rats weighed 180 ± 20 g were purchased from Changsha Tianqin Bio-Technology Co., Ltd. (Changsha, China, Certificate No. SCXK2009-0012). These animals were specifically pathogen-free and acclimated for at least a week in their environmentally controlled quarters (24 ± 1 °C and 12/12 h light/dark cycle) with free access to standard chow and water. The rats were fasted overnight but were supplied with water *ad libitum* before the experiments. All experimental protocols were conducted in accordance with the Guide for the Care and Use of Laboratory Animal (National Institutes of Health Publication 85-23, revised edition 1985) and were permitted by Ethic Committee of the University.

2.3. Instrument and conditions

Chromatographic separations were performed on an Accela 1250 UHPLC system (Thermo Fisher Scientific Inc., Waltham, MA, U.S.) equipped with an Accela 1250 photo diode array (PDA) detector, an Accela HTC PAL autosampler, and an Accela 1250 binary pump. Kinetex XB-C18 column (2.1 mm × 150 mm, 1.7 μm) from Phenomenex Inc. (Torrance, California, U.S.) was used for chromatographic separations. The mobile phases consisted of water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B). The gradient elution program was as follows: 0–4 min, 2% B; 4–5 min, 2 → 25% B; 5–10 min, 25% B. The column temperature was maintained at 25 °C. The flow rate was 200 μL/min and the injection volume was 10 μL.

Mass spectrometric analyses were performed on a TSQ quantum ultra triple-quadrupole mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, U.S.) equipped with an electro-spray ionization (ESI) interface in negative mode. The MS instrument parameters were as follows: sheath gas flow rate, 40 (arbitrary units); auxiliary gas flow rate, 15 (arbitrary units); spray voltage, 2500 V; vaporizer temperature, 500 °C; capillary temperature, 350 °C. Helium was used as the collision gas for collision-induced dissociation (CID). Quantification was performed using multiple reactions monitoring (MRM) mode under unit mass-resolution conditions. Data acquisition and processing was performed using Xcalibur 2.1 data system and LC quan 2.6 quantification software.

2.4. Preparation of GR extract

For preparation of GR extract, 15 g of the dried powder (60 mesh) was accurately weighted into a 250 mL-conical flask and extracted with 180 mL of water with ultrasonic extraction at 60 °C for 30 min. The extract was centrifuged at 5000 rpm for 15 min and then the supernatant was evaporated to dryness for intragastric administration.

2.5. Preparation of stock and working solutions

Stock solutions of gastrodin, parishin were prepared in acetonitrile–water (4:96, v/v) and further diluted into 0.31–49 μg/mL, 0.00026–4.18 μg/mL, respectively, as working solutions. All the solutions were stored at 4 °C before analysis.

2.6. Preparation of standard and quality control (QC) samples

The calibration standard solutions were prepared by freshly spiking 20 μL of the appropriate mixed working solutions into 100 μL of blank plasma to yield the concentrations of 0.10, 0.39, 1.96, 5.23, 7.84, 10.45, 13.07 and 15.68 μg/mL for gastrodin, 0.000083, 0.1670, 0.2783, 0.4448, 0.6688, 0.8896, 1.1136 and 1.3376 μg/mL for parishin, 0.09408 μg/mL for IS, and processed as described in “sample preparation”.

QC samples used for intra- and inter-day accuracy and precision, extraction recovery and stability studies were prepared at concentrations of 1.96, 7.84 and 13.07 μg/mL for gastrodin, 0.00083, 0.6688 and 1.1136 μg/mL for parishin, and 0.09408 μg/mL for IS.

2.7. Preparation of plasma samples

To 100 μL of the plasma sample in a 1.5 mL-Eppendorf tube (EP tube), 20 μL of IS solution (0.294 μg/mL) and 20 μL of 1% formic acid aqueous solution were individually added. After being mixed for 15 s, 300 μL of methanol was added to precipitate protein. Subsequently, the mixture was vortexed for 60 s and then was centrifuged at 12,000 × *g* for 10 min at 4 °C. The supernatant was transferred into another EP tube and evaporated to dryness under the stream of nitrogen in a water bath at 40 °C. The residue was dissolved in 50 μL of acetonitrile–water (4:96, v/v) and then centrifuged at 12,000 × *g* for 10 min. The supernatant was transferred into an auto-sample vial, and a 10 μL aliquot was injected into UHPLC-MS/MS system for analysis.