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Determination of geniposide in adjuvant arthritis rat plasma by ultra-high performance liquid chromatography tandem mass spectrometry method and its application to oral bioavailability and plasma protein binding ability studies

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ABSTRACT

A specific, sensitive and high throughput ultra-high performance liquid chromatography–electrospray ionization tandem mass spectrometric method (UHPLC–ESI-MS/MS) was established and validated to assay geniposide (GE), a promising anti-inflammatory drug, in adjuvant arthritis rat plasma: application to pharmacokinetic and oral bioavailability studies and plasma protein binding ability. Plasma samples were processed by de-proteinised with ice-cold methanol and separated on an ACQUITY UPLC™ HSS C18 column (100 mm × 2.1 mm i.d., 1.8 μm particle size) at a gradient flow rate of 0.2 mL/min using acetonitrile–0.1% formic acid in water as mobile phase, and the total run time was 9 min. Mass detection was performed in selected reaction monitoring (SRM) mode with negative electro-spray ionization includes the addition of paeoniflorin (Pae) as an internal standard (IS). The mass transition ion-pair was followed as *m/z* 387.4 → 122.4 for GE and *m/z* 479.4 → 449.0 for IS. The calibration curves were linear over the concentration range of 2–50,000 ng/mL with lower limit of quantification of 2 ng/mL. The intra-day and inter-day precisions (RSD, %) of the assay were less than 8.4%, and the accuracy was within ±6.4% in terms of relative error (RE). Extraction recovery, matrix effect and stability were satisfactory in adjuvant arthritis rat plasma. The UHPLC–ESI-MS/MS method was successfully applied to a pharmacokinetic study of GE after oral administration of depurated GE at 33, 66, 132 mg/kg and intravenous injection at 33, 66, 132 mg/kg in adjuvant arthritis (AA) rats. In addition, it was found that GE has rapid absorption and elimination, low absolute bioavailability, high plasma protein binding ability in AA rats after oral administration within the tested dosage range. It suggested that GE showed slow distribution into the intra- and extracellular space, and the binding rate was not proportionally dependent on plasma concentration of GE when the concentration of GE was below 5.0 μg/mL.

1. Introduction

Rheumatoid arthritis (RA) is a chronic, inflammatory and systemic autoimmune disease, which is characterized by an inflammatory process in synovium resulting in progressive destruction of cartilage and bone in affected joints [1–3]. In addition, it is the most common inflammatory arthritis and a major cause of disability [4].

GE (Fig. 1A), a promising anti-inflammatory drug, is a water-soluble iridous glycoside purified from gardenia fruit. In recent years, studies have focused on the pharmacological activities of GE, such its anti-thrombotic [5], anti-inflammatory [6,8], antitumor [9,10], immunosuppression [11], neuroprotection [12,13], hypoglycemic effect [14,15]. The anti-flammatory activity of GE has attracted special attention, and several in vivo experiments involving the compound have been carried out in animals and human [7,16]. AA is widely used as an experimental model, which shares some features with human RA in some pathological, histological and immunological aspects [17].

Previous studies had reported pharmacokinetics of GE in rat after oral administration, such its pharmacokinetics study [18,19],

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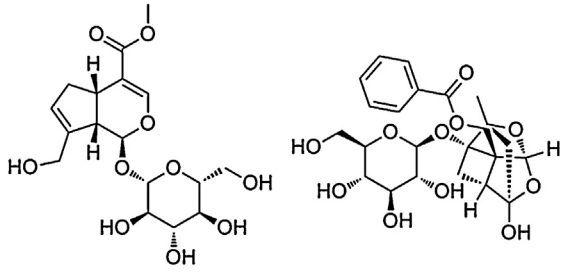

A geniposide **B** paeoniflorin

Fig. 1. Structure of geniposide (A) and paeoniflorin (B).

metabolites [20–22], absolute oral bioavailability [23,24] in normal rats or mice. More detailed information of pharmacokinetic and oral bioavailability and protein binding ability in AA rat is still lacking. Therefore, this study goes a step forward into the rat models with adjuvant arthritis, in addition, the analytical strategies developed for quantitative LC–QqQ-MS (AB SCIEX Triple Quad 4500) with accurate mass information by utilization of a potential IS. Paeoniflorin (Pae, Fig. 1B) was used as the IS because of its nearly identical chemical and physical properties to the target analyte.

To date, ultra high performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry is widely used for the quantitative bioanalysis of some small molecule drugs in the pharmaceutical industry due to its superior sensitivity and selectivity. However, the ever-increasing demands for high-throughput bioanalysis have often caused LC–MS/MS methods with minimum sample preparation and chromatography, where large amounts of endogenous matrix components and sampling error affect the accuracy of the method [25]. One method for controlling the matrix effect during LC/MS/MS experiments is through the utilization of an IS in the form of a stable analog because it has nearly identical chemical and physical properties to the target analyte [26]. In theory, since the IS was almost identical in structure to and co-elutes with the analyte, the degree of ionization suppression or enhancement caused by the co-eluting matrix components should be the same for the IS and analyte. Therefore, although the absolute response or injection may be affected, the analyte to IS peak area ratio should be unaffected and the bioanalytical method should be accurate, precise and rugged.

2. Experimental

2.1. Materials

GE (purity > 99.7%, determined by UFLC XR, Shimadzu, Kyouto, Japan), standard of Pae (purity > 99.7% determined by UFLC XR, Shimadzu, Kyouto, Japan) were supplied by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). LC grade formic acid was obtained from ROE (Newark, NJ, USA). Deionized water was prepared by Milli-Q Ultrapure water purification system (Millipore, Bedford, MA, USA). Acetonitrile and methanol, HPLC grade, were obtained from Fisher (Shanghai, China). Physiological saline samples (brand A, plastic bag) were purchased from the Tian Bao Biopharmaceutical, Ltd., Guangdong Province (Guangzhou, China).

2.2. Apparatus

Instrumentation-MS/MS analysis was constitute of an AB SCIEX Triple Quad™ 4500 Mass Spectrometers (AB SCIEX, Framingham, MA, USA) interfaced to an Agilent 1290 binary pump HPLC system (Agilent Technologies Inc., Santa Clara, CA, USA) fitted with an ACQUITY UPLC™ HSS C18 column (100 mm × 2.1 mm i.d., 1.8 μm particle size, Waters, Milford, MA, USA). Centrifuge (Eppendorf 5430R, Hamburg, Germany) was purchased from Eppendorf. Centrifuge (Heraeus Multifuge X1R, Waltham, MA, USA) was obtained from Thermo Scientific. Centrifugal filter units (Centrifree YM-30 regenerated cellulose membrane, MWCO 30K) were purchased from Millipore (Billerica, MA, USA). pH meter (SevenMulti™ S40, Zurich, Switzerland) was obtained from Mettler-Toledo.

2.3. UHPLC–ESI-MS/MS condition

2.3.1. Chromatographic conditions

Mobile phases consisted of 0.1% formic acid (pH = 2.70 ± 0.05) in water (A) and acetonitrile (B). The gradient applied was as follows: from 0 to 1 min 90% A and 10% B, from 1 to 4 min to 70% A, from 4 to 7 min to 90% A, and from 7 min 90% A and 10% B. Run time was 9 min followed by a 1 min delay prior to the next injection. Column temperature was kept at 20 °C. Flow rate was 0.2 mL/min and injection volume was 2 μL.

2.3.2. Mass spectral conditions

The mass spectrometer equipped with ESI source was operated in the SRM mode. Sample introduction and ionization was in the negative ion mode. The ionspray voltage and source temperature were maintained at −4500 V and 400 °C, respectively. High-pure nitrogen was used as nebulizing gas (75 psi), auxiliary gas (65 psi) and curtain gas (30 psi). The optimized SRM parameters for GE and IS are listed in Table 1. The data acquisition was ascertained by Analyst® 1.6.1 Software.

2.4. Animals

Sprague-Dawley (SD) rats (♂, 180 ± 20 g. Grade II, Certificate No. 011) were obtained from the Animal Department of Anhui University of Chinese Medicine (Hefei, China) and acclimatized to our animal house for at least a week in advance to the experiments. All experiments using rats were performed in accordance with Guidelines for Animal Experiments of Anhui University of Chinese Medicine and euthanized under anesthesia condition. Rats were randomly divided into eight groups, namely, normal group, AA group, low-dosage AA group (i.g.), middle-dosage AA group (i.g.), high-dosage AA group (i.g.), low-dosage AA group (i.v.), middle-dosage AA group (i.v.) and high-dosage AA group (i.v.) and there were six rats in each group. AA was induced as previously described [20]. In brief, AA rats were immunized on day 0 by a single intradermal injection into the left hind paw of 100 μL Freund's complete adjuvant (FCA) for each rat, and normal rats were given an equal volume of physiological saline instead. All the rats were assessed daily for signs of arthritis by two independent observers who were not aware of the treatment. Non-injected hind paw volume was determined with YLS-7A volume meter (Shandong Academy of Medical Sciences Equipment Station, China). The severity of arthritis in each paw (paw swelling) was graded on a scale of 0–4: 0 for no swelling, 1 for isolated swelling of finger joints, 2 for swelling of phalanx joint and digits, 3 for severe inflammation of the entire paws, 4 for deformity or ankylosis. The maximum joint score was 12 including three secondary arthritis paws for each rat.

2.5. Collection of samples

Day 18 after immunization, all the rats were fasted for 12 h, and then given intragastrically GE at 132, 66 and 33 mg/kg (in physiological saline) for low-dosage AA group (i.g.), middle-dosage AA group (i.g.) and high-dosage AA group (i.g.), respectively, while high-dosage AA group (i.v.), middle-dosage AA group (i.v.) and low-dosage AA group (i.v.) were bolus injected into the blood at