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Determination and pharmacokinetics of orientin in rabbit plasma by liquid chromatography after intravenous administration of orientin and *Trollius chinensis* Bunge extract

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Abstract

A high-performance liquid chromatography (HPLC) method was developed and validated for the determination of orientin in rabbit plasma using ultraviolet (UV) absorbance detection. Orientin is the active constituent of purified herbal extract (TRO PE) from the flower of *Trollius chinensis* Bunge. Protein precipitation was used as the sample preparation technique. A Diamonsil C_{18} column (150 mm × 4.6 mm, 5 μm) was equilibrated with a mobile phase composed of 0.1% acetic acid/methanol/acetonitrile (80/5/15, v/v/v). The calibration curve of orientin in rabbit plasma was linear in the concentration range of 0.530–53.0 μg/mL. This validated method was successfully applied to a pharmacokinetic study in rabbits after the intravenous administrations of orientin and TRO PE at three different doses.

Keywords: Orientin; Liquid chromatography with ultraviolet detection; Rabbit plasma; Pharmacokinetics

1. Introduction

Orientin exists widely in plants of many families [1–6] and has a wide variety of biological activities such as radioprotection, vasorelaxant, antioxidative property, free-radical-inhibiting and antiviral activity [2,5,7–10]. For example, literatures indicate that very low non-toxic dose of orientin provides efficient protection of normal tissues in radiotherapy [7]. In addition, pharmacological studies suggest that orientin protects against foetal irradiation-induced genomic damage and instability, thereby reducing the delayed chromosomal abnormalities and tumorigenesis in adult [9].

The dried flower of *Trollius chinensis* Bunge listed in the medical literature in *Zhong Hua's Herbal Classic*, possesses antimicrobial and antiviral actions and has been used widely for a long time to treat cold, fever, chronic tonsillitis and acute tympanitis [11]. The purified herbal extract (TRO PE) from the flower of *T. chinensis* Bunge is being developed as an anti-inflammatory and anti-febrile agent. Orientin, as one of the main active constituents of TRO PE, is responsible for pharmacological effects of the herbal extracts.

Several analytical methods, including liquid chromatography –mass spectrometric method (LC–MS) [12–15], thin-layer chromatography (TLC) [16], high-speed counter-current chromatography (HSCCC) [17], high-performance thin layer chromatography (HPTLC) [18] and high-performance liquid chromatography (HPLC) [19–23], have been used for identification, determination and isolation of orientin from various herbal medicines and foods. However, to our knowledge, there is no published method for the determination of orientin in biological fluids and little literature information on the pharmacokinetics of orientin in humans or animals. It is plausible that an elucidation of the pharmacokinetics study of orientin would lead to a better understanding of the mechanism of action and facilitate further research and development of orientin, *T. chinensis* Bunge and Chinese herbal compound formulations in which *T. chinensis* Bunge is the major ingredient.

The aim of this study is to develop a validated method for determination of orientin in rabbit plasma and apply this method to the pharmacokinetic study of orientin in rabbit plasma after intravenous administration of orientin and TRO PE at three doses, respectively. Therefore, the present study represents the

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first in vivo study in which the pharmacokinetics of orientin was characterized after orientin and TRO PE dosing.

2. Experimental

2.1. Drugs and reagents

Orientin (purity >98% by HPLC) was isolated from *Trollius ledibouri* Reichb. in our laboratory [12]. The internal standard (I.S.), puerarin (99% purity by HPLC) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The chemical structures of orientin and puerarin are given in Fig. 1. Acetonitrile and methanol used for the mobile phase (HPLC grade) and other reagents used for the sample preparation (analytical grade) were obtained from Yuwang Reagent Company (Shandong, China). Double distilled water was used for all the preparations. The dried flower of *T. chinensis* Bunge was collected from the Good Agricultural Practices (GAP) Bases of Traditional Chinese Medical Materials in Chengde (Chengde, Hebei, China) and a voucher specimen (JLHCD0409) is deposited in Shenyang Pharmaceutical University for future reference. TRO PE was prepared as follows in our laboratory. The dried flower of *T. chinensis* Bunge (100 g) was refluxed with boiling water (1.2 L) for 1 h. The extraction was repeated for three times and the extract was filtered, concentrated under reduced pressure, and then precipitated with aqueous ethanol. The precipitate was discarded and the supernatant was loaded on an AB-8 macroporous resin (Nankai University, Tianjin, China) column (70 cm × 3.0 cm i.d.) eluting with aqueous ethanol. The eluate was lyophilized to obtain the TRO PE. The yield of the extract prepared by the above procedure was 7% (w/w). The content of orientin in the dried flower of *T. chinensis* Bunge was determined as 2.2% and that in the TRO PE was 20% using HPLC method.

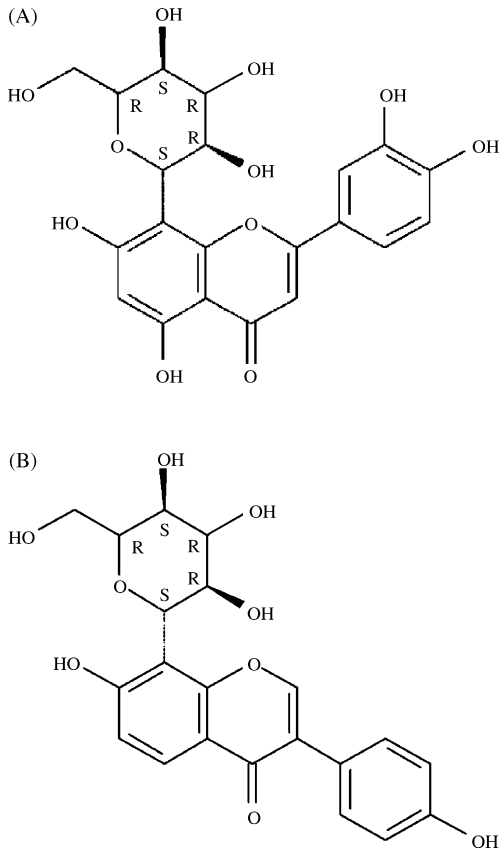

Fig. 1. Chemical structures of orientin (A) and puerarin (I.S.) (B).

2.2. Chromatographic conditions

The HPLC system consisted of a Model LC-10AP vp pump coupled to a Model SPD-10A vp ultraviolet–visible detector (Shimadzu, Kyoto, Japan). Chromatographic separation of orientin and the internal standard was achieved on a Diamonsil C_{18} column (150 mm × 4.6mm, 5 μm) from Dikma Technologies (Beijing, China) protected by a guard column (Phenomenex SecurityGuard™, C_{18} ODS, 4 mm × 3.0 mm, Cheshire, UK). Detection was performed at a wavelength of 340 nm at room temperature. The mobile phase consisted of 0.1% acetic acid/methanol/acetonitrile (80/5/15, v/v/v) at a flow rate of 1.0 mL/min. A 20 μL volume of sample was injected for each separation. Data acquisition and management were achieved with a CBM-102 chromatographic workstation (Shimadzu, Kyoto, Japan).

2.3. Preparation of standard and quality control samples

Stock solution of orientin was prepared in methanol at the concentration of 212 μg/mL and stored at 4 °C and it was further diluted in methanol to make working standards. Solution of internal standard was prepared and diluted to 40 μg/mL with methanol. Calibration curves were prepared by addition of working standards of orientin to blank plasma giving final concentrations of 0.53, 1.06, 2.12, 5.30, 13.3, 26.3 and 53.0 μg/mL. The QC samples were separately prepared in blank plasma at the concentrations of 1.06, 5.30 and 42.4 μg/mL. The spiked plasma samples were stored at −20 °C prior to analysis.

2.4. Preparation of plasma samples

To 100 μL of plasma, 50 μL of internal standard solution (puerarin, 40 μg/mL in methanol) and 250 μL methanol was added and vortexed for 1 min. The mixture was centrifuged at 10,000 × *g* for 10 min and 20 μL of the supernatant was injected into the HPLC.

2.5. Method validation

2.5.1. Selectivity

Selectivity was investigated by comparing chromatograms of blank plasma obtained from rabbits prior to dosing with those of corresponding standard plasma sample spiked with orientin and I.S. (40 μg/mL) and plasma sample from rabbits after intravenous doses of orientin and TRO PE. Blank samples of all matrixes were extracted to ensure the absence of interfering peaks.