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The pharmacokinetics and oral bioavailability studies of columbianetin in rats after oral and intravenous administration

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ABSTRACT

Ethnopharmacological relevance: The roots of *Angelica pubescens* Maxim. *f. biserrata* Shan et Yuan (RAP) has been used as Traditional Chinese medicine to treat rheumatic disease in China since ancient times, but its action mechanisms was not well understood. Columbianetin is one of the main active constituents isolated from RAP, which has been shown to have various biological activities, but the absorption characteristics and oral bioavailability dose proportionality of columbianetin *in vivo* were not studied.

Materials and methods: Male Sprague Dawley rats (210–230 g) received either an intravenous (i.v. 5, 10 and 20 mg kg^{-1}) or oral (5, 10 and 20 mg kg^{-1}) dose of columbianetin. The levels of columbianetin in plasma were measured by a simple and sensitive reversed-phase high-performance liquid chromatography (HPLC) method. The simple liquid–liquid extraction with ethyl acetate was used for sample preparation. Osthole was selected as internal standard (IS).

Results: The chromatographic separation was accomplished on a C_{18} column at a flow rate of 1 mL min^{-1}, where water–methanol was used as mobile phase. The calibration curve of the method was linear in the concentration range of 0.05–2000 μg mL^{-1}. The intra and inter-day accuracy for columbianetin in rat plasma samples were within 8% and the variation was less than 8.3%. This method was suitable for the determination and pharmacokinetic study of columbianetin in rat plasma after both intravenous and oral administration. The results indicated that maximum plasma concentrations(C_{max}) for the columbianetin (17–42 μg mL^{-1}) were achieved at 0.3–0.5 h post-oral dosing and the apparent volume of distribution (V/F) ranged from 0.38 to 0.44 L. Absolute bioavailability of columbianetin was assessed to be 81.13 ± 45.85, 81.09 ± 33.63 and 54.30 ± 23.19%, respectively. Terminal elimination half-life ($T_{1/2}$) of the columbianetin after oral dosing was 60–90 min and were 2.5–3.3 fold longer than those observed for the i.v. dosing.

Conclusions: The pharmacokinetic properties of columbianetin in rat after oral administration were characterized as rapid oral absorption, quick clearance and good absolute bioavailability. The bioavailability of columbianetin ranged from 54 to 81% for 5, 10 and 20 mg kg^{-1} oral doses. The bioavailability of columbianetin is independent of the doses studied. Columbianetin showed dose proportionality over the dose range 5–20 mg kg^{-1}. The results clearly demonstrated that columbianetin was one of the material bases of RAP. Furthermore, an HPLC method was demonstrated in this study for the research of traditional Chinese medicine.

1. Introduction

Traditional Chinese medicines (TCMs) have been used in clinical practice for several thousand years and a large number of TCMs are proved to have beneficial effect. TCMs play an indispensable role in the prevention and treatment of diseases. Nowadays, the active compounds from natural medicines have attracted more and more attention. The pharmacokinetic and bioavailability studies on active constituents in TCMs would have an important impact in evaluating their mechanism of action, and also a good way in appreciating us to elaborate the efficacy of TCMs.

The roots of *Angelica pubescens* Maxim. *f. biserrata* Shan et Yuan (RAP), officially listed as an anodyne and remedy for rheumatic disease in the Chinese Pharmacopeia, has been widely used as an important component in various prescriptions in TCM in China

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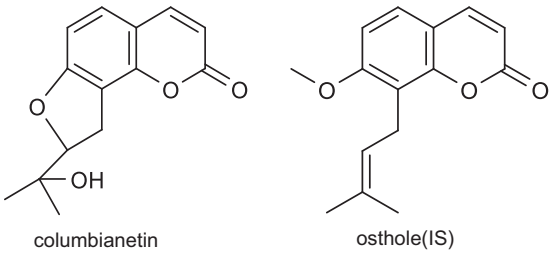

Fig. 1. Chemical structures of columbianetin and osthole (IS).

since ancient times. Columbianetin (Fig. 1) is one of the main active constituents isolated from RAP, which has been shown to have various biological activities, including antioxidation (Ng et al., 2000), cytotoxic (Hideji et al., 1994) and anti-inflammatory (Jeong et al., 2009) activities. For example, columbianetin can significantly reduce NO production in LPS-stimulated murine macrophage cell line RAW264.7, suggesting that columbianetin is potential candidate for the research and development of anti-inflammatory agent (Zhang et al., 2012). Recent studies demonstrated that columbianetin possessed the protective effect against UVB-induced human keratinocyte damage and a potential dermatological and cosmetological value (Ahn et al., 2012). The above research suggested that columbianetin could be an effective natural compound in further new drug investigation.

In view of these beneficial effects, detailed *in-vivo* pharmacokinetics and bioavailability studies of columbianetin were required. Pharmacokinetic studies have been reported for columbianetin after oral administration of RAP extract in rats from previous studies (Chang et al., 2011). However, the pharmacokinetic behavior of columbianetin after oral administration of pure columbianetin was not reported. From available literature reviews, the bioavailability of columbianetin in rats or other experimental animals has not been studied. Columbianetin is presently undergoing preclinical development. Therefore, it is necessary to evaluate the pharmacokinetics and oral bioavailability of columbianetin for drug development and clarify the action mechanism of RAP.

In these present studies, a simple and sensitive high-performance liquid chromatographic (HPLC) method with DAD detection has been developed for the quantitative determination of columbianetin in rat plasma using osthole as an internal standard (IS). This analytical method has been successfully applied to the pharmacokinetics and oral bioavailability studies of columbianetin after oral administration to healthy rats.

2. Experimental

2.1. Chemicals and reagents

Methanol (Tianjin Concord Science Co. Ltd., Tianjin, China) was of HPLC grade. RAP was obtained from Anguo city in Hebei province. The procedure for the identification was executed by Professor Lin Ma of Tianjin University of Traditional Chinese Medicine. The voucher specimens were deposited in the Herbarium of Pharmacognosy, Tianjin University of Traditional Chinese Medicine (Tianjin, China). Columbianetin (purity > 98%) and osthole (IS, purity > 98%) were isolated from *radix angelicae pubescentis* in our laboratory. Their structures were confirmed by 1H NMR, IR, HPLC and MS spectra. Deionized water was purified with a Milli-Q Academic ultra-pure water system (Millipore, Milford, MA, USA).

2.2. Instrumentation and chromatographic conditions

The HPLC analysis was carried out on an Agilent 1260 series liquid chromatographic system (Agilent Technologies, Santa Clara, CA, USA) equipped with G1311C quaternary pump, G1316A thermostatted column compartment, G1329B auto-sampler and G1315D diode array detector (DAD). Chromatographic separation was performed on an analytical column (Agilent ZORBAX SB-C18, 4.6 × 250 mm I.D., 5 μm particle size). The mobile phase was methanol (A) and (B) water, using a gradient elution of 65% (v) A at 0–6 min; 65–81% A at 6–8 min; 81% A at 8–15 min; the re-equilibration time of gradient elution was 5 min. The flow rate was set at 1 mL min^{-1}. The chromatograms were monitored at 325 nm. The column temperature was maintained at 25 °C. The injection volume was 20 μL.

2.3. Isolation of osthole and columbianetin

The dried roots of RAP were finely cut and soaked 8 h before percolation, extracted with 75% ethanol at solid–liquid ratio of 10:1. The ethanol extract was obtained by evaporating the solvent under reduced pressure. The ethanol extract was subjected to normal-phase silica column chromatography (petroleum ether–ethyl acetate (7:2–3:1, v/v)) to give 12 fractions. Fraction 5 and 7 were osthole and columbianetin, respectively. The purity of osthole and columbianetin were more than 98%.

2.4. Preparation of stock solution

The stock solution was prepared by dissolving appropriate columbianetin in methanol to achieve a concentration of 2.0 mg mL^{-1}. The stock solution of IS was also dissolved in methanol and diluted with methanol to a final concentration of 10 μg $mL^{-1}$and stored at 4 °C until analysis.

2.5. Preparation of samples and quality control samples

To an aliquot of 100 μL sample, 10 μL IS solution was added. Samples were vortex-mixed for 30 s, extracted with 1 000 μL ethyl acetate and then centrifuged for 1 min at 14000 rpm. Supernatant was transferred into another centrifuge tube and evaporated to dryness by nitrogen gas. The dried residue was reconstituted in by 100 μL methanol. The solution was swirled and ultrasonicated for 1 min. It was centrifuged at 14,000 rpm for 10 min. A 20 μL of the solution was injected into the HPLC system for analysis.

Quality control (QC) samples (0.2, 20 and 200 μg mL^{-1}) were prepared by spiking blank rat plasma with appropriate standard solutions of columbianetin to the required plasma concentrations, followed by the same sample preparation and extraction method described above.

2.6. Method validation

2.6.1. Specificity and sensitivity

The specificity was tested by comparing the chromatograms of six different batches of blank rat plasma samples with the corresponding spiked plasma. The lower limit of quantification (LLOQ) for determination of columbianetin in plasma, defined as the lowest concentration in the standard curve at which the signal to noise ratio (*S*/*N*) was preliminary found to be larger than 5 with accuracy within ±20% and the relative standard deviation (RSD) (n=6) was within 20% (Guidance for Industry, 2001).