Pharmacokinetic UPLC–MS/MS Studies on Byakangelicol after Oral and Intravenous Administration to Rats

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Byakangelicol is one of coumarins from Baizhi and has been shown to inhibit the release of PGE2 from human lung epithelial A549 cells in a dose-dependent manner. A sensitive ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) method was developed and full validated for the quantification of byakangelicol in rat plasma. The pharmacokinetics of byakangelicol after both intravenous (5 mg/kg) and oral (15 mg/kg) administrations were studied. Chromatographic separation was performed on an ultra-performance liquid chromatography ethylene bridged hybrid (UPLC BEH) C18 column with acetonitrile and 0.1% formic acid as the mobile phase at a flow rate of 0.4 mL/min; fargesin was used as the internal standard (IS). The following quantitative analysis of byakangelicol was utilized in the multiple reaction monitoring mode. The samples were extracted from rat plasma via protein precipitation using acetonitrile. In the concentration range of 1–2000 ng/mL, the method correlated linearity (r > 0.995) with a lower limit of quantitation (LLOQ) of 1 ng/mL. Intra-day precision was less than 11%, and inter-day precision was less than 12%. The accuracy was between 92.0% and 108.7%, the recovery was better than 89.6%, and the matrix effect was between 85.9% and 98.6%. The method was successfully applied to a pharmacokinetic study of byakangelicol after intravenous and oral administration, and the absolute bioavailability was 3.6%.

Keywords: byakangelicol, pharmacokinetics, bioavailability, rat, UPLC–MS/MS

Introduction

Herbal medicines play an important role for disease prevention in China. Remedies of Traditional Chinese medicine (TCM) are typically formulated in a unique way and contain multiple herbs [1, 2]. Angelica dahurica (Fisch. ex Hoffm.) Benth. et Hook. f. var. formosana (Boiss.) Shan et Yuan, with the Chinese name Baizhi, has been widely used as a herb and spice for more than 2000 years [3, 4]. Significant pharmacological activities were found in Baizhi, such as anti-inflammation, analgesia, inhibition of pathogenic microorganisms, anti-tumor, and liver protection [5–7]. Therefore, Baizhi attracted wide scientific attention [8–10]. In recent years, it has been reported that Baizhi could be applied for the scavenging of free radicals, whitening, and treating vitiligo. Coumarins are one type of the main chemical constituents of Baizhi and are renowned for their diverse biological activities [11].

Byakangelicol is one of coumarins from Baizhi and has been shown to inhibit the release of PGE2 from human lung epithelial A549 cells in a dose-dependent manner. This effect may be achieved by the selective inhibition of the expression of COX-2 and the activity of COX-2 [12, 13]. As a drug for the treatment of respiratory tract inflammation, byakangelicol has a promising application prospect. Over recent years, natural drugs have greatly contributed to the prevention and treatment of diseases [14, 15]. The pharmacological research of byakangelicol and its potential clinical application should be further explored. The pharmacokinetic studies of byakangelicol might further provide physiologically relevant clues for mechanistic study and target identification.

Rapid, simple, and reliable high-performance liquid chromatography with ultraviolet detection (HPLC/UV) and liquid chromatography with electrospray ionization tandem mass spectrometry (LC–ESI-MS/MS) methods for the simultaneous determination of 5 active coumarins of Angelicae dahuricae Radix, byakangelicol, oxypeucedanin, imperatorin, phellopterin, and isoimperatorin were developed and validated [16]. Taken together, the shorter analysis time involved makes these HPLC/UV and LC–ESI-MS/MS methods valuable for the commercial quality control of Angelicae dahuricae Radix extracts and its pharmaceutical preparations [16]. To the best of our knowledge, no report on the pharmacokinetics of byakangelicol has been published. Here, a rapid and simple UPLC–MS/MS method was established for determination of byakangelicol in rat plasma. The pharmacokinetics of byakangelicol after oral (PO) and intravenous (IV) administration was studied.

Materials and Methods

Chemicals and Animals. Byakangelicol (purity >98%, Figure 1A) and fargesin (purity >98%, IS, Figure 1B) were purchased from Mansite (Chengdu, China). LC-grade methanol...
and acetonitrile were purchased from Merck (Darmstadt, Germany). Deionized water was obtained using a Millipore Milli-Q purification system (Bedford, USA). Sprague–Dawley rats (male, weight 200–220 g) were purchased from the Animal Experiment Center of Wenzhou Medical University (Wenzhou, China).

Instrument and Conditions. The UPLC–MS/MS system consisted of the following components: an ACQUITY H-Class UPLC instrument, a XEVO TQS-micro triple-quadrupole mass spectrometer (Waters Corp., Milford, USA), and Masslynx 4.1 software (Waters Corp.) for data collection and instrument control.

Prepared samples were separated at 30 °C, using an UPLC BEH C18 (2.1 mm × 50 mm, 1.7 μm). The mobile phase consisted of acetonitrile and 0.1% formic acid in gradient elution at a flow rate of 0.4 mL/min. The initial solvent was 10% acetonitrile and was maintained for 0.2 min, linearly increased to 80% in 1.3 min, maintained for 0.5 min, and then returned to the initial condition of 10% within 0.5 min. The column was equilibrated for 1.5 min until the following injection was started; the elution time was 4 min.

Nitrogen was used as both desolvation gas (800 L/h) and cone gas (50 L/h). Mass spectrometer parameters were ion source temperature of 150 °C, deionization temperature of 400 °C, and ion spray voltage of 2.5 kV. The instrument was equipped with an electrospray ionization source (ESI) in the positive ion detection mode. Multiple reaction monitoring modes (MRM) of m/z 371.0 → 233.1 for byakangelicol and m/z 371.0 → 353.2 for fargesin (IS) were obtained for quantitative analysis.

Preparation of Stock Solutions. Byakangelicol (1.0 mg/mL) and fargesin (0.1 mg/mL) stock solutions were prepared with acetonitrile. Working solutions of byakangelicol were prepared with stock solution via dilution with acetonitrile. The working solution of fargesin (50 ng/mL) was prepared via dilution with the fargesin stock solution using acetonitrile. All of these solutions were stored at 4 °C until the assay.

Preparation of the Calibration Curve. Calibration standards were prepared by spiking appropriate working solution in blank rat plasma (1, 2, 5, 10, 50, 100, 200, 500, 1000, and 2000 ng/mL), which covers the range between 1–2000 ng/mL. Quality control (QC) samples were prepared with 4 concentrations (1, 4, 450, and 1800 ng/mL) in the same manner.

Sample Preparation. The 50-μL rat plasma sample was pipetted into a 1.5-mL polypropylene tube, and 150 μL acetonitrile (IS 50 ng/mL) was added. The mixture was vortex-mixed for 1 min, and then centrifuged at 13000 rpm for 10 min (4 °C). The supernatant (100 μL) was transferred into a liner pipe in vial, and then, 2 μL of the supernatant was analyzed via UPLC–MS/MS.

Method Validation. The verification method was established in accordance with the US Food and Drug Administration (FDA) bioanalytical method validation guidelines. Validation projects include selectivity, matrix effects, linearity, precision, accuracy, recovery, and stability.

Pharmacokinetics. Twelve rats were randomly divided into 2 groups: 6 rats for intravenous administration of a single dose of byakangelicol (5.0 mg/kg) and 6 rats for oral administration (15.0 mg/kg). One-hundred-fifty-microliter blood samples were collected in 1.5-mL polypropylene tubes with heparin from the tail vein of each rat at 0.0833, 0.25, 1, 2, 3, 4, 6, 8, 12, and 24 h. The samples were centrifuged at 3000 rpm for 10 min, and 50 μL plasma was finally obtained and cryopreserved at −20 °C.

The following pharmacokinetic parameters were processed with DAS 2.0 software: the area under the plasma concentration-time curve (AUC), the mean residence time (MRT), the apparent distribution volume (V), the plasma clearance (CL), the maximum plasma concentration (Cmax), and the half-life (t1/2).

Results and Discussion

Method Development. In positive electrospray ionization, byakangelicol is more sensitive than in negative ionization. Different chromatographic conditions were used to optimize the sensitivity, chromatographic peak type, and analysis speed of the method [22–24]. In this work, we used acetonitrile–10 mmol/L ammonium acetate (containing 0.1% formic acid), acetonitrile–0.1% formic acid, methanol–0.1% formic acid, and methanol–10 mmol/L ammonium acetate (containing 0.1% formic acid) as mobile phase. Finally, acetonitrile–0.1% acid was chosen, yielding both satisfactory chromatographic peak and retention time.

Prior to analysis, the removal of protein and potential interferences is a key point of the method [22–24]. A single-step protein precipitation was involved in this study, and acceptable recovery and matrix effects were obtained when protein precipitation used acetonitrile.

Method Validation. Interference between endogenous substance and impurity was not found, which indicated that the method had good selectivity.

The calibration curve of byakangelicol in rat plasma remained within a concentration range of 1–2000 ng/mL. The linear function was \( y = (0.0227 \pm 0.0018)x + (0.0078 \pm 0.0013) \) (\( r = 0.9962 \)), where \( y \) represents the peak area ratio of byakangelicol and IS, and \( x \) represents the byakangelicol in plasma; the lower limit of quantification (LLOQ) of byakangelicol was 1 ng/mL.

As shown in Table 1, the intra-day and inter-day precision of byakangelicol in the rat plasma was below 11% and 12%,
Table 1. Accuracy, precision, matrix effect, and recovery of byakangelicol in rat plasma

| Concentration (ng/mL) | Accuracy (%) | Precision (RSD%) | Matrix effect (%) | Recovery (%) |
|-----------------------|--------------|------------------|------------------|--------------|
|                       | Intra-day    | Inter-day        | Intra-day        | Inter-day    |
| 1                     | 92.0         | 94.8             | 10.3             | 11.4         | 85.9 ± 9.8 | 89.6 ± 7.4 |
| 4                     | 99.8         | 108.7            | 6.4              | 7.6          | 89.6 ± 4.2 | 89.6 ± 8.4 |
| 450                   | 101.1        | 95.1             | 6.3              | 5.8          | 95.0 ± 4.2 | 95.0 ± 4.2 |
| 1800                  | 100.1        | 100.7            | 5.7              | 0.9          | 98.6 ± 3.2 | 96.5 ± 2.1 |

Table 2. Main pharmacokinetic parameters of rats after byakangelicol administration

| Parameters | Unit | IV 5 mg/kg | PO 15 mg/kg |
|------------|------|------------|-------------|
| AUC(0–∞)  | ng/mL*h | 1355.2 ± 236.0 | 144.7 ± 73.9 |
| AUC(0–t)  | ng/mL*h | 1357.7 ± 237.0 | 146.1 ± 74.5 |
| MRT(0–t)  | h     | 1.4 ± 0.1  | 1.5 ± 0.3   |
| MRT(0–∞)  | h     | 1.5 ± 0.2  | 1.6 ± 0.3   |
| t1/2     | h     | 5.2 ± 5.9  | 1.3 ± 0.5   |
| T max    | h     | 0.083      | 0.8 ± 0.4   |
| CL/F     | L/h/kg | 3.8 ± 0.8   | 128.3 ± 68.1 |
| Vc/F     | L/kg   | 27.8 ± 28.9 | 255.6 ± 223.0 |
| C max    | ng/mL  | 783.9 ± 92.1 | 77.2 ± 20.7 |
| F        | %     |            | 3.6%        |

respectively. The accuracy ranged from 92.0% to 108.7%, the recovery exceeded 89.6%, and the matrix effect ranged between 85.9% and 98.6%.

Samples stability was determined under 3 different storage conditions. The short-term temperature stability was assessed via thawing at room temperature (25 °C) for 6 and 24 h. Long-term stability was assessed by storing samples at −20 °C for 30 days. The freeze–thaw stability was evaluated after 3 freeze–thaw cycles (within a range from −20 to 25 °C) on consecutive days. Byakangelicol was found to be stable within ±13% (accuracy), and the %RSD was below 12%.

Pharmacokinetics. A non-compartmental model was used to assess the main pharmacokinetic parameters, as shown in Table 2. The concentration–time curves of byakangelicol after oral administration (15 mg/kg) and intravenous administration (5 mg/kg) are shown in Figure 2. The results indicate that the AUC and C max after intravenous administration far exceeded that of oral administration, and the bioavailability of oral administration was 3.6%. The oral utilization rate is low, which limits its application to a certain extent.

Conclusions

This is the first report regarding determination of byakangelicol in plasma, only 50-μL plasma was used, and the linear range was 1–2000 ng/mL. A single-step acetonitrile direct preparation method was used to process the samples. The UPLC–MS/MS method was successfully applied to a pharmacokinetic study of byakangelicol in rat plasma.

Conflict of Interest Statement

The author(s) declare that there is no conflict of interest regarding the publication of this paper.

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