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Short communication

Determination of imperatorin in rat plasma by reversed-phase high-performance liquid chromatography after oral administration of Radix Angelicae dahuricae extract

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Abstract

A simple high-performance liquid chromatographic (HPLC) method has been developed for the determination of imperatorin in rat plasma and applied to a pharmacokinetic study in rats after administration of Radix Angelicae dahuricae extract. After addition of fluocinonide as an internal standard (IS), plasma samples were extracted with diethyl ether. HPLC analysis of the extracts was performed on a Diamonsil C18 analytical column using methanol–water (70:30, v/v) as the mobile phase. The UV detector was set at 254 nm. The standard curve was linear over the range 0.04–4.0 μg/mL. The lower limit of quantification was 0.04 μg/mL. The HPLC method developed could be easily applied to the determination and pharmacokinetic study of imperatorin in rat plasma after giving the animals Radix Angelicae dahuricae extract.

Keywords: Imperatorin; Radix Angelicae dahuricae; HPLC

1. Introduction

Radix Angelicae dahuricae, the dried radix of *Angelica dahurica* (Fisch. Ex hoffm.) Benth. Et Hook. f. and *A. dahurica* (Fisch. Ex hoffm.) Benth. Et Hook. f. var. formosana (Boiss.) Shan et Yuan is a well known traditional Chinese medicine (TCM) [1]. It has been widely used in China for over 2000 years for the treatment of headache, toothache, nose congestion resulting from cold and the reduction of swelling and pain from sores and wounds. Coumarins comprise the major constituents in Radix Angelicae dahuricae and imperatorin (Fig. 1) is the one of the active compounds [2]. It has been reported that imperatorin triggers apoptosis of HL-60 cells at micromolar concentrations [3] and significantly inhibits T cell receptor-mediated proliferation in human primary T cells in a concentration-dependent manner [4]. It is nontoxic, and showed high activity in the inhibition of the mutagenicity of benzopyrene [5]. For this reason, imperatorin is used as one of the marker compounds for characterizing the Radix Angelicae dahuricae.

As a knowledge of the pharmacokinetics can help us explain and predict a variety of events related to the efficacy and toxicity of herbal preparations, it is important to investigate the pharmacokinetics of their active constituents. A number of published papers have reported the analysis and pharmacokinetics of active constituents from a number of herbal medicines. LC–MS methods are particularly useful for determining compounds at low levels in biological fluids [6]. While GC–MS methods are more suitable for determining compounds with small molecular weights with low boiling points [7]. Compared with LC–MS methods, HPLC–UV methods have several inherent limitations—lower sensitivity and lack of specificity. Nevertheless HPLC–UV methods are very popular and sensitive enough for the determination of a number of active compounds in some preclinical studies [8,9].

Some methods have been used to study the contents of coumarin and imperatorin in plants [10–12]. To date, there have been no published reports of the assay of imperatorin in rat plasma after oral administration of Radix Angelicae dahuricae extract. The present study reports for the first time, the

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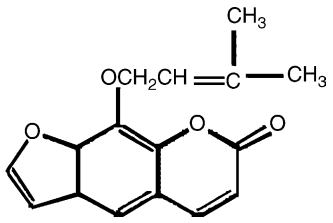

Fig. 1. Chemical structure of imperatorin.

development and validation for the determination of imperatorin concentrations in rat plasma and for its pharmacokinetic study following oral administration of Radix Angelicae dahuricae extract.

2. Experimental

2.1. Materials and reagents

The dried radix of *A. dahurica* (Fisch. Ex hoffm.) Benth. Et Hook. f. was purchased from Zhongxin drug store (Tianjin, China), a voucher specimen (no. AM200321) was deposited in the herbarium of Tianjin University of Traditional Chinese Medicine for future reference. Methanol was of HPLC grade and provided by Shandong Yuwang Chemical Factory (Shandong, China), while all other reagents were of analytical grade. Healthy Wistar rats were obtained from the Experimental Animals Centers of Tianjin University of traditional Chinese medicine. The TGL–16C centrifuge used in this investigation was from Shanghai Anting Science Instrument Factory (Shanghai, China). Imperatorin and fluocinonide used as internal standard (IS) were supplied by the National Institute for the Control of Pharmaceutial and Biological Products (Beijing, China).

2.2. Chromatographic system

The analysis was carried out on an HPLC system (Shimadzu, Japan) equipped with a LC-10ATVP pump, SPD-10AVP UV detector and Antastar workstation. The analyte was determined at room temperature on an analytical column Diamonsil C18 (150 mm × 4.6 mm, i.d., 5 μm). The mobile phase consisted of a mixture of methanol–water (70:30, v/v). The mobile phase was filtered under vacuum through a 0.45 μm membrane filter, and degassed before use. The analysis was carried out at a flow rate of 1.0 mL/min with the detection wavelength set at 254 nm.

2.3. Preparation of Radix Angelicae dahuricae extract

One hundred grams of Radix Angelicae dahuricae was extracted with ethanol (500 mL) by refluxing for 1.5 h in a water-bath at 100 °C, and then filtered. The extraction was repeated twice. The extraction solutions were combined, ethanol was removed under reduced pressure, and the residue was dissolved in water, to give an extract with a concentration of 1 g/mL (expressed as the weight of raw material of Radix Angelicae dahuricae).

2.4. Preparation of standards and quality control samples

Stock standard solutions of imperatorin and the internal standard, fluocinonide (3 μg/mL), were prepared with methanol. Seven calibrators of imperatorin with internal standard were prepared by dilution of stock solutions followed by spiking drug-free plasma, three replicates prepared to calibrators of imperatonin for each concentration. The calibration range was 0.04–4.0 μg imperatorin per millilitre of plasma. Quality control (QC) samples were prepared at low (0.04 μg/mL), medium (0.4 μg/mL) and high (4.0 μg/mL) concentrations in the same way as the plasma samples for calibration.

2.5. Plasma sample preparation

Six Wistar rats (body weight 200 ± 20 g) were not fed for 12 h prior to administration of the drug extract. The rats were then given the extract with an oral dose of 2 g (containing 15 mg of imperatorin)/kg body weight. Animals had free access to water during the experiment. A blood sample (0.4 mL) was collected from the suborbital vein into heparinized tubes at 0, 1, 2, 3, 4, 5, 6, 9, 12 and 24 h following drug administration.

All blood samples were immediately centrifuged for 10 min at 10,000 × *g*, and the plasma was transferred into clean tubes and stored at −20 °C prior to HPLC analysis. To 200 μL of plasma, 100 μL of internal standard, fluocinonide (3 μg/mL), and 400 μL of diethyl ether was added, followed by vortex mixing for 1 min and centrifuging at 10,000 g for 10 min. The extraction was repeated twice with 400 μL diethyl ether. The supernatant was combined and evaporated to dryness under nitrogen at 50 °C. The residue was reconstituted with 100 μL mobile phase and an aliquot (20 μL) was injected into the HPLC system.

3. Results and discussion

3.1. Specificity

Typical chromatogams of blank and spiked plasma with imperatorin and fluocinonide are given in Fig. 2A and B. There were no coeluting peaks in the vicinity of the imperatorin and fluocinonide peaks on the chromatogram of blank plasma. A chromatogram of a plasma samples from a rat at 3 h after oral administration of Radix Angelicae dahuricae extract (2 g/kg body weight) is shown in Fig. 2C.

3.2. Method development

There are three absorption maxima at 219, 254 and 302 nm in the UV spectrum of imperatorin. However, interferences from endogenous substances and constituents of Radix Angelicae dahuricae were observed with detection at 219 and 302 nm. A detection wavelength of 254 nm proved to be the most suitable and was, therefore, selected for the assay.

Diethyl ether was employed as the extraction solvent in our procedure and provided a clean supernatant with a high extraction recovery for imperatorin without any significant

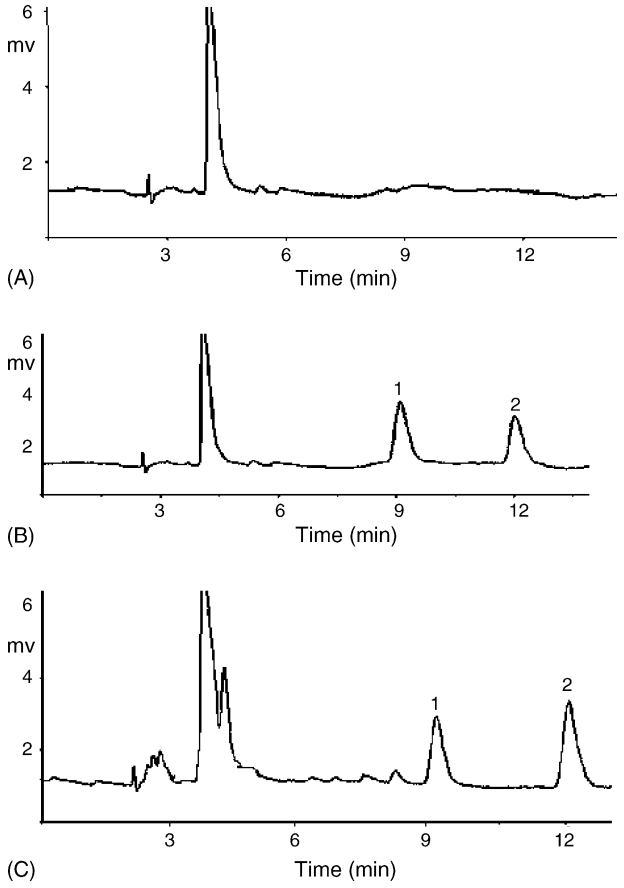

Fig. 2. Representative chromatogram of blank plasma (A); plasma spiked with imperatorin (0.4 μg/mL) (1) and internal standard (3 μg/mL) (2) (B); a plasma sample 3 h after oral administration of Radix Angelicae dahuricae extract (C).

interference. During development of the method, acetonitrile and methanol were tested to deproteinize rat plasma, however, the recovery of imperatorin was unsatisfactory (<50%). Other solvents besides diethyl ether, such as ethyl acetate and chloroform were also tried as the extraction solvent. However, only samples extracted with diethyl ether gave a good resolution and high recovery.

3.3. Calibration and validation

Evaluation of the assay was performed with a seven-point calibration curve over the concentration range 0.04–4.0 μg/mL. Blank plasma was spiked with stock solutions of standard imperatorin to construct the calibration curve. The slope and intercept of the calibration graphs were calculated by weighted least squares linear regression. During the method validation, three sets of calibration standards were prepared and analyzed on three separate days. The regression equation of three standard curves was:

$$y = (1.507 \pm 0.0721)x - (0.00933 \pm 0.00279) \quad (1)$$

where y is the peak area ratio of imperatorin to the internal standard, and x is the plasma concentration of imperatorin. The calibration curve was linear over the concentration range of 0.04–4.0 μg/mL in rat plasma with a mean correlation coefficient of 0.9990.

Table 1
Precision and accuracy of the HPLC-UV method to determine imperatorin in rat plasma ($n = 15$)

Concentration (μg mL^{-1})		RE (%)	Intra-day R.S.D. (%)	Inter-day R.S.D. (%)
Added	Found			
0.04	0.037	−7.5	9.2	7.9
0.4	0.407	1.8	7.7	5.2
4.0	3.89	−2.8	5.9	8.9

The lower limit of quantification (LLOQ) was defined as the lowest drug concentration on the plasma and determined at a signal-to-noise ratio of 10:1. The LLOQ was found to be 0.04 μg/mL for imperatorin in rat plasma with an accuracy (relative error, RE) and precision (relative standard deviation, R.S.D.) not exceeding 20%. The limit of detection (LOD) considering a signal-to-noise ratio 3:1 was estimated to be 0.01 μg/mL for imperatorin in rat plasma. The accuracy and precision of the method were evaluated with QC samples at concentrations of 0.04, 0.4 and 4.0 μg/mL. The results are shown in Table 1. The intra-day and inter-day presicion of the QC samples were satisfactory with R.S.D.s less than 9.2%. The determined values deviated from the nominal concentration with an RE of less than 8.9%.

The extraction recovery was determined by standard addition at three different concentrations (0.04, 0.4 and 4.0 μg/mL) and was calculated by comparing the peak areas of the prepared standard samples with those of the standard solutions. The mean extraction recoveries of imperatorin at the three concentrations was 75.9%, 81.2% and 83.5%, respectively. The extraction recovery of fluocinonide (internal standard) was 80.5%.

The stability of prepared samples at room temperature was examined by comparing the data from samples analyzed immediately with those at 4, 10 and 24 h after sample preparation. The stability of imperatorin in plasma was investiged by using spiked QC samples at three different concentrations prepared in duplicate. The relative errors at the three different concentrations studied were less than 5.8% for imperatorin indicating a stability of at least 24 h at room temperature. The deviation of spiked QC samples stored at −20 °C a week from fresh QC samples were within 8.7%, −7.2% and −6.9%. Imperatorin was stable in rat plasma under these storage conditions.

3.4. Pharmacokinetic applicability

This validated method was applied to monitor the plasma concentrations of imperatorin in rats after a single oral administration of extract at a dose of 2 g (containing 15 mg of imperatorin)/kg body weight. The pharmacokinetic parameters were estimated using the 3P97 computer program (The Chinese Society of Mathematical Pharmacology). The plasma Imperatorin concentration–time curve was fitted at two-compartment open model. The mean plasma concentration–time profile is illustrated in Fig. 3. The pharmacokinetic parameters are presented in Table 2.

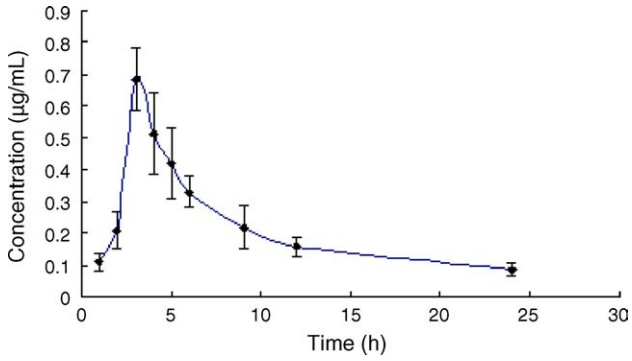

Fig. 3. Plot of the mean concentration of imperatorin in the plasma of rats vs. time after oral administration of Radix Angelicae dahuricae extract.

Table 2
Pharmacokinetic parameters of imperatorin in rats after administration of Radix Angelicae dahuricae extract ($n = 6$)

C_{max} (μg/mL)	0.684 ± 0.099
T_{max} (h)	3.0 ± 0.5
$T_{1/2}$ (h)	8.2 ± 0.5
K_e (h^{-1})	0.0992 ± 0.00778
$AUC_{0-\infty}$ (μg h/mL)	1.18 ± 0.075

The C_{max} of imperatorin was reached after approximately 3.0 h, the $T_{1/2}$ was calculated to be 8.2 h, which indicates this herbal decoction may be administered three times a day. This result explains the suitability of the traditional administration method for Radix Angelicae dahuricae and its compound TCM, three times a day orally. The pharmacokinetic parameters of imperatorin suggest that it may be used as a marker compound to characterize some profiles of the Herbal extract.

4. Conclusion

This paper describes a simple HPLC method with UV detection for the determination of imperatorin in rat plasma. It was applied to the pharmacokinetic study of imperatorins from Radix Angelicae dahuricae extract. It has a potential application in pharmacokinetic studies of traditional Chinese medicines which contain Radix Angelicae dahuricae. A pharmacokinetic study of the active constituents in TCM will play an important role in identifying their mechanisms of action and investigating their synergetic effects.

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