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Pharmacokinetics of honokiol after intravenous administration in rats assessed using high-performance liquid chromatography

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Abstract

A simple and sensitive high-performance liquid chromatographic method for the identification and determination of honokiol in rat plasma has been developed. Up to 0.1 ml of plasma containing honokiol was deproteinized with acetonitrile, which contained an internal standard (paeonol). The supernatant was injected onto a reversed-phase C_{18} column using acetonitrile–water (70:30, v/v, adjusted to pH 2.5–2.8 with orthophosphoric acid) as the mobile phase and ultraviolet detection at 290 nm, followed by UV spectrum identification (between 220 and 380 nm) with a photodiode-array detector. The method was applied to pharmacokinetic studies of honokiol in rat following 5 or 10 mg/kg intravenous administration. A biphasic process consisting of a rapid distribution phase followed by a slower elimination phase was observed from the plasma concentration–time curves. Compartmental analysis yielded a two-compartment model.

1. Introduction

Honokiol is one of the major components of Magnoliaceae [1]. The stem bark of *Magnolia officinalis* (Chinese name: houpo) has been used in traditional Chinese medicine for the treatment of thrombotic stroke, typhoid fever and fever and headache [2]. It was shown that magnolia bark suppresses mitogen-induced proliferation [3], depresses the activity of the central nervous system [4], exhibits muscle relaxant activity [5], inhibits intracellular calcium mobilization in platelets caused by collagen, even in the presence of indomethacin [6], and relaxes vascular smooth muscles by releasing a endothelium-derived relaxing factor and by inhibiting calcium influx through voltage-gated calcium channels [7], and shows antihaemostatic and antithrombotic effects [8]. Recent studies indicate that honokiol has an antagonistic effect on calmodulin [9].

Several methods for the determination of honokiol or magnolol have been described in the literature. They include ion-pair HPLC [10], HPLC photodiode-array detection of honokiol [1], and identification of magnolol and its metabolites by liquid chromatography–mass spectrometry [11,12]. In this work, we report an HPLC method coupled with photodiode-array detection, using paeonol as internal standard, for the determination of honokiol in rat plasma and the application of this method to a pharma-

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cokinetic study involving intravenous (i.v.) bolus doses at 5 and 10 mg/kg.

2. Experimental

2.1. Chemicals and reagents

Honokiol and paeonol (Fig. 1) were extracted from Magnoliaceae [13] and Moutan cortex [14] respectively. Authentic honokiol and paeonol were purchased from Nacalai Tesque (Kyoto, Japan). Identification and purity were compared with the authentic compounds using ^{13}C-NMR (Bruker Model AC300p, Karlsruhe, Germany) and HPLC coupled with photodiode-array detection. Acetonitrile and orthophosphoric acid (85%) were obtained from E. Merck (Darmstadt, Germany). Triple-deionized water (Millipore, Bedford, MA, USA) was used for all preparations.

2.2. Chromatographic apparatus

The HPLC system consisted of an injector (Rheodyne 7125, Cotati, CA, USA), a Model 990 photodiode-array detector (Waters, Milford, MA, USA) and a chromatographic pump (Waters, Model 510). Separation was achieved on a reversed-phase Cosmosile (Nacalai Tesque, Kyoto, Japan) 5C18-AR column (250 × 4.6 mm I.D., particle size 5 μm) at room temperature. The mobile phase was acetonitrile–water (70:30, v/v; adjusted to pH 2.5–2.8 with orthophosphoric acid) at a flow-rate of 1.0 ml/min.

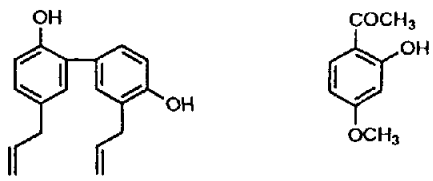

Fig. 1. Structure of honokiol and paeonol.

2.3. Animals

Male Sprague–Dawley rats (250–300 g) were obtained from the Laboratory Animal Center at the National Yang-Ming Medical College. These animals were specifically pathogen free and kept in our own environmentally controlled quarters (temperature maintained at 24 ± 1°C and 12 h:12 h light–dark cycle) for at least 1 week before use. Water and standard laboratory chow were given *ad libitum* until 18 h before the experiments, after which only water was given.

2.4. Blood sampling and treatment

Rats were anesthetized by intraperitonial injection of chloral hydrate at 400 mg/kg. Only one-fourth of the dose of chloral hydrate was administered during the experiment as required. Blood samples (0.3 ml) were collected from the rat by cardiopuncture at 2.5, 5, 10, 15, 20, 30, 45, 60, and 120 min after i.v. administration of honokiol. Blood samples were transferred to a heparinized microfuge tube and centrifuged at 8000 *g* for 3 min (Eppendorf, Model 5402, Germany). The resulting plasma (0.1 ml) was then mixed with 0.2 ml of acetonitrile containing 1 μg/ml paeonol as internal standard. The denatured protein precipitate was separated by centrifugation at 8000 *g* for 3 min. An aliquot (20 μl) of the supernatant was directly injected onto the HPLC apparatus for analysis. The same sample handling process was used to determine the recovery and precision of the assay.

2.5. Recovery

Plasma samples were spiked with honokiol at concentrations of 1.0, 2.0, and 5.0 μg/ml. The resulting peak-area ratios (honokiol/internal standard) were compared with those of the standards prepared in acetonitrile.

2.6. Precision

Precision over the entire working dose range was determined by replicate analyses of plasma samples ($n = 4$) spiked with three concentrations

(1.0, 5.0, and 10.0 μg/ml) of honokiol. To determine the intra-day variance, quadruplicate assays were carried out on the same samples at different times on the same day. Inter-day variance was determined by assaying the spiked samples in quadruplicate on days 1, 2, 4, and 6 after spiking. Coefficients of variation (C.V.s) were calculated from these values.

2.7. Data analysis

A calibration curve was constructed based on the analysis of various concentrations of honokiol spiked in rat plasma by HPLC. The concentrations of honokiol in rat plasma after i.v. administration were determined from the peak area by using the equation for linear regression from the calibration curve. All data were subsequently processed by the computer program PCNONLIN (SCI Software, Lexington, KY, USA). Statistical analysis was performed using ANOVA with the level of significance set at $p < 0.05$.

3. Results and discussion

Under the conditions described above, the retention times of paeonol and honokiol were found to be 4.14 and 4.94 min, respectively (Fig. 2). The pH value of the mobile phase was a very important factor in the separation. In the optimum pH range 2.5–2.8, the separation was improved and the retention time was shortened. The spectrum obtained for the compounds in mobile phase showed absorption maxima at 275 and 290 nm for paeonol and honokiol, respectively.

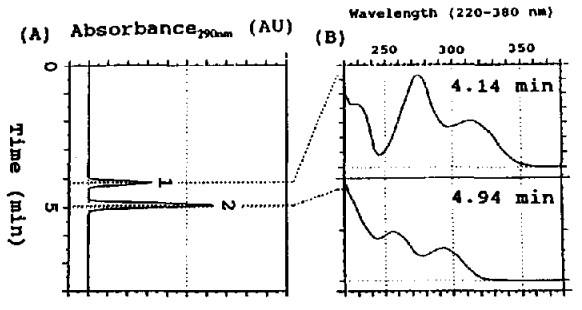

Fig. 2. Chromatogram (A) and UV spectra (B) of 1 μg of authentic paeonol (1) and honokiol (2).

The recoveries of honokiol from rat plasma were found to be 101.64, 96.57, and 100.49% for concentrations of 1.0, 2.0, and 5.0 μg/ml, respectively.

To determine the linearity and the detection limit of the HPLC method, rat plasma samples spiked with six concentrations of honokiol (0.1–20.0 μg/ml) were analyzed. The peak-area ratios (honokiol to paeonol) were linearly related to the concentration of drug (correlation coefficient, $r^2 = 0.999$) and the equation for the regression line for honokiol was found to be $y = 2.866x - 0.069$. The detection limit for honokiol, at a signal-to-noise ratio of 3:1, was 0.1 μg/ml in rat plasma.

The reproducibility of the method can be defined by examining both intra-day and inter-day variabilities. The intra-day C.V.s for honokiol at concentrations of 1.0, 5.0, and 20.0 μg/ml were 0.89, 1.22, and 0.19%, respectively, and the inter-day C.V.s for honokiol at the same concentrations were 2.75, 0.45, 0.41%, respectively (Table 1)

Fig. 3A shows a chromatogram of blank rat plasma. No discernible peaks were observed within the time frame in which honokiol and paeonol were detected. Fig. 3B shows the chromatogram of rat plasma spiked with honokiol and paeonol. Fig. 3C shows the chromatogram

Table 1
Intra-day and inter-day precision for honokiol determination in rat plasma

Spiked concentration (μg/ml)	Measured concentration (Mean ± S.E.M., $n = 4$) (μg/ml)	C.V. (%)
Intra-day		
1.0	0.96 ± 0.01	0.89
5.0	5.02 ± 0.06	1.22
20.0	19.97 ± 0.04	0.19
Inter-day		
1.0	1.01 ± 0.03	2.75
5.0	5.05 ± 0.02	0.45
20.0	20.02 ± 0.08	0.41

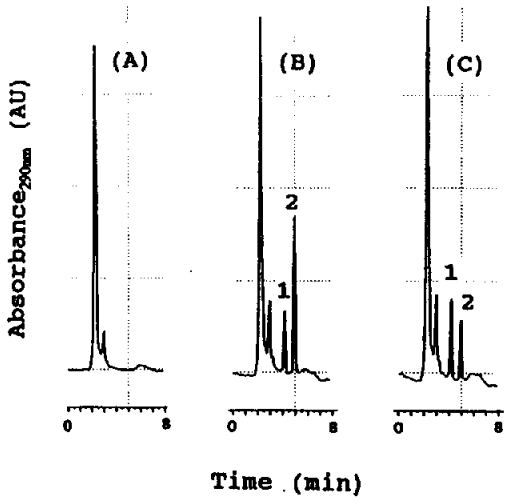

Fig. 3. Chromatograms of honokiol in rat plasma: (A) blank plasma; (B) blank plasma spiked with honokiol (1) (3.0 μg/ml) and internal standard (2) (paeonol); (C) rat plasma sample at 10 min after administration of a 10 mg/kg i.v. dose of honokiol (2) (1.52 μg/ml).

of a plasma sample obtained 10 min after i.v. administration of honokiol (10 mg/kg) to a rat.

The plasma concentration–time profiles in rats after i.v. administration of various doses of honokiol (5 or 10 mg/kg) are shown in Fig. 4. Sampling for the different doses was carried out over the same time period. However, the later concentration values for the profiles of the 5 mg/kg dose were omitted because they were below the detection limit. The initial values provided by samples taken within the first 30 min post dose, showed different patterns with increasing doses. Since all the animals were handled the same way, it is unlikely that anesthesia significantly affected the observations.

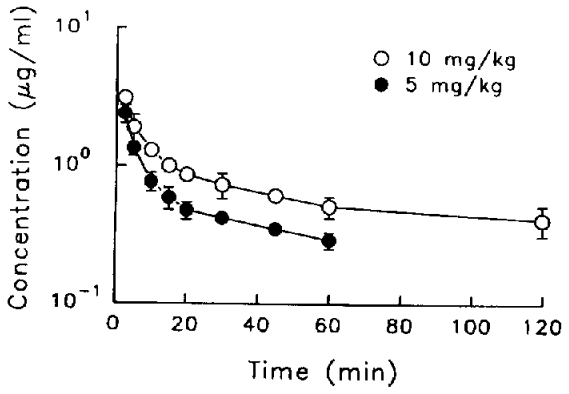

Fig. 4. Plasma concentration–time profiles after i.v. administration of honokiol to rats at doses of 5 and 10 mg/kg.

As shown in Fig. 4, the data obtained for each of the doses fitted best into a two-compartment open model using the PCNONLIN computer program. The following equation applies to a two-compartment pharmacokinetic model:

$$C = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

In eq. (1), A and B are the concentration (C) intercepts for the fast and slow disposition phases, respectively, and α and β are their corresponding rate constants. Analysis of data after i.v. bolus injection of honokiol at 5 or 10 mg/kg yields eqs. (2) and (3), respectively:

$$C = 3.99e^{-0.33t} + 0.69e^{-0.02t} \quad (2)$$

$$C = 5.36e^{-0.35t} + 1.01e^{-0.02t} \quad (3)$$

The pharmacokinetic parameters, as derived from these data and calculated by PCNONLIN program, are shown in Table 2. The parameters K_{12} and K_{21} are the rate constants between the central and peripheral compartments, and K_{10} is the elimination rate constant.

When the i.v. bolus dose of honokiol was increased from 5 to 10 mg/kg, there were no

Table 2
Pharmacokinetic parameters of honokiol in the rat after administration of 5 and 10 mg/kg bolus i.v. doses

Parameter	Value [a]	
	5 mg/kg	10 mg/kg
A (μg/ml)	3.99 ± 0.55	5.36 ± 1.29[b]
B (μg/ml)	0.69 ± 0.06	1.01 ± 0.11[b]
α (1/min)	0.33 ± 0.02	0.35 ± 0.08
β (1/min)	0.02 ± 0.001	0.02 ± 0.002
K_{10} (1/min)	0.08 ± 0.01	0.06 ± 0.02
K_{12} (1/min)	0.20 ± 0.01	0.23 ± 0.05
K_{21} (1/min)	0.06 ± 0.01	0.06 ± 0.01
$t_{1/2,\beta}$ (min)	49.22 ± 6.78	56.24 ± 7.30
CL (ml/min kg)	86.16 ± 4.78	80.54 ± 9.81
AUC (μg min/ml)	58.87 ± 4.19	133.89 ± 16.26[b]

[a] Data are expressed as mean ± S.E.M. ($n = 5$).
[b] Significantly different ($p < 0.05$) from the 5 mg/kg dose.

significant differences in the apparent total body clearance (CL) and the elimination half-life ($t_{1/2,\beta}$). The area under the curve (AUC) of honokiol appears to increase proportionally from 5 to 10 mg/kg. These results suggest that the pharmacokinetics of honokiol are linear.

In contrast, other compounds used in Chinese medicine, *e.g.* glycyrrhetinic acid [15], asarone [16], or glycyrrhizin [17], appear to exhibit nonlinear pharmacokinetics, with CL decreasing and $t_{1/2,\beta}$ increasing significantly with increases in the administered dose [18]. Obviously, the nonlinear pharmacokinetics for these drugs suggest that large dose administration may lead to retardation of their elimination and prolongation of their effects.

4. Conclusion

The UV spectrum identification, plasma sample extraction and chromatographic procedures described in this study allow the quantitation of honokiol in rat plasma. The pharmacokinetic study of honokiol (5 or 10 mg/kg, i.v.) was characterized by a two-compartment model. The dose-related response indicates that the pharmacokinetics of honokiol are linear.

5. Acknowledgement

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6. References

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