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High-performance liquid chromatographic determination of rutaecarpine in rat plasma: application to a pharmacokinetic study

Han-Chieh Ko[a,b], Tung-Hu Tsai[a,c], Cheng-Jen Chou[a,c], Shin-Yuan Hsu[a,c], Shyh-Yuan Li[b], Chieh-Fu Chen[a,c,d,*]

[a]*National Research Institute of Chinese Medicine, Taipei, Taiwan*
[b]*Institute of Applied Chemistry, Chinese Culture University, Taipei, Taiwan*
[c]*Institute of Pharmacology, National Yang-Ming Medical College, Taipei 112, Taiwan*
[d]*Institute of Traditional Medicine, National Yang-Ming Medical College, Taipei 112, Taiwan*

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Abstract

A simple and sensitive high-performance liquid chromatographic method for determination and identification of rutaecarpine in rat plasma has been developed. Up to 0.1 ml of plasma containing rutaecarpine was deproteinized by acetonitrile, which contained an internal standard (paeonol). The supernatant was injected onto a reversed-phase column using a acetonitrile–water–orthophosphoric acid (85%) (60:40:0.1, v/v/v, pH 2.5–2.8) as the mobile phase and ultraviolet detection at 344 nm. It was applied to the pharmacokinetic study of rutaecarpine in rat after a 2 mg/kg intravenous administration. A biphasic process with a rapid distribution followed by a slower elimination phase was observed from the plasma concentration–time curve. Compartmental analysis yielded a two-compartment model.

1. Introduction

Rutaecarpine is one of the major bioactive components of Rutaece [1]. The Chinese herbal drug Wu-Chu-Yu, dried unripen fruit of *Evodia rutaecarpa* (Juss.) Benth., has been used in traditional Chinese medicine for the treatment of abdominal pain, gastrointestinal disorders, headache, dysentery, postpartum haemorrhage and amenorrhea [2]. The cardiovascular effects of its bioactive components, dehydroevodiamine [3–5] and evodiamine [6] and an assay method for dehydroevodiamine [7] have been previously reported. In this paper, we report a HPLC separation coupled with ultraviolet or photodiode-array detection of rutaecarpine in rat plasma and the application of this method to a pharmacokinetic study.

Recently, several studies on the quantitation and pharmacokinetics of bioactive components from Chinese herbal drugs have been reported by us [8–11]. The present paper is an approach

* Corresponding author. Address for correspondence: Institute of Pharmacology, National Yang-Ming Medical College, Taipei 112, Taiwan.

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to the interpretation of experimental results and to therapeutic applications of herbal drugs.

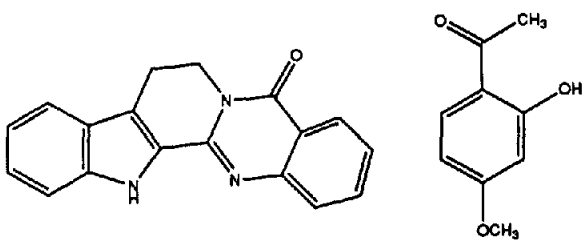
2. Experimental

2.1. Chemicals and reagents

Rutaecarpine and paeonol (Fig. 1) were extracted from *Evodia rutaecarpa* (Juss.) Benth. [1] and Moutan cortex [12], respectively. Authentic rutaecarpine and paeonol were purchased from Nacalai Tesque (Kyoto, Japan). Identity and purity of the extracted compounds were examined by comparison with authentic compounds using ^{13}C NMR (Bruker, Germany) and HPLC coupled with photodiode-array detection (Waters Model 990, Milford, MA, USA); purity of both compounds was *ca.* 98%. Acetonitrile and orthophosphoric acid (85%) were obtained from E. Merck (Darmstadt, Germany). Triple-deionized water (Millipore, Bedford, MA, USA) was used for all preparations. Rutaecarpine stock solutions were made in acetonitrile at a concentration of 1 mg/ml and stored at 4°C. Quality remained reliable for at least one month. Rutaecarpine stock solutions were diluted to 0.1, 0.01 and 0.001 mg/ml with acetonitrile before use.

2.2. Apparatus and chromatography

The HPLC system consisted of an autosampler (SIC Model 23, Tokyo, Japan), a variable-wavelength UV-Vis detector (Soma, Tokyo, Japan) and a chromatographic pump (Waters Model 6000). Separation was achieved on a reversed-phase Cosmosile (Nacalai Tesque) $5C_{18}$-AR column (150 × 4.6 mm I.D., particle size 5 μm) at room temperature. The mobile phase was acetonitrile–water–orthophosphoric acid (60:40:0.1, v/v/v, pH 2.5–2.8) at a flow-rate of 1.0 ml/min.



Rutaecarpine Paeonol

Fig. 1. Structure of rutaecarpine 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 with 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 time only water was given.

2.4. Blood sampling

Rats were anesthetized with chloral hydrate (400 mg/kg, intraperitoneal). Only one-fourth of the chloral hydrate dose was administrated during the experiment upon demand. Blood samples (0.3 ml) were directly collected from the rat by cardial puncture and sampled from the same animals at 2.5, 5, 10, 15, 20, 30, 45, 60 and 120 min after intravenous administration of rutaecarpine. Data from these sample times were used to construct pharmacokinetic profiles by plotting concentration of drug in plasma *versus* time.

2.5. Treatment of plasma samples

Each blood sample was transferred to a heparinized microfuge tube and centrifuged at 8000 *g* for 3 min (Eppendorf Model 5402, Hamburg, Germany). The resulting plasma (0.1 ml) was then mixed with 0.2 ml of acetonitrile containing paeonol (1 μg/ml) as internal standard. The denatured protein precipitation 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 in plasma.

2.6. Recovery

Plasma samples were spiked with rutaecarpine at concentrations 0.05, 0.1, and 0.5 μg/ml. After protein denaturation, fixed amounts of paeonol (internal standard) were added to plasma for normalization. The values were compared with corresponding values of standards and internal standards carried in acetonitrile to provide the recovery values.

2.7. Precision

Precision over the entire working dose range was determined by replicate analyses of plasma samples ($n = 4$) spiked with three concentrations (0.05, 0.5 or 2 μg/ml) of rutaecarpine. To determine intra-day variance, quadruplicate assays were carried out on the same samples at different times on the 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.

2.8. Data analysis

A calibration curve was constructed based on the analysis by HPLC of various concentrations of rutaecarpine spiked in rat plasma. The concentrations of rutaecarpine in rat plasma after intravenous administration were determined from the peak height 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 by 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 rutaecarpine were found to be 4.66 and 6.98 min, respectively (Fig. 2). Further, the spectrum obtained in the mobile phase showed absorption maxima at 275 and 344 nm for paeonol and rutaecarpine, respectively.

The recoveries of rutaecarpine from rat plasma were found to be 102.28, 99.44 and 98.41% for concentrations 0.05, 0.1 and 0.5 μg/ml, respectively.

To determine the linearity and the detection limit of the HPLC method, rat plasma samples spiked with six concentrations of rutaecarpine (0.01–4 μg/ml) were analyzed. The peak height ratios (rutaecarpine to paeonol) were linearly related to the concentration of drug (correlation coefficient, $r^2 = 0.999$) and the equation for the regression line for rutaecarpine was found to be $y = 0.751x - 0.011$. The detection limit for rutaecarpine, at a signal-to-noise ratio of 4, was 0.01 μg/ml in rat plasma.

The reproducibility of the method can be

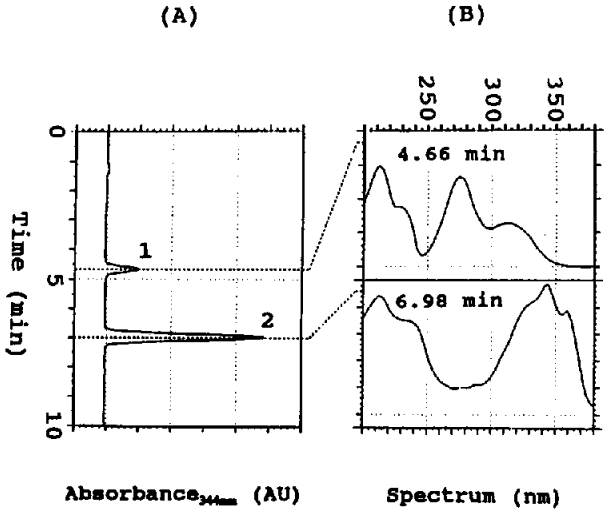


Fig. 2. Peaks (A) and UV spectra (B) of authentic paeonol and rutaecarpine, measured by Waters photodiode-array detector (Model 990). Peaks: 1 = paeonol; 2 = rutaecarpine.

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

Spiked concentration (μg/ml)	Measured concentration (mean ± S.E.M., $n = 4$) (μg/ml)	C.V. (%)
Intra-day		
0.05	0.05 ± 0.055	9.09
0.5	0.49 ± 0.005	0.97
2	1.96 ± 0.024	1.22
Inter-day		
0.05	0.06 ± 0.006	9.84
0.5	0.49 ± 0.003	0.66
2	1.95 ± 0.042	2.15

defined by examining both intra-day and inter-day variabilities. The intra-day C.V.s for rutaecarpine at concentrations of 0.05, 0.5 and 2 μg/ml were 9.09, 0.97 and 1.22%, respectively, and the inter-day C.V.s for rutaecarpine at the same concentrations were 9.84, 0.66 and 2.15%, respectively (Table 1).

Fig. 3A shows the chromatogram of blank rat plasma. No discernible peaks were observed within the time frame in which rutaecarpine and paeonol were detected. Fig. 3B shows the chromatogram of rat plasma spiked with rutaecarpine (0.2 μg/ml) and paeonol. Fig. 3C shows the chromatogram of rutaecarpine (0.44 μg/ml) sample obtained 30 min after intravenous administration of rutaecarpine (2 mg/kg) to a rat.

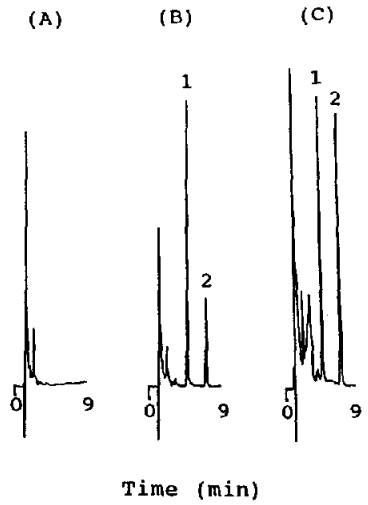


Fig. 3. Chromatograms of rutaecarpine in rat plasma: (A) blank plasma; (B) blank plasma spiked with rutaecarpine (0.2 μg/ml) and internal standard (paeonol); (C) plasma sample 30 min after a 2 mg/kg intravenous dose of rutaecarpine (0.44 μg/ml). Peaks: 1 = paeonol; 2 = rutaecarpine.

The curves of concentration in plasma *versus* time after intravenous administration of rutaecarpine (2 mg/kg) are shown in Fig. 4. The initial values were from samples taken within the first 30 min. Since all the animals were handled the same way, it is unlikely that anesthesia significantly affected the observations. As shown in Fig. 4, the curves generally exhibited a biexponential decline for the administration. The data from the dose fitted best to a two-compartment open model by the computer program PCNONLIN. The following equation applies to a two-compartment pharmacokinetic model:

$$C = A\,e^{-\alpha t} + B\,e^{-\beta t} \quad (1)$$

In Eq. 1, A and B are the concentration C intercepts for fast and slow disposition phases, respectively. And α and β are disposition rate constants for fast and slow disposition phase, respectively. Analysis of data after intravenous bolus injection of rutaecarpine at 2 mg/kg yield Eq. 2:

$$C = 2.52\,e^{-0.27t} + 0.59\,e^{-0.05t} \quad (2)$$

The pharmacokinetic parameters, as derived from these data and calculated by PCNONLIN

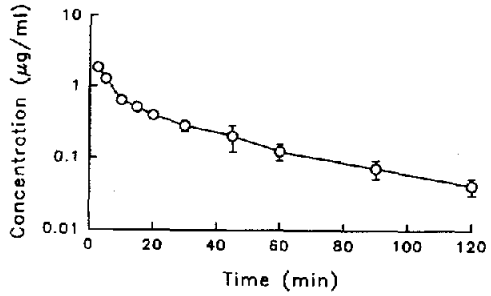


Fig. 4. Curves of concentration in plasma *versus* time after intravenous administration of rutaecarpine to rats of a dose of 2 mg/kg.

Table 2
Pharmacokinetic parameters of rutaecarpine in rats after administration of intravenous bolus (2 mg/kg) dose

Parameter	Estimate (mean ± S.E.M., $n = 6$)
A (μg ml^{-1})	2.52 ± 0.22
B (μg ml^{-1})	0.59 ± 0.12
α (min^{-1})	0.27 ± 0.04
β (min^{-1})	0.05 ± 0.02
K_{10} (min^{-1})	0.10 ± 0.01
K_{12} (min^{-1})	0.12 ± 0.02
K_{21} (min^{-1})	0.08 ± 0.02
$t_{1/2,\beta}$ (min)	29.29 ± 4.25
CL (ml min^{-1} kg^{-1})	63.46 ± 5.39
Volume (ml kg^{-1})	655.15 ± 43.93
AUC (μg min ml^{-1})	32.93 ± 3.39

program, are shown in Table 2. K_{12} and K_{21} are rate constants between the central and peripheral compartments and K_{10} the elimination rate constant.

In conclusion, the UV spectrum identification, plasma sample extraction and chromatographic procedures described in this study allow the quantization of rutaecarpine from rat plasma. The pharmacokinetic study of rutaecarpine (2 mg/kg, intravenous) was characterized by the two-compartment model.

4. Acknowledgement

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