ELSEVIER

Journal of Chromatography B, 681 (1996) 277–281

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Determination of dicentrine in rat plasma by high-performance liquid chromatography and its application to pharmacokinetics

Tung-Hu Tsai[a,b,*], Tong-Rong Tsai[c], Cheng-Jen Chou[a], Chieh-Fu Chen[a,d]

[a]*Department of Pharmacology, National Research Institute of Chinese Medicine, Taipei 11221, Taiwan*
[b]*Institute of Traditional Medicine,* [d]*Department of Pharmacology, National Yang-Ming University, Taipei 11221, Taiwan*
[c]*Graduate Institute of Pharmaceutical Sciences, Kaohsiung Medical College, Kaohsiung 807, Taiwan*

Received 10 August 1995; revised 23 November 1995; accepted 30 November 1995

Abstract

A simple high-performance liquid chromatographic method was developed to study the pharmacokinetics of dicentrine in rat plasma after 10 mg/kg intravenous administration. After addition of an internal standard (coumarin), plasma was deproteinized by acetonitrile for sample clean-up. The drugs were separated on a reversed-phase Nucleosil C_{18} column (250 × 4 mm I.D., particle size 5 μm) and detected by photodiode-array detection at a wavelength of 308 nm. Acetonitrile–water (35:65, v/v, pH 2.5–2.8, adjusted with orthophosphoric acid) was used as the mobile phase. A biphasic phenomenon with a rapid distribution followed by a slower elimination phase was observed from the plasma concentration–time curve.

Keywords: Dicentrine

1. Introduction

Dicentrine, a chemical compound isolated from the root of *Lindera megaphylla* [1] or *Glaucium flavum* Grantz [2], is an apophine alkaloid found to have strong α1-adrenoceptor-blocking activity [1,3,4], antiarrhythmic action [5] and anti-platelet effect [1]. Although hyperlipidaemia [6] and cardiovascular effects of dicentrine have been reported, the spectrum identification of dicentrine from plasma and its pharmacokinetic properties have not been studied. In the present study, we developed a high-performance liquid chromatographic (HPLC) method with photodiode-array and UV absorbance detection to determine the concentration of dicentrine in rat plasma and its pharmacokinetic profile.

2. Experimental

2.1. Chemicals and reagents

Dicentrine (Fig. 1) was extracted from the roots of *Lindera megaphylla*. Identification and purity of dicentrine was compared with an authentic compound by ^{13}C NMR (Bruker Model AC300p, Karlsruhe, Germany), infra-red and HPLC/photodiode-array detection (Fig. 2). Acetonitrile and orthophosphoric acid (85%) were obtained from Merck (Darmstadt, Germany). Triple deionized

*Corresponding author.

0378-4347/96/$15.00
SSDI 0378-4347(95)00553-6

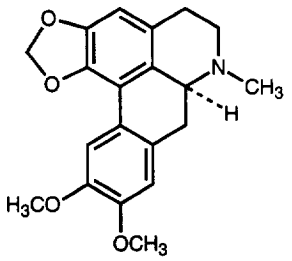

Fig. 1. Chemical structure of dicentrine.

water (Millipore, Bedford, MA, USA) was used for all preparations.

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 510). Separation was achieved on a reversed-phased Nucleosil C_{18} column (250 × 4 mm I.D., particle size 5 μm, Macherey-Nagel, Duren, Germany). The mobile phase was acetonitrile–water (35:65, v/v; pH 2.5–2.8, adjusted with orthophosphoric acid), and the flow-rate was 1.0 ml/min. Dicentrine was monitored at a wavelength of 308 nm throughout the experiments. The system was operated at room temperature (25°C).

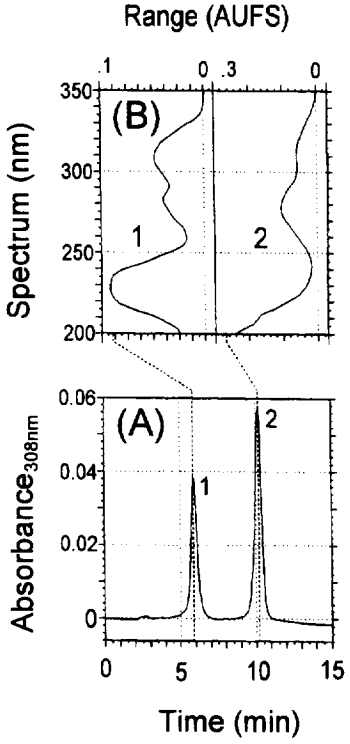

Fig. 2. Chromatogram (A) and UV spectra (B) of authentic dicentrine and coumarin (internal standard), measured by a photodiode-array detector (Waters, Model 990). Peaks: 1 = dicentrine (10 ng); 2 = coumarin (10 ng).

2.3. Animals

Male Sprague–Dawley rats (250–300 g) were obtained from the Laboratory Animal Center at the National Yang-Ming University. 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 one week before use. Standard laboratory food and water were available at all times, with the exception that food was withdrawn 18 h prior to experimentation.

2.4. Blood sampling

Rats were anaesthetized with intraperitoneal chloral hydrate (400 mg/kg). An additional dosage of 100 mg/kg chloral hydrate was given when the rat showed signs of awakening during the experiment. Blood samples (0.3 ml) were collected via cardiac puncture 2.5, 5, 10, 15, 20, 30, 45, 60, 90, 120 and 180 min after intravenous administration of dicentrine (10 mg/kg). Data from these samples were used to construct pharmacokinetic profiles by plotting the concentration of the drug in plasma versus time. Sigmaplot for Windows (version 1.01, Jandel Scientific, Corte Madera, CA, USA) was used to plot standard curves and to perform least squares regression analysis on the calibration data.

2.5. Treatment of plasma samples

Each collected blood sample was transferred to a heparinized microcentrifuge 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 coumarin (1 μg/ml) as the internal standard. The

denatured protein precipitate was separated by centrifugation at 8000 *g* for 3 min and a 20-μl aliquot of the supernatant was directly injected onto the HPLC system. The same sample handling process was used for recovery and precision determination.

2.6. Recovery

Recovery has been defined as a measure of the efficiency of the extraction of the analyte from the sample matrix. In the experiment, recovery of dicentrine was determined at low, moderate and high concentrations (0.1, 0.5 and 1 μg/ml, respectively) from rat plasma. Two groups of samples were used to assess extraction recovery (i.e. test and control groups). The samples in the test group was spiked with dicentrine in rat plasma to yield final concentrations of 0.1, 0.5 and 1 μg/ml, whereas the samples in the control group were spiked with dicentrine after the extraction. The extraction recovery was calculated as the ratio of the measured concentration of the test samples over the measured concentration of the control samples at the low, moderate and high concentrations. Quadruplicate assays have been performed at the same concentrations.

2.7. Precision

Precision over the entire working dose range was determined by replicate analyses of plasma samples (n = 4) spiked with three different concentrations (0.1, 0.5 or 1 μg/ml) of dicentrine. To determine intra-assay variance, quadruplicate assays were carried out on the same samples at different times during the day. Inter-assay variance was determined by assaying the spiked samples, in quadruplicate, on days one, two, four and six, 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 dicentrine spiked in rat plasma. The concentrations of dicentrine in rat plasma after i.v. administration were determined from the peak area by using the equation for linear regression from the calibration curve.

2.9. Pharmacokinetic analysis

All data were subsequently processed by the computer program PCNONLIN (Version 4.0, SCI Software, Lexington, KY, USA). The data for the area under the curve of concentration in plasma versus time (AUC_{0-inf}) were calculated by the trapezoidal method and extrapolated to infinite time.

3. Results

3.1. Performance of the HPLC system

Under the conditions described above, the retention times of dicentrine and coumarin (internal standard) were found to be 5.91 and 10.23 min, respectively (Fig. 2A). The main characteristic spectral data obtained in the mobile phase showed absorption maxima at 226, 283 and 308 nm for dicentrine and at 208, 277 and 310 nm for coumarin (Fig. 2B).

3.2. Recovery and precision

The recoveries of dicentrine from rat plasma were found to be 95.49, 94.41 and 96.21% for the concentrations 0.1, 0.5, and 1 μg/ml, respectively. The reproducibility of the method was defined by examining both intra- and inter-assay variabilities. The intra-assay variation for the determination of dicentrine at concentrations of 0.1, 0.5, and 1 μg/ml were acceptable with C.V.s of less than 7% (Table 1). The inter-assay C.V.s for dicentrine at the same concentrations were less than 9% (Table 1).

3.3. Linearity and reproducibility

To determine the linearity, rat plasma samples spiked with six different concentrations of dicentrine (0.05–2 μg/ml) were analyzed. The peak-area ratios (dicentrine to coumarin) were linearly related to the concentration of drug and the equation for the regression line for dicentrine was found to be $y = 0.809x + 0.016$ ($r^2 = 0.999$). The slopes of the

Table 1
Intra- and inter-assay precision and accuracy for dicentrine determination

Nominal concentration (μg/ml)	Concentration found (mean ± S.D.) (μg/ml)	C.V. (%)	Accuracy (%)
Intra-assay (n = 4)			
0.1	0.108 ± 0.0069	6.43	8.0
0.5	0.494 ± 0.021	4.25	−1.2
1	1.02 ± 0.036	3.53	2.0
Inter-assay (n = 4)			
0.1	0.091 ± 0.0081	8.65	−9.0
0.5	0.505 ± 0.027	5.43	1.0
1	0.96 ± 0.046	4.75	−4.0

Precision [C.V.(%)] = [Standard deviation (S.D.)/mean concentration] × 100.
Accuracy (%) = [(mean concentration − actual concentration)/actual concentration] × 100.

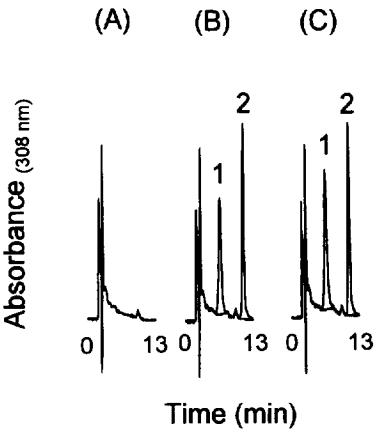

Fig. 3. Chromatograms of dicentrine in rat plasma: (A) blank plasma; (B) spiked with dicentrine (0.5 μg/ml) and coumarin (internal standard) and (C) plasma sample 5 min after a 10 mg/kg i.v. administration of dicentrine (0.62 μg/ml). 1 = dicentrine; 2 = coumarin.

calibration graphs were reproducible throughout the study: 0.792 ± 0.058 (mean ± S.D.) with a C.V. of 7.32%.

3.4. Limits of detection and quantification

The limit of detection is defined as the lowest concentration of the standard that can be measured with acceptable precision (C.V. < 20%). Under the procedure described in Section 2, the limit of detection for dicentrine (signal-to-noise ratio of three) was 0.01 μg/ml in rat plasma. The lower practical limit of quantification was 0.05 μg/ml.

3.5. Plasma interference and selectivity

Fig. 3A shows the chromatogram of a blank rat plasma. No discernible peaks were observed within the time frame in which dicentrine and coumarin were detected. Fig. 3B shows the chromatogram of rat plasma spiked with dicentrine (0.5 μg/ml) and internal standard. Fig. 3C shows the chromatogram of a dicentrine (0.62 μg/ml) sample obtained 5 min after i.v. administration of dicentrine (10 mg/kg) to a rat.

3.6. Pharmacokinetic application

The data from each of the doses fitted best to a two-compartment open model by the computer program PCNONLIN. 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 fast and slow disposition phases, respectively, and α and β are disposition rate constants for fast and slow disposition phases, respectively. The distribution half-life ($t_{1/2,\alpha}$) and the elimination half-life ($t_{1/2,\beta}$) of dicentrine, as shown in the initial phase and the terminal phase of the plasma concentration–time curve, were determined by the equations $0.693/\alpha$ and $0.693/\beta$, respectively. Analysis of data after i.v. injection of dicentrine at 10 mg/kg yields Eq. 2 (Fig. 4).

$$C = 0.55e^{-0.20t} + 0.29e^{-0.017t} \quad (2)$$

The pharmacokinetic parameters, derived from these data and calculated by the PCNONLIN program, are shown in Table 2.

4. Discussion

The complementary use of the photodiode-array detection for identifying the analyzed compound by its retention time/wavelength absorption is more

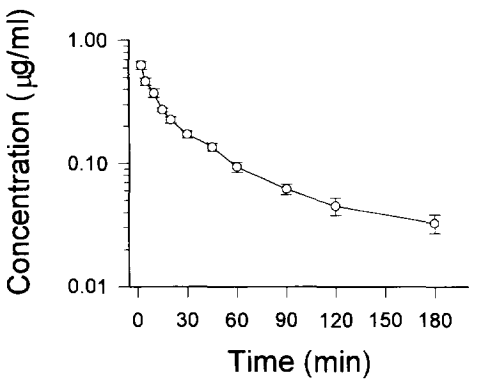

Fig. 4. Plasma concentration–time curve after i.v. administration of dicentrine to rats at a dose of 10 mg/kg.

reliable than the UV absorbance detector which gives only the retention time [6–9]. Photodiode-array detection allowed the observation of the full UV spectrum of each peak as it eluted from the chromatographic column. Hence, the detection of other components could be observed.

A statistical non-linear regression program was accessed through the JANA and PCNONLIN programs for the kinetic analysis. The pharmacokinetic models (one- vs. two-compartment) were compared according to Akaike information criterion (AIC) [10] and Schwartz criterion (SC) [11] and, with minimum AIC and SC values, were regarded as the best representation of the plasma concentration–time course data. A two-compartment open model with elimination from the central compartment was proposed and validated through the program, to explain the apparent biphasic disposition of dicentrine in rat plasma after i.v. administration.

In conclusion, the present method allows a high selectivity and reliability. The relative simplicity permits its use for pharmacokinetic studies. Analysis of data after i.v. injection of dicentrine at 10 mg/kg yields a two-compartment pharmacokinetic model.

Table 2
Pharmacokinetic parameters of dicentrine (10 mg/kg, i.v., $n = 6$) in rats

Parameter	Estimate
Vol (l/kg)	12.58±1.11
$t_{1/2,\alpha}$ (min)	4.32±0.85
$t_{1/2,\beta}$ (min)	45.20±7.85
C' (l/kg/min)	0.51±0.046
AUC (μg min/ml)	20.95±2.28
AUMC (μg min^2/ml)	1262±354
MRT (min)	55.18±8.52

Data are expressed as mean ± S.E.M.; Vol = volume of distribution; Cl = clearance; AUC = area under the concentration–time curve; AUMC = area under the first moment curve; MRT = mean residence time.

Acknowledgments

This study was supported by research grant NSC-85-2331-B-077-004 from the National Science Council, Taiwan to T.H.T.

References

[1] C.C. Chen, Y.L. Huang, J.C. Ou, M.J. Su, S.M. Yu and C.M. Teng, Planta Med., 57 (1991) 406.
[2] I. Lalezari, A. Shafiee and M. Mahjour, J. Pharm. Sci., 65 (1976) 923.
[3] C.M. Teng, S.M. Yu, F.N. Ko, C.C. Chen, Y.L. Huang and T.F. Huang, Br. J. Pharmacol., 104 (1991) 651.
[4] S.M. Yu, S.Y. Hsu, F.N. Ko, C.C. Chen, Y.L. Huang, T.F. Huang and C.M. Teng, Br. J. Pharmacol., 106 (1992) 797.
[5] M.L. Young, M.J. Su, M.H. Wu and C.C. Chen, Br. J. Pharmacol., 113 (1994) 69.
[6] T.H. Tsai, C.M. Chen and C.F. Chen, J. Pharm. Pharmacol., 44 (1992) 620.
[7] T.H. Tsai, C.J. Chou and C.F. Chen, Drug Metab. Dispos., 22 (1994) 518.
[8] T.H. Tsai, C.J. Chou and C.F. Chen, J. Pharm. Sci., 83 (1994) 1307.
[9] T.H. Tsai, C.J. Chou, F.C. Cheng and C.F. Chen, J. Chromatogr. B., 655 (1994) 41.
[10] K. Yamoaka, T. Nakagawa and T. Uno, J. Pharmacokin. Biopharm., 6 (1978) 165.
[11] G. Schwartz, Ann. Stat., 6 (1978) 461.