Determination and Pharmacokinetic Study of Dauricine in Rat Plasma by UPLC–MS/MS

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Dauricine is the major bioactive component isolated from the roots of *Menispermum dauricum* D.C., a bisbenzylisoquinoline alkaloid derivative, and has shown multiple pharmacological properties. In this work, a sensitive and selective ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) method was developed for determination of dauricine in rat plasma and its application to pharmacokinetic study of dauricine after intravenous and oral administration in rats. After addition of daurisolone as an internal standard (IS), protein precipitation by acetonitrile was used to prepare samples. Chromatographic separation was achieved on a UPLC BEH C18 column (2.1 mm × 50 mm, 1.7 μm) with 0.1% formic acid and acetonitrile as the mobile phase with gradient elution. An electrospray ionization source was applied and operated in positive ion mode; multiple reactions monitoring (MRM) mode was used for quantification. Calibration plots were linear throughout the range 2–600 ng mL⁻¹ for dauricine in rat plasma. Relative standard deviation (RSD) of intra-day and inter-day precision was less than 13%. The accuracy of the method was between 95.8% and 105.9%. Matrix effect of dauricine in rat plasma ranged from 88.0% to 90.3%. Mean recoveries of dauricine in rat plasma ranged from 91.5% to 95.1%. The method was successfully applied to pharmacokinetic study of dauricine after intravenous and oral administration in rats. The bioavailability of dauricine was found to be 55.4% for the first time.

Keywords: Dauricine, UPLC–MS/MS, pharmacokinetics, rat, plasma

Introduction

Dauricine (Figure 1) is a bisbenzylisoquinoline alkaloid isolated from the root of *Menispermum dauricum* D.C. The herb is often included in remedies for the treatment of antiplatelet aggregation, throat swelling, and chronic bronchitis [1, 2]. Dauricine has been suggested for the treatment of various diseases, including cardiac ischemia, angina, and inflammation [3–5]. The alkaloid has shown varieties of bioactive properties, including antiarrhythmic, antitumor, anti-inflammatory, and neuron-protective effects. Thus, dauricine has attracted substantial attention due to its multiple pharmacologic activities and abundance in natural source [6, 7]. Therefore, it is necessary to develop a sensitive and fast bioanalytical method to characterize the pharmacokinetics.

There was a liquid chromatography method developed for the quantification of dauricine in beagle dog plasma to characterize the pharmacokinetics [8]. There, a sensitive and selective liquid chromatography–tandem mass spectrometry method had been developed and validated for simultaneous quantitation of 10 alkaloids (daurisolone, dauricine, *N*-desmethyl daurisolone, dauricolone, dauriporphinoline, bianfugecine, dauricoside, stepholidine, acutumine, and acutumidine) from *Rhizoma Menispermum* in rat plasma, and the validated method was successfully applied to a comparative pharmacokinetic study of 10 alkaloids in rat plasma after oral administration of *Rhizoma Menispermum* extract [9]. Liu et al. developed a liquid chromatography–tandem mass spectrometry method for quantitative determination of dauricine in human plasma and its application to pharmacokinetic study, with liquid–liquid extraction for sample preparation [10]. However, the bioavailability of dauricine was not reported in these literatures.

In comparison with conventional analytical techniques, ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC–MS/MS) is documented to possess im-

proved sensitivity, selectivity, and specificity in quantitative determination of the active compound of herbal drug in biological samples [11–14]. Consequently, in the present study, a UPLC–MS/MS method was established for the determination of dauricine in rat plasma samples and was successfully applied to the pharmacokinetic after oral and intravenous administration in rats. The UPLC–MS/MS method in this study was validated for selectivity, linearity, accuracy, precision, recovery, and stability with a total run time of 3 min.

Experimental

Chemicals and Reagents. Dauricine (purity, >98%; Figure 1a) and daurisolone (IS, purity, >98%; Figure 1b) were purchased from the Chengdu Mansite Pharmaceutical Co. Ltd. (Chengdu, China). Liquid chromatography (LC)-grade acetonitrile and methanol were purchased from Merck Company (Darmstadt, Germany). Ultra-pure water was prepared by Millipore Milli-Q purification system (Bedford, MA, USA). Rat blank plasma samples were supplied by drug-free rats (Laboratory Animal Center of Wenzhou Medical University).

Instrumentation and Conditions. A UPLC–MS/MS system with ACQUITY I-Class UPLC and a XEVO TQD triple quadrupole mass spectrometer (Waters Corp., Milford, MA, USA), equipped with an electrospray ionization (ESI) interface, was used to analyze the compounds. The UPLC system was comprised of a binary solvent manager (BSM) and a sample manager with flow-through needle (SM-FTN). Masslynx 4.1 software (Waters Corp.) was used for data acquisition and instrument control.

Dauricine and daurisolone (IS) were separated using a UPLC BEH C18 column (2.1 mm × 50 mm, 1.7 μm, Waters, USA) maintained at 40 °C. The initial mobile phase consisted of acetonitrile and water (containing 0.1% formic acid) with gradient elution at a flow rate of 0.4 mL min⁻¹. Elution was in a linear

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(a)

(b)

400, and 600 ng mL⁻¹, each by adding 10 µL of the appropriate working solution to 100 µL of blank rat plasma, followed by short vortex mixing. Quality-control (QC) samples were prepared in the same manner as the calibration standards, in three different plasma concentrations (4, 150, and 500 ng mL⁻¹). The calibration standards and QC samples protein were precipitated with acetonitrile before UPLC-MS/MS analysis.

Sample Preparation. Before analysis, the plasma sample was thawed to room temperature. An aliquot of 10 μL of the IS working solution ($0.5 \mu\text{g mL}^{-1}$) was added to 100 μL of the collected plasma sample in a 1.5-mL centrifuge tube, followed by the addition of 200 μL of acetonitrile. The tubes were vortex mixed for 1.0 min. After centrifugation at 14,900g for 10 min, the supernatant (2 μL) was injected into the UPLC-MS/MS system for analysis.

Method Validation. Rigorous tests for selectivity, linearity, accuracy, precision, recovery, and stability, according to the guidelines set by the United States Food and Drug Administration (FDA) and European Medicines Agency (EMA) were conducted in order to thoroughly validate the proposed bioanalytical method [14–24]. Validation runs were conducted on three consecutive days. Each validation run consisted of one set of calibration standards and six replicates of QC plasma samples.

Pharmacokinetic Study. Male Sprague-Dawley rats (200–220 g) were obtained from the Laboratory Animal Center of Wenzhou Medical University to study the pharmacokinetics of dauricine. All twelve rats were housed at the Laboratory Animal

Center of Wenzhou Medical University. All experimental procedures and protocols were reviewed and approved by the Animal Care and Use Committee of Wenzhou Medical University and were in accordance with the Guide for the Care

and Use of Laboratory Animals. Diet was prohibited for 12 h before the experiment, but water was freely available. Blood samples (0.3 mL) were collected from the tail vein into heparinized 1.5 mL polythene tubes at 0.0333, 0.15, 0.5, 1, 1.5,

Figure 2. Representative UPLC–MS/MS chromatograms of dauricine and daurisolone (IS). a, Blank plasma; b, blank plasma spiked with dauricine and IS; c, a rat plasma sample 0.5 h after intravenous administration of single dosage 2 mg kg⁻¹ dauricine

and 2 h after intravenous administration (2 mg kg^{-1}), and at 0.0833, 0.15, 0.5, 1, 1.5, and 2 h after oral administration (5 mg kg^{-1}) of dauricine. The samples were immediately centrifuged at $3000g$ for 10 min. The plasma as obtained ($100 \mu\text{L}$) was stored at -20°C until analysis.

Plasma dauricine concentration versus time data for each rat was analyzed by DAS (Drug and Statistics) software (version 2.0, Wenzhou Medical University). The maximum plasma concentration (C_{max}) was observed directly from the concentration–time curve. The area under the plasma concentration–time curve (AUC) was estimated by the trapezoidal rule. The plasma clearance (CL), apparent volume of distribution (V), and the half-life ($t_{1/2}$) were estimated using non-compartmental calculations performed with DAS software.

Results and Discussion

Selectivity and Matrix Effect. Figure 2 shows typical chromatograms of a blank plasma sample, a blank plasma sample spiked with dauricine and IS, and a plasma sample. There were no interfering endogenous substances observed at the retention time of the dauricine and IS.

To evaluate the matrix effect, blank rat plasma was extracted and spiked with the analyte at 4, 150, and 500 ng mL^{-1} concentrations ($n = 6$). The corresponding peak areas were then compared to those of neat standard solutions at equivalent concentrations; this peak area ratio is defined as the matrix effect. The matrix effect of the IS was evaluated in a similar manner. The matrix effect for dauricine at concentrations of 4, 150, and 500 ng mL^{-1} was measured between 88.0% and 90.3% ($n = 6$) (Table 1). The matrix effect for IS (50 ng mL^{-1}) was 93.6% ($n = 6$). As a result, matrix effect from plasma is considered negligible in this method.

Calibration Curve and Sensitivity. Linear regressions of the peak area ratios versus concentrations were fitted over the concentration range of $2\text{--}600 \text{ ng mL}^{-1}$ for dauricine in rat plasma. The equation used to express the calibration curve is the following: $y = (0.000203 \pm 0.0000134)x + (0.000212 \pm 0.000032)$, $r = 0.9985 \pm 0.0013$, where y represents the ratios of dauricine peak area to that of IS, and x represents the plasma concentration. The lower limit of quantification (LLOQ) was defined as the lowest concentration on the calibration curves. The precision and accuracy at LLOQ should be less than 20%, and accuracy should be between 80% and 120%. The LLOQ for the determination of dauricine in plasma was 2 ng mL^{-1} . The precision, accuracy, and recovery at LLOQ were 16.4%, 90.6% and 92.2%, respectively. The LOD, defined as a signal/noise ratio of 3, was 0.5 ng mL^{-1} for dauricine in rat plasma.

Precision, Accuracy, and Recovery. The precision of the method was determined by calculating relative standard deviation (RSD) for QCs at three concentration levels over 3 days of validation tests. Intra-day precision was 13% or less, and inter-day precision was 11% or less at each QC level. The accuracy of the method was between 95.8% and 105.9% at each QC level. Mean recoveries of dauricine in rat plasma ranged from 91.5% to 95.1% (Table 1). The recovery of the IS (50 ng mL^{-1}) was 86.4%.

Carry-over. None of the analytes showed any significant peak ($\geq 20\%$ of the LLOQ and 5% of the IS) in blank samples

injected after the upper limit of quantification (ULOQ) samples. Adding 0.4 extra minutes to the end of the gradient elution effectively washed the system between samples, thereby eliminating carry-over [25].

Stability. Results from the autosampler showed that the analyte was stable under room temperature, freeze–thaw, and long-term (20 days) conditions, which was confirmed because the bias in concentrations was within $\pm 12\%$ of their nominal values. To this effect, the established method is suitable for pharmacokinetic study.

Application. The method was applied to a pharmacokinetic study in rats. The mean plasma concentration–time curve after intravenous (2 mg kg^{-1}) and oral (5 mg kg^{-1}) administration of dauricine is shown in Figure 3. Primary pharmacokinetic parameters, based on non-compartment model analysis, are summarized in Table 2.

The bioavailability of dauricine was found to be 55.4% for the first time.

Conclusion

In the present study, a simple, precise, and accurate UPLC–MS/MS method for the quantitation of dauricine in rat plasma was established, using $100 \mu\text{L}$ of plasma with an LLOQ of 2 ng mL^{-1} . The UPLC–MS/MS method was successfully applied to a pharmacokinetic study of dauricine after intravenous

Figure 3. Mean plasma concentration time profile after intravenous (2 mg kg^{-1}) and oral (5 mg kg^{-1}) administration of dauricine in rats

Table 2. Primary pharmacokinetic parameters after intravenous and oral administration of dauricine in rats ($n = 6$)

Parameters	Unit	i.v. 2 mg kg^{-1}	p.o. 5 mg kg^{-1}
$\text{AUC}_{(0-t)}$	$\text{ng mL}^{-1} \cdot \text{h}$	55.3 ± 15.3	76.7 ± 21.5
$\text{AUC}_{(0-\infty)}$	$\text{ng mL}^{-1} \cdot \text{h}$	57.7 ± 55.4	86.4 ± 29.3
$\text{MRT}_{(0-t)}$	h	0.3 ± 0.1	0.5 ± 0.1
$\text{MRT}_{(0-\infty)}$	h	0.4 ± 0.2	0.7 ± 0.4
$t_{1/2}$	h	0.5 ± 0.3	0.7 ± 0.3
CL	$\text{L h}^{-1} \text{ kg}^{-1}$	36.7 ± 10.1	63.8 ± 26.3
V	L kg^{-1}	29.5 ± 22.7	57.9 ± 12.3
C_{max}	ng mL^{-1}	209.7 ± 57.8	168.4 ± 35.9

Table 1. Precision, accuracy, recovery, and matrix effect for dauricine of QC sample in rat plasma ($n = 6$)

Concentration (ng mL^{-1})	Precision (CV%)		Accuracy (%)		Matrix effect	Recovery
	Intra-day	Inter-day	Intra-day	Inter-day		
10	12.5	8.3	102.9	96.8	90.0	92.6
100	7.6	10.4	104.7	95.8	90.3	95.1
500	10.9	7.3	99.9	105.9	88.0	91.5

and oral administration. The bioavailability of dauricine was found to be 55.4%.

Disclosure of Conflict of Interest

The authors declare no conflict of interest.

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