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Pharmacokinetics of palmatine in rat after oral and intravenous administration by UPLC-MS/MS

JIANBO LI[1†], YUJIE HU[2†], YAJIN WU[2], TIANTIAN FENG[2], CONGCONG WEN[2*] and XIAJUAN JIANG[3*]

[1] The Second Affiliated Hospital Zhejiang University School of Medicine Yuhang Campus, Hangzhou, China

[2] Laboratory Animal Centre, Wenzhou Medical University, Wenzhou, China

[3] The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China

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ABSTRACT

Palmatine is a compound with good water solubility extracted from *Coptis chinensis, Fibraurea recisa Pierre, Cortex Phellodendri Chinensis*. Palmatine has good antibacterial activity and mainly used for the treatment of bacterial dysentery, gynecological inflammation, surgical infection, and conjunctivitis. It has anti-diabetic, anti-oxidant, and cognitive-enhancing activities. In this study, we used UPLC-MS/MS to determinate palmatine in rat plasma, and investigated its pharmacokinetics. Coptisine was utilized as an internal standard (IS), and acetonitrile precipitation method was used to process the plasma samples. Chromatographic separation was achieved using a UPLC BEH C18 column using mobile phase of acetonitrile- 0.1% formic acid with gradient elution. Electrospray ionization (ESI) tandem mass spectrometry in multiple reaction monitoring (MRM) mode with positive ionization was applied. The results indicated that within the range of 1–500 ng/mL, linearity of palmatine in rat plasma was acceptable ($r > 0.995$), and the lower limit of quantification (LLOQ) was 1 ng/mL. Intra-day and inter-day precision RSD of palmatine in rat plasma were less than 14%. Accuracy range was between 93.7 and 107.1%, and matrix effect was between 101.6 and 109.4%. The method was successfully applied in the pharmacokinetics of palmatine in rats after oral and intravenous administration. The absolute bioavailability of the palmatine was 15.5% in rats.

KEYWORDS

palmatine, pharmacokinetics, bioavailability, rat, UPLC-MS/MS

INTRODUCTION

Natural products and traditional Chinese medicines have enormous structural and chemical diversity, which is good active material source for drug-based molecules and drug discovery after evolutionary optimization [1–3]. One example is palmatine, which is a compound with good water solubility, extracted from *Coptis chinensis, Fibraurea recisa Pierre, Cortex Phellodendri, Chinensis* [4–7]. Palmatine has good antibacterial activity and is mainly used for the treatment of bacterial dysentery, gynecological inflammation, surgical infection, and conjunctivitis [8]. In addition, palmatine has anti-diabetes, anti-oxidation and cognitive activities.

There has been several LC-MS/MS method reported for determination of palmatine in plasma [9–20]. Compared to HPLC and LC-MS/MS, UPLC-MS/MS is faster, possessing significant advantages in the investigation on pharmacokinetics of drugs [21–28]. Meanwhile, the powerful separation and analysis capacity is applicable to analyze the in *vivo* metabolism of complex compound systems [29, 30]. Yang et al. developed a UPLC-ESI-MS/MS method for simultaneous determination of anemoside B4, phellodendrine, berberine, palmatine,

†These authors contributed equal to this work.

*Corresponding authors.
E-mail: bluce494949@163.com (C. Wen), 421717014@qq.com (X. Jiang).

obakunone, esculin, esculetin in rat plasma by and its application to a comparative pharmacokinetic study in normal and ulcerative colitis rats, however, the run-time needed 10 min.

In this study, we established an UPLC-MS/MS method for palmatine in rat plasma, and investigated its pharmacokinetics in rats, using the simple and fast one-step acetonitrile precipitation method to process the plasma samples, and it only needed 3 min. The bioavailability of palmatine was then calculated, providing scientific basis for clinical application.

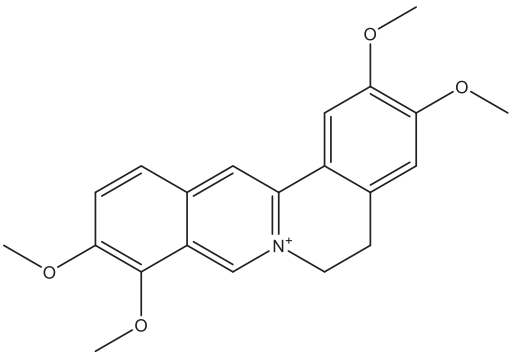
MATERIALS AND METHODS

Chemical and animals

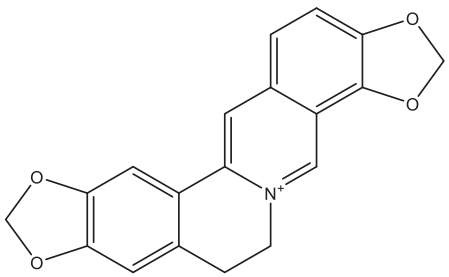
Palmatine (purity >98%, Figure 1A) and coptisine (purity >98%, Figure 1B) were purchased from Chengdu Mansite Bio-Technology Co., Ltd (Chengdu, China). HPLC-grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). HPLC-grade formic acid was obtained from Tedia (Ohio, USA). Ultrapure water was prepared by Millipore Milli-Q water system (Bedford, MA, USA). Sprague Dawley (SD) rats (male, body weight 200–220 g) were obtained from Animal Experimental Center of Wenzhou Medical University.

Instrument and condition

ACQUITY H-Class UPLC and XEVO TQS-micro triple quadrupole mass spectrometer (Waters Corp, Milford, MA, USA) were used in this study. Masslynx 4.1 software (Waters Corp.) was used to collect data and control the equipment.



(A)



(B)

Figure 1. Chemical structure of (A) palmatine and (B) coptisine

UPLC BEH C18 (2.1 mm × 50 mm, 1.7 μm) was used at 40 °C. Mobile phase was composed of acetonitrile and 0.1% formic acid in gradient elution, and the flow rate was set at 0.5 mL/min. The gradient elution was as follows: 0–0.2 min, 10% acetonitrile; 0.2–1.4 min, 10–80% acetonitrile; 1.4–2.0 min, acetonitrile 80%; 2.0–2.1 min, 80–10% acetonitrile; 2.1–3.0 min, 10% acetonitrile. The total run time was 3.0 min. Acetonitrile-water (10/90, v/v) was used for wash solution.

Nitrogen was used as desolvation gas (800 L/h) and nebulizing gas. Capillary voltage was set as 2.4 kV, ion source temperature was 150 °C, and desolvation temperature was 400 °C. ESI in MRM mode with positive ionization was used: m/z 352.2 → 308.0 and 320.2 → 292.0 for palmatine and IS, respectively.

Preparation for control solutions

Stock solutions of palmatine (1.0 mg/mL) and coptisine (1.0 mg/mL) were prepared by methanol. Palmatine standard solutions with different concentrations were prepared by diluting the stock solutions with acetonitrile. Coptisine at a concentration of 50 ng/mL was diluted with acetonitrile from coptisine stock solution. All of the solutions were stored at 4 °C.

Preparation for standard curve

The blank rat plasma was spiked with moderate amounts of working solutions of palmatine to prepare standard solutions with concentrations of 1, 5, 20, 100, 200, and 500 ng/mL. Quality control samples at concentrations of 1, 3, 180, and 450 ng/mL were prepared using the same method.

Plasma processing

In a 1.5 mL centrifuge tube, 50 μL plasma, acetonitrile (150 μL, containing IS 50 ng/mL) was added into plasma, mixed by a vortexer for 1.0 min, and centrifuged at 13,000 rpm for 10 min at 4 °C. The supernate (100 μL) was transferred into a liner pipe in vial, and injection volume was set at 2 μL.

METHOD VALIDATION

The validation method was established in accordance with the guidance of the US FDA (FDA) [31].

To explore whether endogenous substance would interfere with detection of the compound and internal standard (IS), blank rat plasma samples from 6 different sources and LLOQ samples prepared with corresponding blank rat plasma were detected by UPLC-MS/MS. The detected concentration of LLOQ should be the baseline value of blank plasma plus concentration of added palmatine, and the deviation should be within ±20%.

Six detected concentrations (1–500 ng/mL) were used as x axis, and the peak area ratio of the sample to IS was used as

y axis. Standard curve (linear regression equation) was obtained by weighted least-squares method ($W = 1/x^2$).

QC samples at low, medium, and high concentrations (3, 180, and 450 ng/mL) were detected for six times within 3 days. Intra-and inter-day precision and accuracy were calculated according to the concentrations of QC samples calculated by the intra-day standard curve.

Recovery was assessed by the comparison of peak areas between QC samples (at three concentrations) and standard samples. By spiking standard solutions into processed blank plasma, three solutions with low, medium, and high concentrations (3, 180, and 450 ng/mL) were prepared. The matrix effect was evaluated by comparing between detected peak areas of the three solutions and those of standard solutions (3, 180, and 450 ng/mL) diluted with acetonitrile-0.1% formic acid (1:1, v/v).

Stability of palmatine was detected in the plasma samples that were placed at room temperature for 6 h, processed by protein precipitation and placed at room temperature for 24 h, frozen-thawed for three times, and placed at −70 °C for 30 days.

Pharmacokinetics

Twelve rats were randomly divided into two groups. One group was given palmatine (10 mg/kg) by oral (po) administration, and the other group was given palmatine (2 mg/kg) by intravenous (iv) administration, six rats for each group. 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 (Wydw2013-0071). The blood (150 μL) was withdrawn from the caudal vein at 5 min, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24 h after administration, and collected into 1.5 mL centrifuge tubes containing heparin. Plasma (50 μL) was collected after centrifuging at 3,000 rpm for 10 min and stored at −20 °C.

Pharmacokinetic parameters were all analyzed using DAS 2.0 software (China Pharmaceutical University).

RESULTS AND DISCUSSION

Method development

The choice of positive and negative electrodes for electrospray ESI is often evaluated in methodology [28, 32]. Palmatine is more suitable for ESI positive electrode detection. Selection of IS was also an important task in the process of establishment. Finally, coptisine was selected as IS because it and palmatine have similar structure and mass spectrometric ionization mode.

As far as possible, the internal interfering substances are separated from the retention time by HPLC, mobile phase, and chromatographic column determine the chromatographic behavior [33]. We tried acetonitrile-0.1% formic acid, acetonitrile-10 mmol/L ammonium acetate solution (containing 0.1% formic acid), methanol-0.1% formic acid, methanol-10 mmol/L ammonium acetate solution (containing 0.1% formic acid) and gradient elution. Using a gradient elution method, the residual impurities of each sample could more complete eluted from the column compared to isocratic elution [34, 35]. Acetonitrile-0.1% formic acid in water with gradient elution could provide more beautiful peak and better chromatographic separation than others.

Method validation

Figure 2 illustrates the UPLC-MS/MS chromatograms of blank plasma samples spiked with palmatine and IS. There were no obvious impurities and endogenous substances that had intervened in the detection of palmatine, suggesting the selectivity of the method was acceptable.

The concentration of palmatine standard curve in rat plasma was within the range of 1–500 ng/mL. The equation of standard curve is $y = 0.015 \times -0.021$, $r = 0.9982$, where y is the peak area ratio of palmatine to IS and x represents palmatine concentrations in plasma. The LLOQ of palmatine in rat plasma was 1 ng/mL.

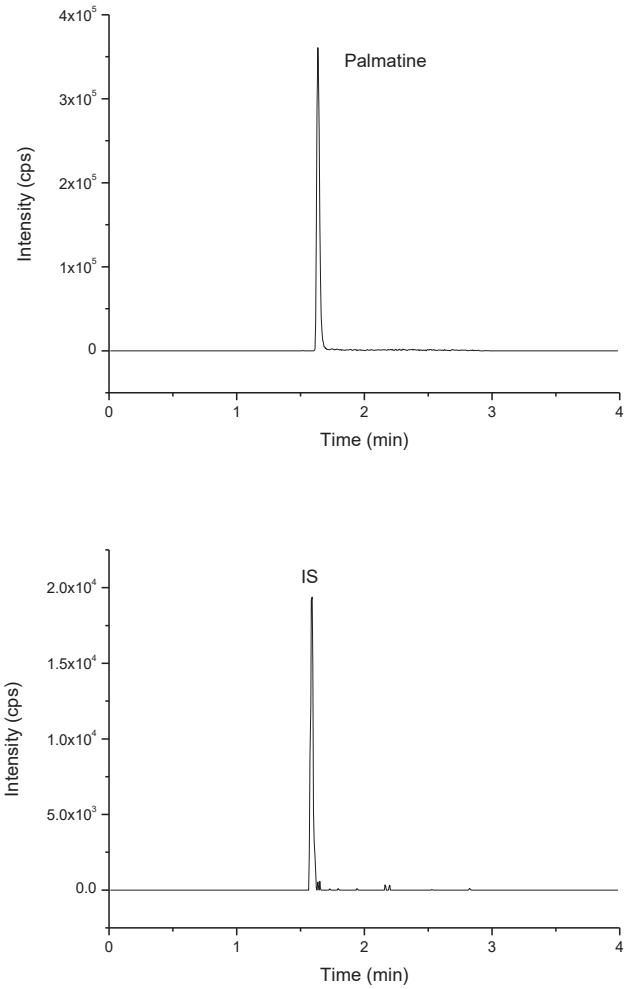


Figure 2. UPLC-MS/MS spectra of palmatine and coptisine (internal standard) in rat plasma

Table 1. Accuracy, precision, matrix effect and recovery of palmatine in rat plasma

Concentration (ng/mL)	Accuracy (%)		Precision (RSD %)		Matrix effect (%)	Recovery (%)
	Intra-day	Inter-day	Intra-day	Inter-day		
1	13.9	11.2	93.7	93.8	109.4	82.4
4	7.8	9.5	107.1	102.4	102.2	79.9
150	3.6	9.1	103.1	99.9	101.6	74.9
450	1.1	4.4	101.0	100.9	108.1	85.2

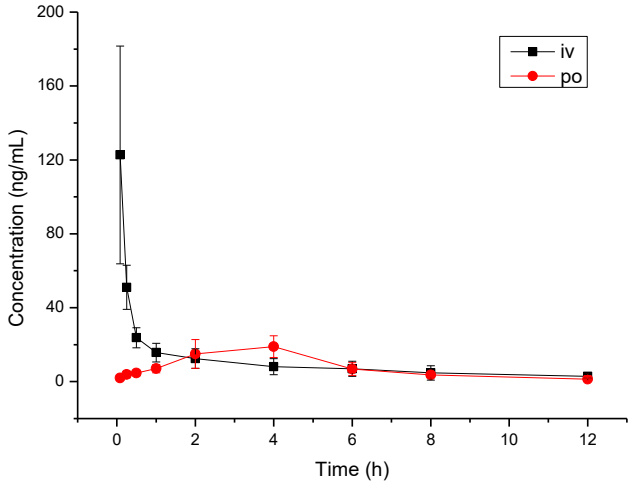


Figure 3. Plasma concentration-time curve of palmatine after oral (10 mg/kg) and intravenous (2 mg/kg) administration

As shown in Table 1, intra- and inter-day precision RSD of palmatine in rat plasma were lower than 14%. Accuracy range was between 93.7 and 107.1%, and matrix effect was between 101.6 and 109.4%. These data met the pharmacokinetic study requirements of palmatine.

Pharmacokinetic

Figure 3 displays the plasma concentration-time curves of palmatine. The main pharmacokinetic parameters analyzed by the non-compartment model were listed in Table 2. The bioavailability of palmatine is 15.5% after oral administration. The oral utilization rate is low, which limits its application to a certain extent. The $t_{1/2}$ was 5.65 ± 2.22 h after intragastric administration of *Pulsatillae Decoction formula* extract 10 g/kg in rat plasma. It was 215.7 ± 33.5 min after an oral administration of coptis–evodia powder (6:1, g/g) 1.086 g/kg body weight [9]. It was 14.2 ± 1.32 after oral administration of 300 mg/kg *Yiqing Capsule* and 600 mg/kg *Gegen-Qinlian Tablet* [10]. It was3.91 ± 0.80 h and 2.62 ± 0.52 h in rats after the single oral administration of *Rhizoma coptidis* and Jiao-Tai-Wan extract [15]. It was 12.84 ± 4.58 h after oral administration of *Rhizoma Corydalis Decumbentis* extract at 2.0 g/kg to rats [17]. While $t_{1/2}$ was 5.8 ± 1.8 h after oral administration of palmatine in rats.

Table 2. Main pharmacokinetic parameters of palmatine in rats

Parameters	Unit	iv 2 mg/kg	po 10 mg/kg
$AUC_{(0-t)}$	ng/mL*h	123.4 ± 54.8	95.5 ± 15.1
$AUC_{(0-\infty)}$	ng/mL*h	193.0 ± 146.1	100.9 ± 9.8
$MRT_{(0-t)}$	h	3.1 ± 0.3	4.3 ± 0.7
$MRT_{(0-\infty)}$	h	11.0 ± 8.5	5.0 ± 1.6
$t_{1/2z}$	h	11.1 ± 1.3	5.8 ± 1.8
V_z	L/kg	9.7 ± 7.3	2.2 ± 1.4
CL_z	L/h/kg	163.1 ± 125.2	326.3 ± 222.2
C_{max}	ng/mL	13.8 ± 6.2	99.9 ± 9.6
Bioavailability			15.5%

CONCLUSION

In this study, we developed a fast UPLC-MS/MS method with good selectivity for palmatine in rat plasma. The linear range was 1–500 ng/mL, and 50 µL plasma was used and processed by the acetonitrile precipitation method, and it only needed 3 min for the runtime. This method was applied in pharmacokinetics of palmatine in rats, and bioavailability was reported to be 15.5% after oral administration.

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