Determination and pharmacokinetics of calycanthine in rat plasma by UPLC-MS/MS

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ABSTRACT

Calycanthine is an important class of alkaloids extracted and isolated from the roots, leaves, flowers and fruits of *Chimonanthus praecox*. In this work, the UPLC-MS/MS method was used for determination of calycanthine in rat plasma, and the pharmacokinetics in rats were investigated. Midazolam was used as an internal standard (IS), and methanol precipitation method was used to pretreatment the rat plasma samples. Chromatographic separation was achieved on a UPLC BEH C18 (50 × 2.1 mm, 1.7 μm) column with the mobile phase of methanol- 0.1% formic acid aqueous solution with gradient elution. Multiple reaction monitoring (MRM) mode with positive ionization was applied for quantitative analysis, m/z 347.3 → 246.7 and 326.2 → 291.4 for calycanthine and IS, respectively. The results indicated that within the range of 1–200 ng/mL, linearity of calycanthine in rat plasma was good ($r > 0.995$), and the lower limit of quantification (LLOQ) was 1 ng/mL. Accuracy range was between 90.6 and 109.4%, precision (RSD) of calycanthine was less than 14%. The matrix effect was between 97.9% and 105.4%, the recovery was better than 85.6%. The developed UPLC-MS/MS method was successfully applied in the pharmacokinetics of calycanthine in rats after oral and intravenous administration. The absolute bioavailability of the calycanthine was 37.5% in rats.

KEYWORDS

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

INTRODUCTION

The *Chimonanthus* belongs to the family *Calycanthaceae*, which has large fragrant flowers and beautiful leaves [1, 2]. *Chimonanthus* contains volatile oil, coumarins, alkaloids, alkene, sesquiterpenoids, flavonoids [3–6]. The main medicinal components are alkaloids [7–10]. Calycanthine is an important class of alkaloids extracted and isolated from the roots, leaves, flowers and fruits of *Chimonanthus praecox* [11, 12]. Calycanthine may mediate its convulsant action predominantly by inhibiting the release of the inhibitory neurotransmitter GABA as a result of interactions with L-type Ca²⁺ channels and by inhibiting GABA-mediated chloride currents at GABA(A) receptors [13]. In the in vitro tests, calycanthine showed significant inhibitory activities against five plant pathogenic fungi *Exserohilum turcicum*, *Bipolaris maydis*, *Alternaria solani*, *Sclerotinia sclerotiorum*, and *Fusarium oxysporum*.

Pharmacokinetics is a discipline that quantitatively studies the laws of drug absorption, distribution, metabolism, and excretion in living organisms [14–18]. It has also been extended to the mechanism research of the entire treatment process in *vivo* absorption, distribution, metabolism, and excretion (ADME), the interaction of complex drug systems, and drug action substances. In order to further understand the absorption and distribution of calycanthine in the body, it is necessary to conduct a systematic study of pharmacokinetics to provide a theoretical basis for drug development. Therefore, the study of the pharmacokinetics of calycanthine is of great significance for the clinical application.

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As far as I known, there was no reports about the quantitative analysis and pharmacokinetic study of calycanthine. In this study, an UPLC-MS/MS method was developed for determination of calycanthine in rat plasma, and pharmacokinetics in rats was investigated.

MATERIALS AND METHODS

Chemical and animals

Calycanthine (purity > 98%, Fig. 1A) and midazolam (purity > 98%, Fig. 1B) were purchased from Chengdu Mansite Bio-Technology Co., Ltd (Chengdu, China). Ultrapure water was prepared by Millipore Milli-Q water system (Bedford, MA, USA). HPLC-grade methanol was purchased from Merck (Darmstadt, Germany). HPLC-grade formic acid was obtained from Tedia (Ohio, USA).

Instrument and condition

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

(Waters Corp.) was used to control the equipment and collect data.

UPLC BEH C18 (50 mm × 2.1 mm, 1.7 μm) was used at 40 °C. Mobile phase was composed of methanol and 0.1% formic acid aqueous solution 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% methanol; 0.2–1.4 min, 10–80% methanol; 1.4–2.0 min, methanol 80%; 2.0–2.1 min, 80–10% methanol; 2.1–3.0 min, 10% methanol. The total run time was 3.0 min.

Capillary voltage was set as 2.4 kV, ion source temperature was 150 °C, and desolvation temperature was 400 °C. Nitrogen was used as desolvation gas (800 L/h) and nebulising gas. ESI in Multiple reaction monitoring (MRM) mode was used in positive mode: m/z 347.3 → 246.7 and 326.2 → 291.4 for calycanthine and internal standard (IS), respectively.

Stock and working solutions

Stock solutions of calycanthine (1.0 mg/mL) and midazolam (1.0 mg/mL) were prepared by methanol. Calycanthine working solutions with different concentrations (10, 50, 100, 200, 500, 1,000, 2,000 ng/mL) were prepared by diluting the stock solutions with methanol. Midazolam working solution of 50 ng/mL was diluted with methanol from midazolam stock solution.

Standard curve

The blank rat plasma was spiked with suitable amounts of working solutions of calycanthine to prepare standard solutions with concentrations of 1, 5, 10, 20, 50, 100, 200 ng/mL. Quality control samples at concentrations of 1, 3, 18, and 180 ng/mL were prepared by the same means.

Plasma pretreatment

In a 1.5 mL centrifuge tube, 50 μL plasma, methanol (200 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 (150 μL) was transferred into a liner pipe in vial, and 2 μL was inject into UPLC-MS/MS for analysis.

Method validation

The validation method was established in accordance with the guidance of the US Food and Drug Administration (FDA) [19, 20].

Pharmacokinetics

Twelve Sprague Dawley (SD) rats (male, body weight 200–220 g) were obtained from Animal Experimental Center of Wenzhou Medical University. Six rats was given calycanthine (10 mg/kg) by oral (po) administration, and the other six rats was given calycanthine (2 mg/kg) by intravenous (iv) administration. The blood (100

Fig. 1. Chemical structure of (A) calycanthine and (B) midazolam

μL) was withdrawn from the caudal vein at 5 min, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24 h after administration. Plasma (50 μL) was collected after centrifuging at 3,000 rpm for 10 min

and stored at $-20\text{ }^{\circ}\text{C}$. DAS 2.0 software (China Pharmaceutical University) was used to analyze pharmacokinetic parameters.

Fig. 2. UPLC-ESI-MS/MS of calycanthine and midazolam (IS) in rat plasma. (A) blank plasma, (B) blank plasma spiked calycanthine (1 ng/mL) and IS, (C) a rat plasma after oral administration of calycanthine in rats (62.3 ng/mL)

RESULTS AND DISCUSSION

Method development

The choice of positive and negative ionization mode for electrospray ESI is often evaluated in methodology [21–26]. In positive ion mode the charging generally occurs via protonation, and in negative ion mode charging occurs via deprotonation of the analyte. Calycanthine is an alkaloid compound and belongs to weakly basic drugs. Generally speaking, weakly basic drugs are more suitable for positive ionization mode. In our work, calycanthine has higher sensitivity in positive ionization ESI mode than negative mode. The choice of IS is very important in bioanalytical methodology, related to the quantitative accuracy of the method. Midazolam was selected as IS because it and calycanthine have similar mass spectrometric ionization mode.

UPLC conditions were as far as possible separating endogenous interfering from the analyte and IS [27, 28]. We tried acetonitrile, methanol, 0.1% formic acid aqueous solution, ammonium acetate solution as mobile phase. As a result, the mobile phase of methanol-0.1% formic acid aqueous solution, using gradient elution, could obtain satisfactory chromatographic peaks and retention time. UPLC-MS/MS was faster and higher sensitive than traditional HPLC. It only takes 3 min to analyze one plasma sample, which could save a lot of time and solvents. In addition, the relatively low LLOQ (1 ng/mL) for calycanthine could be used to determine lower plasma concentrations at the last sampling time point.

Method validation

The representative UPLC-MS/MS chromatograms of calycanthine and IS in rat plasma were shown in Fig. 2. The absence of obvious endogenous substances interfered with the detection, indicating that the method was specificity.

The concentration of calycanthine standard curve in rat plasma was within the range of 1–200 ng/mL. Linearity of analytical method was determined by least square linear regression by using $1/x^2$ as a weighting factor. The equation of standard curve is $y = (0.001748 \pm 0.000125)x - (0.00763 \pm 0.000645)$, $r = 0.9984$, where y is the peak area ratio of calycanthine to internal standard and x represents calycanthine concentrations in rat plasma. The LLOQ of calycanthine in rat plasma was 1 ng/mL.

As shown in Table 1, accuracy range was between 90.6 and 109.4%, intra-day and inter-day precision (RSD) were

less than 14%, the matrix effect was between 97.9 and 105.4%, the recovery was better than 85.6%.

The rat plasma at room temperature for 2 h, -20°C for 30 days and freeze-thaw stability test, the variation of calycanthine was within $\pm 12\%$ and CV was less than 13%, it indicated the stability of calycanthine was acceptable.

Pharmacokinetics

UPLC-MS/MS was applied to the quantitative detection of calycanthine in plasma, which was faster and sensitivity than traditional HPLC. The plasma concentration-time curves of calycanthine was shown in Fig. 3. The main pharmacokinetic parameters of calycanthine analyzed by the non-compartment model were listed in Table 2. The bioavailability of calycanthine is 37.5% after oral administration. Calycanthine with $t_{1/2}$ of intravenous and oral administration were 5.0 ± 3.0 and 3.5 ± 1.8 h, it indicated that calycanthine metabolism was not fast in rats. The $\text{AUC}_{(0-t)}$ of intravenous and oral administration were 304.1 ± 108.2 and 570.9 ± 187.9 ng/mL·h, it indicated that the oral absorption was good. The apparent distribution volumes (V) of intravenous and oral administration were 35.9 ± 26.9 and 85.8 ± 23.6 L/kg, it could be speculated that they were widely distributed in the organs. As far as I known, there was no reports about the quantitative analysis and pharmacokinetic study of calycanthine in rats. The study the pharmacokinetics of calycanthine *in vivo*, it

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

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

Concentration (ng/mL)	Accuracy (%)		Precision (RSD %)		Matrix effect (%)	Recovery (%)
	Intra-day	Inter-day	Intra-day	Inter-day		
1	90.6	107.9	13.6	9.0	97.9	87.3
3	109.4	105.2	5.9	7.7	99.0	89.9
18	98.7	100.8	7.7	10.1	105.4	85.6
180	96.9	96.7	8.6	3.6	103.9	91.2

Table 2. Main pharmacokinetic parameters of calycanthine in rats

Parameters	Unit	iv 2 mg/kg	po 10 mg/kg
AUC _(0–t)	ng/mL·h	304.1 ± 108.2	570.9 ± 187.9
AUC _(0–∞)	ng/mL·h	374.9 ± 146.7	589.5 ± 217.5
MRT _(0–t)	h	3.7 ± 0.3	5.6 ± 1.6
MRT _(0–∞)	h	7.2 ± 6.6	6.1 ± 2.5
t _{1/2Z}	h	5.0 ± 3.0	3.5 ± 1.8
V _z	L/kg	35.9 ± 26.9	85.8 ± 23.6
CL _z	L/h/kg	6.0 ± 2.1	18.8 ± 6.9
C _{max}	ng/mL	90.2 ± 8.2	81.0 ± 12.4
Bioavailability			37.5%

could help to better understand the mechanism of pharmacology.

CONCLUSION

In this study, a simple and fast UPLC-MS/MS method with good selectivity for determination of calycanthine in rat plasma was developed. The linear range was 1–200 ng/mL, and 50 µL plasma was used for sample treatment. This developed UPLC-MS/MS method was applied in pharmacokinetics of calycanthine in rats, and bioavailability was determined to be 37.5% after oral administration.

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