Pharmacokinetics of Isocorynoxine in Rat Plasma after Intraperitoneal Administration by UPLC–MS/MS

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Isocorynoxine is one of the main alkaloids in Chinese medicinal herbs, and has pharmacological activities such as antihypertensive, sedative, anticonvulsant, and neuronal protection. It is an effective component of *Uncaria* for the treatment of hypertension. In this study, we used a fast and sensitive ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) to detect isocorynoxine in rat plasma and investigated its pharmacokinetics in rats. Six rats were given isocorynoxine (15 mg/kg) by intraperitoneal (i.p.) administration. Blood (100 μ L) was withdrawn from the caudal vein at 5 and 30 min and 1, 2, 4, 6, 8, 12, and 24 h after administration. Chromatographic separation was achieved using a UPLC BEH C18 column using a mobile phase of acetonitrile–0.1% formic acid with gradient elution. Electrospray ionization (ESI) tandem mass spectrometry in the multiple reaction monitoring (MRM) mode with positive ionization was applied. Intra-day and inter-day precisions (relative standard deviation, %RSD) of isocorynoxine in rat plasma were lower than 12%. The method was successfully applied in the pharmacokinetics of isocorynoxine in rats after intraperitoneal administration. The $t_{1/2}$ of isocorynoxine is 4.9 ± 2.1 h, which indicates quick elimination.

Keywords: isocorynoxine, pharmacokinetics, rat, UPLC–MS/MS

Introduction

Uncaria rhynchophylla is a plant of the family Rubiaceae, which grows in valleys and streams [1–3]. It is widely distributed in the southern provinces of China [4, 5]. It is used in traditional Chinese medicine for headache, dizziness, cold, convulsions, convulsions, and pregnancy [6–10], as well as in treatment of conditions such as eclampsia and hypertension. Alkaloids are the main medicinal ingredients of *U. rhynchophylla*, and the changes and distribution characteristics of medicinal ingredients in the body are closely related to their pharmacological effects [11–15]. Isocorynoxine is one of the main tetracyclic oxindole alkaloids found in *U. rhynchophylla* and has pharmacodynamic effects, such as antihypertensive, sedative, hypnotic, and anti-age dementia, showing a certain development value [16–18]. Although the pharmacokinetic characteristics of isocorynoxine given by oral administration in rat had been revealed in recent years, no study on the i.p. administration in vivo until now has been investigated [18, 19]. In order to further understand the absorption and distribution of isocorynoxine by i.p. administration in vivo, it is necessary to carry out systematic research on pharmacokinetic characteristics to provide a theoretical basis for drug development. Therefore, a validated method for determination of isocorynoxine was needed for analysis and drug exploration in the future.

There have been several LC–MS methods reported for the determination of isocorynoxine in rat plasma [20, 21]. Cai

et al. developed a liquid chromatography–mass spectrometry (LC–MS) method for the determination of isocorynoxine in rat plasma with an LLOQ of 5 ng/mL; it needed 10 min for the runtime, and sample preparation was performed by using liquid–liquid extraction with ethyl acetate [21]. Wang et al. presented the pharmacokinetics and mass balance of isocorynoxine in rats after an oral dose of 40.0 mg/kg and an intravenous dose of 4.0 mg/kg through detection of isocorynoxine and its in vivo metabolites, using an optimized LC–MS [20]; it needed 20 min for the runtime, and tedious plasma samples preparation was used. However, these methods needed too long runtime, and the selective ion monitoring (SIM) mode was used to quantify isocorynoxine in rat plasma in their study.

In this study, we presented a fast and sensitive UPLC–MS/MS method for isocorynoxine in rat plasma in the multiple reaction monitoring (MRM) mode with an LLOQ of 1 ng/mL and investigated its pharmacokinetics in rats, using a simple and fast one-step acetonitrile precipitation method to process the plasma samples, which only needed 50 μ L rat plasma and 3 min for the runtime.

Materials and Methods

Chemicals and Animals. Isocorynoxine (purity >98%, Figure 1) was purchased from Chengdu Mansite Biotechnology Co., Ltd. (Chengdu, China). HPLC-grade methanol and formic acid were purchased from Merck (Darmstadt, Germany). Ultrapure water was prepared using a Millipore Milli-Q water system (Bedford, MA, USA). Sprague Dawley (SD) rats (male, body weight 200–220 g) were

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Figure 1. Chemical structure of isocorynoxine (A) and the IS (B)

obtained from the Animal Experimental Center of Wenzhou Medical University.

Instruments and Conditions. An ACQUITY H-Class UPLC and a XEVO TQS-micro triple quadrupole mass spectrometer (Waters Corp, Milford, MA, USA) were used in this study. A UPLC BEH C18 (2.1 mm × 50 mm, 1.7 μm) was used at 40 °C. The 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, acetonitrile 10%; 0.2–1.0 min, linear acetonitrile from 10% to 80%; 1.0–2.0 min, acetonitrile 80%; 2.0–2.1 min, linear acetonitrile from 80% to 10%; and 2.1–3.5 min, acetonitrile 10%.

Nitrogen was used as the desolvation gas (800 L/h) and nebulizing gas. The capillary voltage was set at 2.2 kV, the ion source temperature was 150 °C, and the desolvation temperature was 400 °C. ESI in the MRM mode with positive ionization was used: 383.2 → 159.9 for isocorynoxine, *m/z* 320.2 → 292.0 for the internal standard (IS).

Plasma Processing. In a 1.5-mL centrifuge tube, 50 μL plasma was added, further diluted with 200 μL acetonitrile (containing the IS of 50 ng/mL), 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 1 μL.

Method Validation. The validation method was established in accordance with the guidance of the US Food and Drug Administration (FDA) [22, 23].

To explore whether endogenous substance would interfere with the detection of the compound and the internal standard, blank rat plasma samples from 6 different sources and the LLOQ samples prepared with the 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 isocorynoxine, and the deviation should be within ±20%.

Eight detected concentrations (1–2000 ng/mL) were used as the *x*-axis, and the peak area ratio of the sample to the internal

standard was used as the *y*-axis. A standard curve (linear regression equation) was obtained using the weighted least-squares method ($W = 1/x^2$).

QC samples at low, medium, and high concentrations (50, 900, and 1800 ng/mL) were detected 6 times within 3 days. Intra- and inter-day precisions and accuracy were calculated according to the concentrations of the QC samples calculated by the intra-day standard curve.

Recovery was assessed by the comparison of peak areas between QC samples (at 3 concentrations) and standard samples. By spiking standard solutions into the processed blank plasma, 3 solutions with low, medium, and high concentrations (50, 900, and 1800 ng/mL) were prepared. The matrix effect was evaluated by comparing between detected peak areas of the 3 solutions and those of standard solutions (50, 900, and 1800 ng/mL) diluted with acetonitrile–0.1% formic acid (1:1, *v/v*).

Stability of isocorynoxine 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 3 times, and placed at –70 °C for 30 days.

Pharmacokinetics. Six rats were given isocorynoxine (15 mg/kg) by intraperitoneal (i.p.) administration. The blood (100 μL) was withdrawn from the caudal vein at 5 and 30 min and 1, 2, 4, 6, 8, 12, and 24 h after administration and collected into 1.5-mL centrifuge tubes containing heparin. Plasma (50 μL) was collected after centrifuging at 3000 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 Optimization. At present, most of the extracts of the genus *Uncaria* reported in the literature use the alkaline ethyl acetate liquid–liquid extraction method. In this paper, acetonitrile protein-precipitation method, perchloric acid protein-precipitation method, ethyl acetate liquid–liquid extraction method, and solid-phase extraction method were investigated. The recovery rate of the alkaloid extraction method was compared with the recovery rates of alkaloids extracted by different methods. It is unstable, and the operation is time-consuming; the solid-phase extraction method is costly; the perchloric acid method is incomplete in addition to protein. Therefore, the acetonitrile protein-precipitation method was chosen as a pretreatment method for samples. The optimization of the extract of target resulted in an acceptable sensitivity and matrix effect [24–28]. As for the complication of the rat plasma samples, the addition of 200 μL acetonitrile could meet the requirements of removing the proteins and other potential interferences out of 50 μL plasma samples.

Liquid chromatography conditions separate isocorynoxine from the endogenous interfering substances as much as possible from the analyte retention time [29–34]. In the investigation of chromatographic conditions, mobile phase systems such as methanol–water, acetonitrile–water, methanol–0.1% formic acid, and acetonitrile–0.1% formic acid were investigated. The results of using acetonitrile–0.1% formic acid system were suitable. It can effectively separate the isocorynoxine and other substances, and the endogenous substances have no interference, and meet the requirements of blood concentration analysis.

Method Validation. Figure 2 illustrates the UPLC–MS/MS chromatograms of blank plasma samples spiked with isocorynoxine. There were no endogenous substances that had intervened in the detection of isocorynoxine, suggesting the selectivity of the method was acceptable.

Figure 2. Representative UPLC–MS/MS of isocorynoxine and the IS in rat plasma

Table 1. Accuracy, precision, matrix effect, and recovery of isocorynoxine in rat plasma ($n = 6$)

Concentration (ng/mL)	Accuracy (%)		Precision (%RSD)		Matrix Effect (%)	Recovery (%)
	Intra- day	Inter- day	Intra- day	Inter- day		
1	102.3	93.8	10.3	11.8	93.1 ± 7.5	87.7 ± 6.7
50	95.5	106.2	9.1	10.7	99.5 ± 8.3	78.9 ± 4.3
900	86.2	98.7	8.5	8.2	94.7 ± 5.3	81.4 ± 5.6
1800	97.0	110.4	4.9	10.6	89.1 ± 4.7	87.7 ± 6.2

The concentration of isocorynoxine standard curve in rat plasma was within the range of 1–2000 ng/mL. The LLOQ of isocorynoxine in rat plasma was 1 ng/mL. The standard curve equation of isocorynoxine in the rat plasma was $Y = 0.0199C + 0.0201$, $r = 0.9964$, where Y represents the peak area ratio of the isocorynoxine to the IS, and C represents the concentration of isocorynoxine in rat plasma.

The intra-day and inter-day precisions (%RSD) of isocorynoxine in rat plasma were lower than 12%. The accuracy range was between 86.2% and 110.4%, the matrix effect was between 89.1% and 99.5%, and the recovery was higher than 78.9% (Table 1).

The rat plasma at room temperature for 2 h, at -20°C for 30 days, and under the freeze–thaw stability test, the variation of isocorynoxine was within $\pm 14\%$, and the RSD was less than 12%, which indicated that the stability of isocorynoxine was acceptable.

Pharmacokinetics. The plasma samples from 6 male rats following a single i.p. dose of 15 mg/kg isocorynoxine were analyzed by using the validated UPLC–MS/MS method. The mean plasma concentration–time curves for isocorynoxine are shown in Figure 3. The main pharmacokinetic parameters analyzed using the non-compartment model are listed in Table 2. The $t_{1/2}$ of isocorynoxine is 4.9 ± 2.1 h, which

Table 2. Main pharmacokinetic parameters of isocorynoxine in rats after intraperitoneal (15 mg/kg) administration ($n = 6$)

Parameters	Unit	i.p. 15 mg/kg
$AUC_{(0-t)}$	ng/mL*h	857.1 ± 426.5
$AUC_{(0-\infty)}$	ng/mL*h	858.6 ± 425.7
$MRT_{(0-t)}$	h	0.8 ± 0.2
$MRT_{(0-\infty)}$	h	0.9 ± 0.2
$t_{1/2z}$	h	4.9 ± 2.1
V_z	L/kg	159.0 ± 109.3
CL_z	L/h/kg	20.9 ± 9.0
C_{max}	ng/mL	1022.3 ± 455.7

Figure 3. Plasma concentration–time curve of isocorynoxine after intraperitoneal (15 mg/kg) administration ($n = 6$)

indicates quick elimination, while it was reported that the elimination $t_{1/2}$ was estimated to be 1.05 h after oral administration [18].

Conclusion

In summary, we first report the pharmacokinetic properties of major isocorynoxine metabolites by i.p. administration in vivo. A UPLC–MS/MS method was developed for the rapid determination of the pharmacokinetic properties of isocorynoxine in rat plasma for i.p. administration at 15 mg/kg. The linear range was 1–2000 ng/mL, and 50 μ L plasma was used and processed by the acetonitrile precipitation method. This method was applied in pharmacokinetics of isocorynoxine in rats. Our study provides helpful information about the pharmacokinetic properties of tetracyclic oxindole alkaloids.

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Reference

1. Ndagijimana, A.; Wang, X.; Pan, G.; Zhang, F.; Feng, H.; Olaleye, O. *Fitoterapia* **2013**, *86*, 35–47.
2. Wang, Y. L.; Dong, P. P.; Liang, J. H.; Li, N.; Sun, C. P.; Tian, X. G.; Huo, X. K.; Zhang, B. J.; Ma, X. C.; Lv, C. Z. *Phytomedicine* **2018**, *51*, 120–127.
3. Zhu, S.; Li, Q.; Chen, S.; Wang, Y.; Zhou, L.; Zeng, C.; Dong, J. *Pharm. Biol.* **2018**, *56*, 548–558.
4. Feng, Z.; Hou, J.; Yu, Y.; Wu, W.; Deng, Y.; Wang, X.; Zhi, H.; Zhang, L.; Wu, W.; Guo, D. A. *Front. Pharmacol.* **2019**, *10*, 845.
5. Geng, C. A.; Yang, T. H.; Huang, X. Y.; Ma, Y. B.; Zhang, X. M.; Chen, J. J. *J. Ethnopharmacol.* **2019**, *232*, 39–46.
6. Liu, A.; Chu, Y. J.; Wang, X.; Yu, R.; Jiang, H.; Li, Y.; Zhou, H.; Gong, L. L.; Yang, W. Q.; Ju, J. *J. Evidence-Based Complementary Altern. Med.* **2018**, *2018*, 9281946.
7. Lopes, A. A.; Chioca, B.; Musquiar, B.; Crevelin, E. J.; Franca, S. C.; Fernandes da Silva, M., and Pereira, A.M.S. *Sci. Rep.* **2019**, *9*, 11349.
8. Navarro, M.; Arnaez, E.; Moreira, I.; Hurtado, A.; Monge, D.; Monagas, M. *Antioxidants* **2019**, *8*.
9. Navarro-Hoyos, M.; Alvarado-Corella, D.; Moreira-Gonzalez, I.; Arnaez-Serrano, E.; Monagas-Juan, M. *Antioxidants* **2018**, *7*.
10. Santo, G. D.; Grotto, A.; Boligon, A. A.; Da Costa, B.; Rambo, C. L.; Fantini, E. A.; Sauer, E.; Lazzarotto, L. M. V.; Bertoncello, K. T.; Junior, O. T.; Garcia, S. C.; Siebel, A. M.; Rosemberg, D. B.; Magro, J. D.; Conterato, G. M. M., and Zanatta, L. *Environ. Sci. Pollut. Res.* **2018**, *25*, 11703–11715.
11. Guo, Q.; Yang, H.; Liu, X.; Si, X.; Liang, H.; Tu, P.; Zhang, Q. *Fitoterapia* **2018**, *127*, 47–55.
12. Wei, X.; Jiang, L. P.; Guo, Y.; Khan, A.; Liu, Y. P.; Yu, H. F.; Wang, B.; Ding, C. F.; Zhu, P. F.; Chen, Y. Y.; Zhao, Y. L.; Chen, Y. B.; Wang, Y. F.; Luo, X. D. *Nat. Prod. Bioprospect.* **2017**, *7*, 413–419.
13. Pan, H.; Yang, W.; Yao, C.; Shen, Y.; Zhang, Y.; Shi, X.; Yao, S.; Wu, W.; Guo, D. *J. Chromatogr. A* **2017**, *1516*, 102–113.
14. Huang, B. Y.; Zeng, Y.; Li, Y. J.; Huang, X. J.; Hu, N.; Yao, N.; Chen, M. F.; Yang, Z. G.; Chen, Z. S.; Zhang, D. M.; Zeng, C. Q. *Int. J. Oncol.* **2017**, *51*, 257–268.
15. Li, L.; Xu, L.; Chen, M.; Zhang, G.; Zhang, H.; Chen, A. *J. Pharm. Biomed. Anal.* **2017**, *141*, 39–45.
16. Li, T.; Xu, K.; He, J.; Jahan, N.; Song, J.; Wang, S. *Toxicol. Appl. Pharmacol.* **2019**, *376*, 95–106.
17. Li, T.; Xu, K.; Che, D.; Huang, Z.; Jahan, N.; Wang, S. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2018**, *391*, 1285–1293.
18. Zhao, L.; Zang, B.; Qi, W.; Chen, F.; Wang, H.; Kano, Y.; Yuan, D. *Fitoterapia* **2016**, *111*, 49–57.
19. Zhao, L.; Qi, W.; Chen, F.; Sun, J.; Yuan, D. *Eur. J. Drug Metab. Pharmacokinet.* **2016**, *41*, 615–626.
20. Wang, W.; Li, B.; Chen, Y. P.; Li, M. H.; Hattori, M. *J. Liq. Chromatogr. Relat. Technol.* **2016**, *39*, 77–86.
21. Cai, J. Z.; Lin, C. L.; Hu, L. F.; Lin, G. Y.; Wang, X. Q.; Ma, J. S. *J. Liq. Chromatogr. Relat. Technol.* **2013**, *36*, 2232–2241.
22. Zimmer, D. *Bioanalysis* **2014**, *6*, 13–19.
23. FDA Guidance for Industry Analytical Procedures and Methods Validation for Drugs and Biologics **2014**, <https://www.fda.gov/oc/oc/2014/02/02-19-14-Guidance.pdf>.
24. Chen, R.; Lu, M.; Tu, X.; Sun, W.; Ye, W.; Ma, J.; Wen, C.; Wang, X.; Geng, P. *Acta Chromatogr.* **2019**, *31*, 146–150.
25. Han, A.; Lin, G.; Cai, J.; Wu, Q.; Geng, P.; Ma, J.; Wang, X.; Lin, C. *Acta Chromatogr.* **2019**, *31*, 99–104.
26. Song, H.; Huang, Y.; Zhu, D.; Tong, S.; Zhang, M.; Wang, X.; Bao, X. *Curr. Pharm. Anal.* **2019**, *15*, 194–199.
27. Wang, S.; Lin, Z.; Su, K.; Zhang, J.; Zhang, L.; Gao, Z.; Wang, Z.; Ma, J.; Wang, X. *Acta Chromatogr.* **2018**, *30*, 26–30.
28. Ye, W.; Sun, W.; Chen, R.; Wang, Z.; Cui, X.; Zhang, H.; Qian, S.; Zheng, Q.; Zhou, Y.; Wan, J.; Xu, J.; Wang, X.; Zhou, Y. *Acta Chromatogr.* **2019**, *31*, 120–125.
29. Chen, L.; You, W.; Chen, D.; Cai, Y.; Wang, X.; Wen, C.; Wu, B. *BioMed Res. Int.* **2018**, *2018*, 6562309.
30. Chen, L.; Zhang, B.; Liu, J.; Fan, Z.; Weng, Z.; Geng, P.; Wang, X.; Lin, G. *BioMed Res. Int.* **2018**, *2018*, 1578643.
31. Geng, P. W.; Luo, J.; Weng, Z. W.; Fan, Z. H.; Zhang, B.; Ma, J. S.; Wang, X. Q.; Zhang, M. L. *Biomed. Chromatogr.* **2018**, *32*, E4273.
32. Li, T.; Ye, W.; Huang, B.; Lu, X.; Chen, X.; Lin, Y.; Wen, C.; Wang, X. *J. Pharm. Biomed. Anal.* **2019**, *168*, 133–137.
33. Geng, P.; Luo, X.; Peng, X.; Lin, Z.; Chen, W.; Zhang, J.; Wen, C.; Hu, L.; Hu, S. *Acta Chromatogr.* **2018**, *30*, 231–235.
34. Geng, P.; Zhang, J.; Chen, B.; Wang, Q.; Wang, S.; Wen, C. *Acta Chromatogr.* **2018**, *30*, 136–140.