Effective Separation and Simultaneous Determination of Corynoxeine and Its Metabolites in Rats by High-Performance Liquid Chromatography with Tandem Mass Spectrometry and Application to Pharmacokinetics and In Vivo Distribution in Main Organs

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An effective separation and simultaneous determination of corynoxeine and its metabolites using high-performance liquid chromatography with tandem mass spectrometry was developed and validated. The method was applied to pharmacokinetics and in vivo distribution investigations in rats after oral (0.105 mmol kg\(^{-1}\)) and intravenous (0.0105 mmol kg\(^{-1}\)) doses of corynoxeine. Its brain uptake index was of \(3.08 \times 10^{-11}\) mol g\(^{-1}\) at 3 h and \(3.75 \times 10^{-11}\) mol g\(^{-1}\) at 74 min after oral and intravenous doses, respectively.

Keywords Corynoxeine, separation, quantification, pharmacokinetics, brain uptake index

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Introduction

Kampo medicines Chotosan and Yokukansan (YKS) containing Uncaria hooks as the main effective component are commonly applied in Asia. Due to multiple components embedding in the hooks, the identification, separation, purification, and quantification of the bioactive alkaloids, including corynoxeine (COR) (Fig. 1), has been of interest, especially using high-performance liquid chromatography with tandem mass spectrometry (LC-MS).\(^1\)\(^-\)\(^5\) The plentiful pharmacological activities and in vivo identification in biosystems of COR have been investigated.\(^6\)\(^-\)\(^13\) We have reported that after oral administration, COR was hydroxylated into 11-hydroxycorynoxeine (M1) and 10-hydroxycorynoxeine (M2), which successively glucuronidated into 11-hydroxycorynoxeine 11-\(\beta\)-D-glucuronide (M3) and 10-hydroxycorynoxeine 10-\(\beta\)-D-glucuronide (M4), respectively.\(^14\) This paper presents the development and validation of an LC-MS method used to separate and determine COR and M1-4 simultaneously, and its application to COR pharmacokinetics and their distribution investigation in the main organs after oral and intravenous doses to rats.

Experimental

Background

The LC-MS equipment was described previously.\(^14\) The gradient elution system consisted of an increment of solvent B (0.01% formic acid in CH\(_3\)OH, v/v) from 10 to 25% in solvent A (0.01% formic acid, v/v) within 20 min, to 50% within 5 min, to 85% within 5 min at 1.0 mL min\(^{-1}\). The sampling of 5.0 \(\mu\)L was set for LC-MS analysis. The constant volume of the sample in methanol for LC-MS analysis was fixed at 4.0 mL.

The chemicals, enzyme, COR, M1-4, and internal standard

Fig. 1 Time course of COR in rat plasma after the oral administration of 0.105 mmol kg\(^{-1}\). COR concentrations in plasma, quantified by LC-MS (EIC) monitored at \(m/z\) 383 \(\pm\) 0.5 in the ESI positive ion mode; data shown as mean \(\pm\) SD (\(n = 3\)).
(IS) rhynchophylline (RHY) were purchased or prepared in the same ways as reported.\textsuperscript{14} Male Sprague Dawley rats at 8 weeks with a body weight of 220 ± 5 g, were obtained from Kunming Medical University Experimental Animals Center, Kunming. The animal husbandry, care, and studies used in this work resembled that of previous work with the guidelines, and were approved by the Animal Care and Use Committee at the same institution.\textsuperscript{14}

**Plasma and main organ samples**

After COR was administered orally, the plasma was separated from blood (0.2 – 0.3 mL) collected from the tail veins of a rat at 0.5, 1, 2, 3, 4, 6, 8, 12, 24, and 36 h, by centrifugation at 2220g for 5 min. Two plasma portions of 0.05 mL at each sampling point were lyophilized. One residue was treated and analyzed using the same method for rat bile, except that digested with \(\beta\)-glucuronidase (200 units).\textsuperscript{14} Another residue was directly reconstituted in methanol and analyzed. An intravenous plasma sample was prepared in the same manners after COR was administered into a tail vein. The rat brain, liver, heart, and kidneys were cut en bloc at \(t_{\text{max}}\) (3 h) after oral administration and being pretreated separately.\textsuperscript{15} The lyophilized livers were mechanically homogenized and sonicated (120 W, 10 min) in methanol (10 mL) in triplicate, but involved 4 mL for others. The rat brain was also isolated at 74 min (the first \(t_{\text{1/2}}\) after intravenous administration, and prepared using the same methods as those mentioned above.

**Validation of LC-MS for COR and M1-4**

Stock standard solutions of COR and M1-4 at 5.0 \(\times\) 10\(^{-7}\) M (\(C_{\text{High}}\), 1.5 \(\times\) 10\(^{-8}\) (\(C_{\text{Methanol}}\), and \(5.0 \times 10^{-10}\) M (\(C_{\text{Low}}\)) were prepared accordingly. Calibration curves of COR and M1-4 were constructed in methanol solution of plasma and organ controls, respectively.\textsuperscript{14} The lower limit of quantification (LLOQ) was defined as \(C_{\text{Low}}\) for the found calibration curves, with a signal-to-noise ratio of 10:1 for MS. The correlation coefficient (\(r\)) for COR and M1-4 in controls varied from 0.9975 to 0.9997 (\(n = 6\)).\textsuperscript{14}

COR and M1-4 were added into related controls with final concentrations of \(C_{\text{Low}}, C_{\text{Methanol}}, \) and \(C_{\text{High}}\) for the matrix effect, stability, assay accuracy and precision determination, respectively. Rat blood (0.1 mL) was taken from the tail veins of a rat after taking oral doses of vehicle at 0.5, 12, 24 and 36 h, respectively. The pooled plasma (0.05 mL \(\times\) 3) was lyophilized and added with COR and IS for analysis by LC-MS. After rat organ controls were taken and pretreated, respectively, each of them was sonicated into methanol added with COR for the brain, M1-2 for the heart and kidneys, and M1-4 for the liver, separately, along with IS. The supernatants were analyzed by LC-MS for matrix factor (MF) determination.\textsuperscript{14} The MF value ranged from 0.893 to 0.975 with RSD being from 0.215 to 0.947% (\(n = 6\)).

Standard solutions of COR and/or M1-4 were evaporated to dryness as well as IS, respectively. The residue was mixed with control plasma (0.05 mL). Stability tests were proceeded according to the same methods for a rat biliary sample,\textsuperscript{14} except that long-term stability was carried out after being stored at \(-22^\circ\)C for 12 months. After the pretreatment, organ controls were reconstituted into analysts and IS standard solutions, mixed well, and made to dryness under an \(N_2\) stream. The recovery of stability tests of COR and M1-4 varied from 92.17 to 102.78% with the RSD being from 2.99 to 8.72% (\(n = 6\)). The accuracy and precision were expressed as mean ± SD and precision as RSD. The accuracy for intra-day (6 measurements in 24 h for each sample) and inter-day (6 measurements in 24 h for each sample and continued for 5 d) assays ranged from 96.62 to 103.38%, with the RSD being from 2.90 to 7.76%.

**Results and Discussion**

To separate and determine simultaneously COR and M1-4, which have quite similar structures and closer retention times, an efficient LC-MS method with electrospray ionization (ESI) in the positive ion mode was created. An extracted ion chromatogram (EIC) declined interference from multiple molecular ion peaks in the total ion chromatogram.

After oral and intravenous administrations, COR was detected in plasma (ESI-MS m/z: 383 [M+H]+), and identified by comparing the retention time and MS\(^1–3\) fingerprint spectra with those of substrate COR.\textsuperscript{14} However, M1-4 had not been identified in plasma after an oral or intravenous dose. This suggests that M1-4 had not joined the systemic circulation, but was excreted directly. COR reached a concentration maximum of 1.01 \(\pm\) 0.220 \(\mu\)g mL\(^{-1}\) (\(C_{\text{max}}\), observed value) after an oral dose. With the same \(t_{\text{max}}\) being at 3 h after the oral dose, COR and RHY seem to have been absorbed competitively, similar to the finding that they would be in direct competition at a single common binding site and interacting with \(\alpha(1A)\) adrenergic receptors.\textsuperscript{15} Plasma and main organ samples digested with \(\beta\)-glucuronidase did not produce different LC-MS yields.

After COR was dosed intravenously to rats, \(t_{\text{1/2}}\) was of 53 min based on a BP-ANN model of pharmacokinetics,\textsuperscript{13} but in this study was of 74 min. The key pharmacokinetic parameters for COR in rats are given in Table 1. After YKS was administered to rats, no COR was detected in plasma by LC-MS/MS, which might be due to the total amounts of oxindole alkaloids in the formulation. But, it could be generally found that \(t_{\text{max}}\) and \(C_{\text{max}}\) of alkaloids in Uncaria hooks increased with the increment of the dosage.\textsuperscript{3} COR was identified in rat liver, heart, and kidneys after oral

### Table 1 Key pharmacokinetic parameters for COR in rats

| Parameter     | Unit | Intravenous administration 0.0105/mmol kg\(^{-1}\) | Oral administration 0.105/mmol kg\(^{-1}\) |
|---------------|------|-----------------------------------------------|------------------------------------------|
| \(k\)         | min\(^{-1}\) | (9.362 ± 0.16) \times 10\(^{-3}\) | 74.02 ± 2.6 |
| \(t_{\text{1/2}}\) | min | | |
| \(V\)         | L kg\(^{-1}\) | (2.21 ± 0.0173) \times 10\(^{-2}\) | (2.07 ± 0.350) \times 10\(^{-4}\) |
| \(CL\)        | L kg\(^{-1}\) min\(^{-1}\) | (2.21 ± 0.0173) \times 10\(^{-2}\) | (2.07 ± 0.350) \times 10\(^{-4}\) |
| \(t_{\text{max}}\) | min | | |
| \(C_{\text{max}}\) | \(\times 10^3\) mol mL\(^{-1}\) | 180 | 1.01 ± 0.220 |
| \(AUC_{0-\infty}\) | \(\times 10^4\) mol min mL\(^{-1}\) | 50.7 ± 5.83 | 0.525 ± 0.0476 |

Data shown as mean ± SD (\(n = 3\)); observed data: \(t_{\text{max}}\) and \(C_{\text{max}}\).
administration. M1–2 (ESI-MS m/z: 399 [M+H]+) were detected in rat heart and kidneys, whereas all of M1–4 (M3–4 with ESI-MS m/z: 575 [M+H]+) were found in rat liver. The distribution of COR and its metabolites in the main organs of rat excised at $t_{\text{max}}$ are given in Table 2.

COR was detected in rat brain after both oral and intravenous administration. The brain uptake index of COR at $t_{\text{max}}$ of 3 h after oral dose and $t_{1/2}$ of 74 min after intravenous dose was $3.08 \times 10^{-11}$ and $3.75 \times 10^{-11}$ mol g$^{-1}$ (dry weight), respectively. The identification of COR in rat brain indicates the material basis in related diseases focus for its pharmacological activity. With the absolute bioavailability being 0.104%, to increase the dosage of COR or Uncaria hooks for improving seems to be reasonable.

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References

1. J. Qu, T. Gong, B. Ma, L. Zhang, Y. Kano, and D. Yuan, *Chem. Pharm. Bull.*, 2012, 60, 23.
2. J. He, S. Han, F. Yang, N. Zhou, and S. Wang, *J. Chromatogr. Sci.*, 2013, 51, 905.
3. H. Kushida, M. Fukutake, M. Tabuchi, T. Katsuhara, H. Nishimura, Y. Ikarashi, M. Kanitani, and Y. Kase, *Biomed. Chromatogr.*, 2013, 27, 1647.
4. S. Xie, Y. Shi, Y. Wang, C. Wu, W. Liu, F. Feng, and N. Xie, *J. Pharm. Biomed. Anal.*, 2013, 81–82, 56.
5. H. Chen, C. Y. Wu, F. Feng, Y. X. Wang, W. Y. Liu, and S. L. Wang, *J. Sep. Sci.*, 2013, 36, 3723.
6. T. J. Kim, J. H. Lee, J. J. Lee, J. Y. Yu, B. Y. Hwang, S. K. Ye, L. Shujuan, L. Gao, M. Y. Pyo, and Y. P. Yun, *Biol. Pharm. Bull.*, 2008, 31, 2073.
7. K. Matsumoto, R. Morishige, Y. Murakami, M. Tohda, H. Takayama, I. Sakakibara, and H. Watanabe, *Eur. J. Pharmacol.*, 2005, 517, 191.
8. Z. Kawakami, H. Kanno, Y. Ikarashi, and Y. Kase, *J. Ethnopharmacol.*, 2011, 134, 74.
9. T. Ueki, A. Nishi, S. Imamura, H. Kanno, K. Mizoguchi, K. Sekiguchi, Y. Ikarashi, and Y. Kase, *Cell. Mol. Neurobiol.*, 2013, 33, 129.
10. L. L. Chen, J. X. Song, J. H. Lu, Z. W. Yuan, L. F. Liu, S. S. Durairajan, and M. Li, *J. Neuroimmune Pharm.*, 2014, 9, 380.
11. Y. Niu, F. Li, C. Inada, K. Tanaka, S. Watanabe, H. Fujiwara, S. Sasaki-Hamada, J. Oka, and K. Matsumoto, *J. Pharm. Biomed. Anal.*, 2015, 104, 21.
12. S. Imamura, M. Tabuchi, H. Kushida, A. Nishi, H. Kanno, T. Yamaguchi, K. Sekiguchi, Y. Ikarashi, and Y. Kase, *Cell Mol. Neurobiol.*, 2011, 31, 787.
13. J. Ma, J. Cai, G. Lin, H. Chen, X. Wang, X. Wang, and L. Hu, *J. Chromatogr. B*, 2014, 959, 10.
14. W. Wang, X. Li, Y. Chen, and M. Hattori, *Biomol. Chromatogr.*, 2014, 28, 1219.
15. W. Wang, C. M. Ma, and M. Hattori, *Biol. Pharm. Bull.*, 2010, 33, 669.