Pharmacokinetics of perlolyrine in rats by stable isotope dilution in conjunction with GC-MS

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KEY WORDS perlolyrine; pharmacokinetics; stable isotope dilution; gas chromatography-mass spectrometry

ABSTRACT

AIM: To determine the pharmacokinetics of perlolyrine in rats. METHODS: The plasma concentration and pharmacokinetic parameters of perlolyrine were determined by gas chromatography-mass spectrometry (GC-MS) with selected ion (m/z 247 and m/z 248) and [2-¹⁵N] perlolyrine (m/z 248) as internal standard. RESULTS: The concentration-time profile of perlolyrine after i.g perlolyrine 2 mg·kg⁻¹ fitted a two-compartment open model in rats. The pharmacokinetic parameters were T1/2α = 0.33 h, T1/2β = 4.52 h, T2 (ka) = 0.14 h, Tmax = 0.35 h, Cmax = 18.84 µg/L, K12 = 0.88 h⁻¹, K21 = 0.42 h⁻¹, K10 = 0.32 h⁻¹, V/F = 109.22 L·kg⁻¹, AUC = 112.68 µg·h·L⁻¹. CONCLUSION: The method was constant, sensitive, and accurate. It provides a useful method for the determination of pharmacokinetics of perlolyrine which are important for clinical use of perlolyrine.

INTRODUCTION

1-(3-hydroxymethyl-2-furyl)-9H-pyrido[3,4-b]indole (perlolyrine, C₁₉H₁₂N₂O₂), an active ingredient of the traditional Chinese herb Ligusticum chuanxiong Hort, has been used in treatment of coronary disease and cerebrovascular disease, after confirmation its potency by pharmacological experiments¹,². The pharmacokinetic parameters of perlolyrine in mice were determined by the radioisotope tracer ([³H] perlolyrine) method², which was, however, not suitable for the study of clinical pharmacokinetics because radioactivity. In this study, we examined the pharmacokinetics of perlolyrine in rats by the stable isotope tracer method in conjunction with gas chromatography-mass spectrometric (GC-MS) technique. The method is suitable for the study of clinical pharmacokinetics, because of its high selectivity, sensitivity and rapid rate of analysis³.

MATERIALS AND METHODS

Materials Perlolyrine and [2-¹⁵N]perlolyrine (internal standard, ¹⁵N abundance > 95 %) was prepared in our own laboratory⁴. N-methyl-N-trimethylsilyl-trifluoracetamide (MSTFA) was purchased from Sigma Co. Buffer used was Na₂CO₃ 0.2 mol·L⁻¹-boric acid 0.2 mol·L⁻¹ (contained KCl 2.00 mol·L⁻¹)-water (57: 43:100) with pH adjusted to 8 - 9. Other reagents and chemicals were of AR. Wistar rats, ♂ 24 and ♀ 24, Trade ™, weighing (200 ± 20) g, were from Animals Center of Chinese Academy of Medical Sciences and Nan Fang Hospital (certificates No 900529 and No 901012).

GC-MS analysis GC-MS analyses were carried out with a Hewlett Packard 5990 Series II gas chromatography and HP 5971 mass selective detector. A cross linked capillary column HP-1 (10 m × 0.22 mm × 0.33 µm) was connected to the ion source. Samples were injected in the split off mode. Helium was used as the carrier gas at the flow rate of 1.0 mL·min⁻¹. The temperature of injection and detector were set at 250 °C and 300 °C, respectively. The column temperature was programmed to start at 70 °C for 1 min, increase at a rate of 15 °C·min⁻¹ up to 300 °C for 15 min. The volume of injection was 1 µL. The mass spectrum peaks of m/z 247 and m/z 248 (containing one nitrogen) during the retention time of perlolyrine trimethylsilyl derivative were detected with selective ion monitoring mode (SIM) in GC-MS.

Standard curve preparation Three mL blood

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samples of the rats were collected from jugular vein into heparinized glass tubes after a 12-h fasting before the medication. The samples were centrifuged at 3000 x g for 15 min to get blank plasma. The standard solutions of per洛lyrine (2.5, 10, 20, 30, and 60 μg/L) were prepared with the blank plasma. The internal standard ([2,15N]per洛lyrine, 30 ng) was added to 1 mL of the standard solution, shaken for 1 min and let to stand for 15 min. After 0.5 g of NaCl and 1 mL of Na2CO3-boric acid-KCl buffer were added and shaken for 1 min, they were extracted with 1 mL of ethyl ether-isopropyl alcohol (10:1) and shaken for 5 min. The organic phase was separated by centrifugation for 10 min and transferred with a Pasteur pipet to a screw-capped centrifuge tube. The extraction was repeated twice with 1 mL of organic solvent. The combined solvent extracts were taken to dryness under a nitrogen stream. The residue was derivatized by treatment with 20 μL of MSTFA, heated at 70 °C for 45 min. Aliquots of 1 μL of the supernatant were injected for GC-MS. Thus, the standard curves for per洛lyrine were prepared. Three per洛lyrine samples of high, medium, and low concentrations (2, 10, and 30 μg·L⁻¹) were prepared with the blank plasma. The internal standard ([2,15N] per洛lyrine, 30 ng) was added to 1 mL of sample, then per洛lyrine was extracted and determined as above. The recovery, the inter-day RSD and the intra-day RSD of per洛lyrine were determined.

**Plasma sampling** Forty-eight rats were randomly assigned to 12 groups and each group contained 2 and 2. The rats, after a 12-h fasting, were given administered per洛lyrine 2 mg·kg⁻¹ with 1 mL water. A uniform diet was supplied after 2 h and a uniform water was supplied after the medication. Blood samples (3 mL) of the rats anesthetized with 3% sodium pentobarbital were collected from jugular vein into the heparinized glass tubes at 0.033, 0.083, 0.167, 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 h after the medication. The samples were centrifuged at 3000 x g for 15 min to get plasma. The internal standard ([2,15N] per洛lyrine, 30 ng) was added realigh to 1 mL of the plasma, then per洛lyrine was extracted and determined as above.

**Pharmacokinetic analysis** The pharmacokinetic parameters were obtained by program 3P87. Statistical inferring was obtained by t test. The linear regression of the results was made by Microsoft Excel.

**RESULTS AND DISCUSSION**

**Standard curve** For a standard curve the ratio (m/z 247:m/z 248) of the mass spectrum peaks, height as ordinate was plotted vs the standard solution of per洛lyrine as abscissa. The standard curve for per洛lyrine was linear over the range of 2, 5, 10, 20, 30, and 60 μg·L⁻¹ in plasma and its regression equation was Y = 6.678X - 0.1096 (r = 0.9960). When the ratio of signal and noise (S:N) was 3, the minimum detection limit of per洛lyrine in plasma was 1 μg·L⁻¹. When the concentration of per洛lyrine samples was 2, 10, and 30 μg·L⁻¹, the absolute recovery of per洛lyrine was 96.21 % ± 6.28 %, 97.87 % ± 5.18 % and 98.95 % ± 4.32 %, and the inter-day RSD of per洛lyrine was 6.79 %, 5.48 %, and 4.66 %, and the intra-day RSD of per洛lyrine was 8.67 %, 6.53 % and 5.44 %, respectively in plasma.

**Pharmacokinetics** After the results were treated by program 3P87, the disposition of per洛lyrine conformed to 2-compartment model. Thus, the plasma concentration-time curves were best fitted by 2-compartment model (Fig 1).

**Fig 1. Concentration-time curve of per洛lyrine in plasma. n = 4 rats. ± s.**

Tab 1 summarizes the pharmacokinetic data of per洛lyrine in plasma in 4 rats. The absorption half-lives (T1/2) and time to peak (Tmax) were very short (0.31 h and 0.34 h, respectively), and the terminal half-lives (T1/2) was only 4.62 h. These results showed that per洛lyrine metabolized very rapidly and its effects disappeared very fast in rats. These experimental data were compared to results which were detected by the radioisotope tracer method except for T1/2 and T1/2 (ka)21. However, the radioisotope tracer method was not suitable for the study of human pharmacokinetics, our stable isotope tracer method in conjunction with GC-MS technique, is not only good for the study of animals pharmacokinetics but also for the determination of clinical pharmacoki-
Tab 1. Pharmacokinetic parameters of perlolyrine in rats after oral administration in rats. \( n = 4 \) rats. \( \pm \) s. \( t^p > 0.05, \ t ^ p < 0.05 \) as the radioisotope tracer method\(^{21}\).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>( T_\frac{1}{2}^{a} / h )</td>
<td>0.33 ± 0.10(^a)</td>
</tr>
<tr>
<td>( T_\frac{2}{3}^{a} / h )</td>
<td>4.52 ± 1.70(^a)</td>
</tr>
<tr>
<td>( T_\frac{1}{2}^{(ka)} / h )</td>
<td>0.14 ± 0.12(^a)</td>
</tr>
<tr>
<td>( T_\frac{max} / h )</td>
<td>0.35 ± 0.18(^a)</td>
</tr>
<tr>
<td>( C_{max} / ng*ml^{-1} )</td>
<td>18.84 ± 3.36</td>
</tr>
<tr>
<td>( K_{12} / h^{-1} )</td>
<td>0.88 ± 0.34(^a)</td>
</tr>
<tr>
<td>( K_{10} / h^{-1} )</td>
<td>0.42 ± 0.21(^a)</td>
</tr>
<tr>
<td>( V/F (L*kg^{-1}) )</td>
<td>109.22 ± 26.34(^b)</td>
</tr>
<tr>
<td>( AUC_{0-\infty} / ng<em>h</em>ml^{-1} )</td>
<td>112.68 ± 20.44</td>
</tr>
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REFERENCES