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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-^{15}N] perlolyrine (m/z 248) as internal standard. **RESULTS**: The concentration-time profile of perlolyrine after ig perlolyrine 2 mg·kg^{-1} fitted a two-compartment open model in rats. The pharmacokinetic parameters were $T_{\frac{1}{2}\alpha}$ = 0.33 h, $T_{\frac{1}{2}\beta}$ = 4.52 h, $T_{\frac{1}{2}}$(ka) = 0.14 h, T_{max} = 0.35 h, C_{max} = 18.84 μg/L, K_{12} = 0.88 h^{-1}, K_{21} = 0.42 h^{-1}, K_{10} = 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-(5-hydroxymethyl-2-furyl)-9H-pyrido[3,4-b]indole (perlolyrine, $C_{16}H_{12}N_2O_2$), 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 experiments[1,2]. The pharmacokinetic parameters of perlolyrine in mice were determined by the radioisotope tracer ([^{3}H]perlolyrine) method[2], 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 analysis[3].

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MATERIALS AND METHODS

Materials Perlolyrine and [2-^{15}N]perlolyrine (internal standard, ^{15}N abundance > 95 %) was prepared in our own laboratory[1]. *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide (MSTFA) was purchased from Sigma Co. Buffer used was Na_2CO_3 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 Ⅱ, weighing (200 ± *s* 20) g, were from Animals Center of Chinese Academy of Medical Sciences and Nan Fang Hospital (certificates № 980521 and № 990728).

GC-MS analysis GC-MS analyses were carried out with a Hewlett Packard 5890 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 ℃ and 300 ℃, respectively. The column temperature was programmed to start at 70 ℃ for 1 min, increase at a rate of 15 ℃·min^{-1} up to 300 ℃ 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

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 × *g* for 15 min to get blank plasma. The standard solutions of perlolyrine (2, 5, 10, 20, 30, and 60 μg/L) were prepared with the blank plasma. The internal standard ([2-^{15}N]perlolyrine, 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 Na_2CO_3-boric acid-KCl buffer were added and shaken for 1 min, they were extracted with 1 mL of ethyl ether-iso-propyl 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 ℃ for 45 min. Aliquots of 1 μL of the supernatant were injected for GC-MS. Thus, the standard curves for perlolyrine were prepared. Three perlolyrine samples of high, medium, and low concentrations (2, 10, and 30 $\mu g \cdot L^{-1}$) were prepared with the blank plasma. The internal standard ([2-^{15}N] perlolyrine, 30 ng) was added to 1 mL of sample, then perlolyrine was extracted and determined as above. The recovery, the inter-day RSD and the intra-day RSD of perlolyrine 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 ig administered perlolyrine 2 $mg \cdot kg^{-1}$ 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 × *g* for 15 min to get plasma. The internal standard ([2-^{15}N]perlolyrine, 30 ng) was added realigh to 1 mL of the plasma, then perlolyrine 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 perlolyrine as abscissa. The standard curve for perlolyrine was linear over the range of 2, 5, 10, 20, 30, and 60 $\mu g \cdot L^{-1}$ in plasma and its regression equation was $Y = 6.678X - 0.1098$ ($r = 0.9960$). When the ratio of signal and noise (S∶N) was 3, the minimun detection limit of perlolyrine in plasma was 1 $\mu g \cdot L^{-1}$. When the concentration of perlolyrine samples was 2, 10, and 30 $\mu g \cdot L^{-1}$, the absolute recovery of perlolyrine was 96.21 % ± 6.28 %, 97.87 % ± 5.18 % and 98.95 % ± 4.32 %, and the inter-day RSD of perlolyrine was 6.79 %, 5.48 %, and 4.66 %, and the intra-day RSD of perlolyrine was 8.67 %, 6.53 % and 5.44 %, respectively in plasma.

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

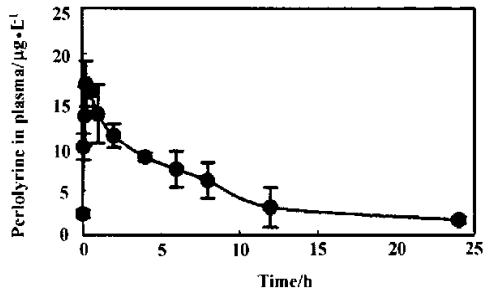

Fig 1. Concentration-time curve of perlolyrine in plasma. $n = 4$ rats. $\bar{x} \pm s$.

Tab 1 summarizes the pharmacokinetic data of perlolyrine in plasma in 4 rats. The absorption half-lives ($T_{\frac{1}{2}\alpha}$) and time to peak (T_{max}) were very short (0.31 h and 0.34 h, respectively), and the terminal half-lives ($T_{\frac{1}{2}\beta}$) was only 4.62 h. These results showed that perlolyrine 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 $T_{\frac{1}{2}\beta}$ and $T_{\frac{1}{2}}$ (ka)[2]. 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-

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netics parameters, because of its high selectivity, sensitivity (1 $\mu g \cdot L^{-1}$) and rapid rate of analysis.

Tab 1. Pharmacokinetic parameters of perlolyrine in rats after oral administration in rats. $n = 4$ rats. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$ *vs* the radioisotope tracer method[2].

Parameters	Calue
$T_{\frac{1}{2}\alpha}$/h	0.33 ± 0.16^a
$T_{\frac{1}{2}\beta}$/h	4.52 ± 1.78^b
$T_{\frac{1}{2}}$(ka)/h	0.14 ± 0.12^b
T_{max}/h	0.35 ± 0.18^a
C_{max}/ng·mL^{-1}	18.84 ± 3.36
K_{12}/h^{-1}	0.88 ± 0.34^a
K_{21}/h^{-1}	0.42 ± 0.21^a
K_{10}/h^{L-1}	0.32 ± 0.12^a
V/F (L·kg^{-1})	109.22 ± 26.34^a
$AUC_{0\to24}$/ng·h·mL^{-1}	112.68 ± 20.44

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稳定同位素结合 GC-MS 法测定大鼠体内川芎哚的药物动力学参数

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关键词 川芎哚; 药代动力学; 稳定同位素法; 气相色谱-质谱法

目的: 测定川芎哚(川芎 III 号碱, perlolyrine)的药动学参数. **方法**: 以[2-^{15}N]川芎哚为内标准及 GC-MS 的 SIM (选择性离子监测)为检测手段, 定量测定大鼠体内川芎哚的含量及其药代动力学参数. **结果**: 大鼠灌胃给予川芎哚 2 mg·kg^{-1}后, 川芎哚在大鼠体内呈二室模型分布, 其药代动力学参数为: $T_{\frac{1}{2}\alpha}$ = 0.33 h, $T_{\frac{1}{2}\beta}$ = 4.52 h, $T_{\frac{1}{2}}$(ka) = 0.14 h, T_{max} = 0.35 h, C_{max} = 18.84 μg/L, K_{12} = 0.88 h^{-1}, K_{21} = 0.42 h^{-1}, K_{10} = 0.32 h^{-1}, V/F = 109.22 L·kg^{-1}, AUC = 112.68 μg·h·L^{-1}. **结论**: 本法灵敏度高、特异性强且准确性好, 为测定川芎哚药代动力学参数提供了实用的分析方法. 本研究为川芎哚临床应用提供了重要的参考资料.

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