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STUDIES ON URINARY METABOLITES OF PERLOLYRINE BY STABLE ISOTOPE TRACER METHOD

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SUMMARY

After oral administration of perlolyrine and deuterated perlolyrine, the rat urines were collected, hydrolyzed with glucuronidase, basified with $NaHCO_3$—Na_2CO_3, extracted with ethyl ether—iso-propyl alcohol. The organic phases (neutral and basic fractions) were concentrated for conversion to TMS derivatives. The aqueous phase were acidified with sulfuric acid, taken to dryness under a nitrogen stream, extracted with methanol (water soluble acidic fractions) and concentrated for conversion to TMS derivatives. The TMS derivatives were determined by gas chromatography-mass spectrometry (GC-MS). Perlolyrine and one metabolite were found from the neutral and basic fractions, and two different metabolites were found from the water soluble acidic fractions. It was proposed that the major possible metabolic pathways of perlolyrine were the hydroxylation of perlolyrine and the oxidition of its hydroxylmethyl group.
Keywords: Perlolyrine, Deuterated perlolyrine, Metabolite, GC-MS

INTRODUCTION

Perlolyrine, 1-(5-hydroxymethyl-2-furyl)-9H-pyrido[3,4-b]indole, an active ingredient from Chuanxiong (the traditional Chinese herb), can be used in treatment of coronary atherosclerotic heart diseases and cerebrovascular diseases, after confirmation of its potency by pharmacological experiments[1,2]. However, it has been reported that perlolyrine metabolizes too rapidly, its bioavailability is too low and its effects disappear too fast in animals. To study the metabolic transformation of perlolyrine in animals, the stable isotope tracer method in conjunction with gas chromatography-mass spectrometric (GC-MS) technique was selected for the identification of perlolyrine and its metabolites from urine, because of its selectivity, sensitivity and rapid rate of analysis.

MATERIALS AND METHODS

Materials

Perlolyrine and deuterated perlolyrine (contains 4 deuterium atoms) were prepared in our own laboratory. N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) and β-glucuronidase were purchased from Sigma Co. Other reagents and chemicals were of AR. Water was distilled. Wistar rats were obtained from Animals Center of Chinese Academy of Medical Sciences.

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Instrumentation

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 into the ion source. Samples were injected in the split off mode. Helium was used as the carrier gas at the flow rate of 1 ml/min. 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 up to 300℃ for 15 min.

Urine Samples

Urine samples were collected after a 12-h fasting and over 24 h following administration of a single dose (20 mg/kg) of perlolyrine and deuterated perlolyrine to a rat, respectively.

Extraction procedure

5 ml urine was transferred to a 12 ml calibrated centrifuge tube with a teflon-lined screw cap, and 100 μl β-glucuronidase was added. The enzymatic hydrolysis was performed at 37℃ overnight. After the addition of powdered anhydrous mixture of $NaHCO_3$—Na_2CO_3, the pH of the sample was adjusted to 8~9 and the sample were extracted with 5 ml of ethyl ether—iso-propyl alcohol (10:1). 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 with 2×5 ml of solvent. The combined solvent extracts (the neutral metabolites) were taken to dryness under a nitrogen stream. The residue was derivatized by treatment with 50 μl N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA), heated at 70℃ for 45 min and used for GC-MS. After extraction of the neutral metabolites, the aqueous layer was carefully acidified (pH 2) with 6 N sulfuric acid, and the acids are extracted with 3×5 ml of ethyl ether—iso-propyl alcohol. The aqueous and organic phase were separated by centrifugation. The organic phase (the ethyl ether-iso-propyl alcohol - soluble acidic metabolites) was removed under a nitrogen stream, derivatized by treatment with 50 μl of MSTFA, heated at 70℃ for 45 min and used for GC-MS.

RESULTS AND DISCUSSION

A GC-MS technique was optimized for analysis of perlolyrine and its metabolites in urine. The extraction yield of perlolyrine was 85% and the minimum detection limit was 15 ng/ml urine. After the administration of perlolyrine and deuterated perlolyrine orally, unchanged perlolyrine M0 (or deuterated perlolyrine) and its metabolites M1, M2 and M3 were identified in urine. The chromatograms of urine samples before and after administration of perlolyrine are shown in Figure 1. Mass fragmentations of perlolyrine, deuterated perlolyrine and their metabolites trimethylsilyl (TMS) derivatives are given Figure 2, respectively. The chromatograms of urine samples before and after administration of deuterated perlolyrine were almost the same as those of perlolyrine and their chromatograms are not shown in Figure 1.

Perlolyrine and its metabolites were analyzed by the stable isotope tracer method combined with GC-MS. There was m/z 1~4 mass units difference between perlolyrine metabolites and deuterated perlolyrine metabolites in their mass spectra, and the mass spectra of deuterated perlolyrine and its metabolites exhibited characteristic ion clusters (Figure 2). Thus, perlolyrine and its metabolites were very easyly identified. Their structures were deduced by comparison of their mass spectra and retention times of perlolyrine and deuterated perlolyrine. Ion fragmentograms of

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trimethylsilyl (TMP) derivatives of perlolyrine and its metabolites displayed molecular ions of 336, 424 and 438(M^+). They were M0, M1 (or M2) and M3, respectively. The deduced structures of perlolyrine and its metabolites were shown in Table 1 and Figure 3. Their definite structues will wait to be further studied.

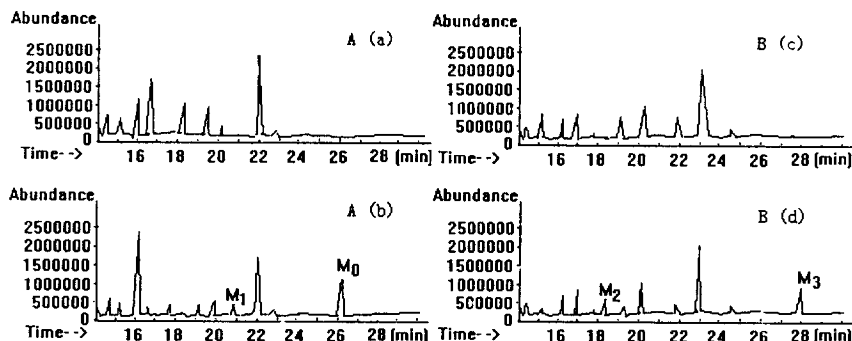

Fig. 1 Chromatograms of urines samples before and after administration of perlolyrine.
A. The neutral and basic fraction extracted with ethyl ether—iso-propyl alcohol
(a) before administration of perlolyrine. (b) after administration of perlolyrine.
B. The water soluble acidic fraction extracted with ethyl ether—iso-propyl alcohol.
(c) before administration of perlolyrine. (d) after administration of perlolyrine.

Excretion rates of perlolyrine (or deuterated perlolyrine) and its metabolites in urine were very rapid, the final recovery amounted to 28.5% of the dose given, 24 h after the administration of perlolyrine. Our data show that the major metabolite of perlolyrine is unchanged perlolyrine. The mole percent distributions of perlolyrine and its metabolites recovered in urine, after the administration of perlolyrine, were: M0 62.4, M1 10.6, M2 11.5, and M3 15.5.

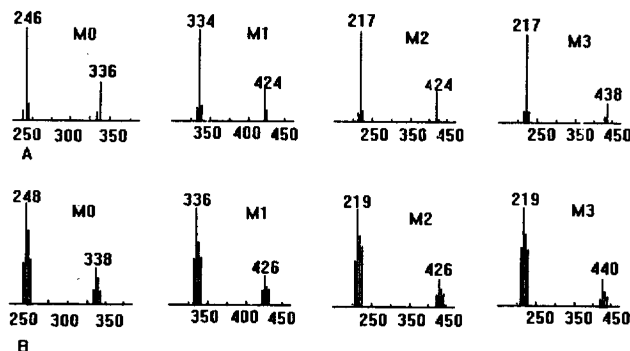

Fig. 2 The characteristic ions mass fragmentations of perlolyrine (or deuterated perlolyrine) and its metabolites.

According to the stuctures of perlolyrine and its metabolites above, the in vivo metabolic pathway of perlolyrine is given in Figure 3. It shows that the major possible metabolic pathway of perlolyrine is the hydroxylation of perlolyrine including the possibility of an N-oxide and the oxidation of hydroxylmethyl group.

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Tab 1 Metabolites of perlolyrine

Compounds	Retention time/min	*H-m/z(M^+)	**D-m/z(M^+)
TMS - M0	26.0~26.3	336	340
M0		264	268
TMS - M1	20.6~21.1	424	428
M1		280	284
TMS - M2	18.1~18.4	424	428
M2		280	284
TMS - M3	27.9~28.1	438	442
M3		294	298

* From the urine sample of the rats treated after administration of perlolyrine.
** From the urine sample of the rats after administration of deuterated perlolyrine.

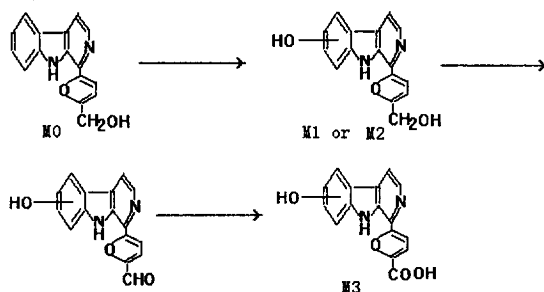

Fig. 3 The proposed metabolic pathway of perlolyrine in rats

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