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Pharmacokinetic Study of A Sleep-Promoting Agent Melatonin

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Abstract: The plasma concentration of melatonin in rabbits are measured by GC/MS with selected ion (m/z 173) and caffeine (m/z 194) as internal standard to determine the pharmacokinetic of melatonin. The concentrtion-time profile of melatonin is abtained after melatonin 30 mg · kg^{-1} administered for a two-compartment open model in rabbits. The pharmacokinetic parameters are $t_{1/2\alpha}$= 0.31 h, $t_{1/2\beta}$= 8.58 h, $t_{1/2Ka}$= 0.34 h, T_{max}= 0.63±0.47 h, C_{max}= 322.23±255.40 ng/mL, K_{12}= 0.81 h^{-1}, K_{21}= 2.80 h^{-1}, K_{10}= 2.10 h^{-1}, AUC= 697.49 (ng/mL) * h, CL (s) = 0.097±0.054 (mg/kg)/h (ng/mL). The method is stable, sensitive and accurate. It is a useful method for the determination of pharmacokinetics of melatonin which is important for clinic.

Key workds: Melatonin; pharmacolinetics; GC/MS; SIM
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At present, the wide-use hypnotics are benzadiazepine, for example diazepam and its derivatives, to play an important role in clinic. However, reseaches about it show that benzodiazepine have long half-life, they would accumulate in bodies. Chronic insomnia patients would have physical dependence on the drug, and a characteristic syndrome will happen, for example rebound insomnia and residual sequelae if insomnia patients stop benzodiazepine administration after a long-term treatment. So a new, effective, low side-effect hypnotics is needed in clinic now.

Melatonin is an indorne hormone secreted by the human pineal gland, it is thought to be consistent to endocrine system, and it has relation with endocrine activities, it regulates the functions of immune system, nervous system, digestive system, cardiovascular system, reproductive system. It has hypnotic secative role, strengthens immunity functions, resists decrepit, controls tumor, et al.

Melatonin's light-dark regulation is according to human sleep-wake cycle, many studies show melatonin has effective hypnotic function, and it is an endogenious substance, so it is hopeful to be a new, effective sleep-promoting agent. In this study, we studied the pharmacokinetics of melatonin in rabbits.

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1 MATERIALS AND METHODS

1.1 Drugs and Chemicals

Melatonin was synthesised in our lab. Melatonin standard was purchased from SIGMA, Chemical Co.

1.2 Animals and Experiments

4 male rabbits, wieghted mor than 2.5 kg, were provided by animal center of AMMS.

The dose was 30 mg/kg, rabbits administered 8 mL physiological saline containing melatonin, blood samples were got after administration 0.25、0.50、0.75、1.0、1.25、1.50、1.75、2.0、2.5、3.0、3.5、4.5、6.0、7.5、9.0、10.5、14.5、17.0、19.5、23.5 h from rabbits ears

1.3 Sample Preparation

Plasma 1 mL was mixed with caffeine 40 μL 0.25 ng/mL (an internal standard), a drop of 0.1 mol·L^{-1} NaOH solution. The sample was extracted with 5 mL CH_2Cl_2 two times and vortexed. After centrifuged, the organic phase was evaporated to dryness under a stream of nitrogen at 37 ℃. The residues were dissolved in 40 μL methanol. Solution 4 μL was injected for analysis.

1.4 GC-MS Analysis

The GC-MS system consisted of an HP5890A Gas chromatography, an PH5970B Mass Selective Detector (MSD). A cross-linked capillary column (OV-1, 25 m × 0.2 mm × 0.33 mm) was connected to the ion source. The data system was a HP MSD Chemstation controller. The injector and GC-MS interface temperatures were set at 260 ℃ and 280 ℃, respectively. Helium was used as carrier gas at the flow rate 15 mL·min^{-1}. Qualtitative method was made in pulse-split mode and quantitative method was made in pulse-splitless mode. The oven temperature was held to start at 150 ℃, then increased to 250 ℃ at 8 ℃ per min. The mass spectrum peaks 173 and 194 were detected as selective ion of melatonin and internal standard caffeine. Melatonin was qualified by selective ion monitoring mode (SM) in GC-MS. The Selective Ion quantified method was set as 194 (3.5～8.8 min), 173 (8.8～15.5 min) and 194 (15.5～20 min).

1.5 Validation Study

The melatonin was quanltified by the peak height ratio using a calibratoin curve. The accuracy and precision of assay were tested. The lower limit of detection 0.5 ng/μL was defined as a signal-to-noise ratio of 3∶1.

1.6 Pharmacokinetics Anaysis

The pharmacokinetic parameters were obtained by program 3P97. The linear regression of the results was made by Microsoft Excel.

2 RESULTS

2.1 GC-MS

No endogenous components interfered with the analysis. Retentive time of Melatonin and caffeine were 14.54 and 8.08 min, respectively, which were obstained from melatonin's total ions GC-MS figure (Fig. 1). The mass spetra of melatonin had base peak at m/z 173, the mass spetra of caffeine had base peak at m/z 194 (Fig. 2). Concentration of melatonin in plasma were quantitafied by base peak ratio of melatonin at 173 to caffeine at 194.

2.2 Calibration Curve

The peak height ratio of melatonin and internal standard caffeine were linear over 10～400 ng/mL weighted linear regression was used in constructing the calibration curves. calibration equation is $Y = 0.05820 + 0.6067X$, $r = 0.9994$ ($n = 5$).

2.3 Validation Test

The average recovery of melatonin ranged from 74.04% to 94.4%. Coefficients of both intra-and inter-day variations (CV) were 6.55% and 14.5%, respectively. The detection limit was 0.5 ng/μL.

2.4 Applications Of The Method

The method was applied to study pharmacokinetic of melatonin in rabbits. The data and curve of concentration-time were shown in Table 1 and Fig. 3; the pharmacokinitic parameters of melatonin were shown in Table 2, re-

spectively.

Table 1 The concenration of Melatonin in rabbits(n= 4)

Time(h)	Concentration(ng/mL)
0.25	98.51±116.58
0.5	121.87±31.48
0.75	166.84±63.92
1.0	113.52±95.36
1.5	41.88±16.09
2.0	13.59±7.98
2.5	8.70±2.63
3.0	11.18±1.38
4.5	11.87±6.04
6.0	11.42±5.47
9.0	9.43±4.31
1.05	9.87±3.15
23.5	7.40±3.79

Table 2 Pharmacokinetic parameters of Melatonin in rabbits after oral administration of Melatonin (n= 4 rabbits)

Parameters	Units	Calue(X±SD)
Cmax	ng/mL	322.23±255.40
Tmax	h	0.63±0.47
$t_{1/2\alpha}$	h	0.31±0.29
$t_{1/2\beta}$	h	8.58±12.15
$t_{1/2Ka}$	h	0.34±0.26
K_{21}	1/h	2.80±4.57
K_{10}	1/h	2.10±2.43
K_{12}	1/h	0.81±1.50
Vc	(mg/kg)/(ng/mL)	0.077±0.061
AUC	(ng/mL) * h	697.49±682.71
CL(s)	(mg/kg)/h/(ng/mL)	0.097±0.054

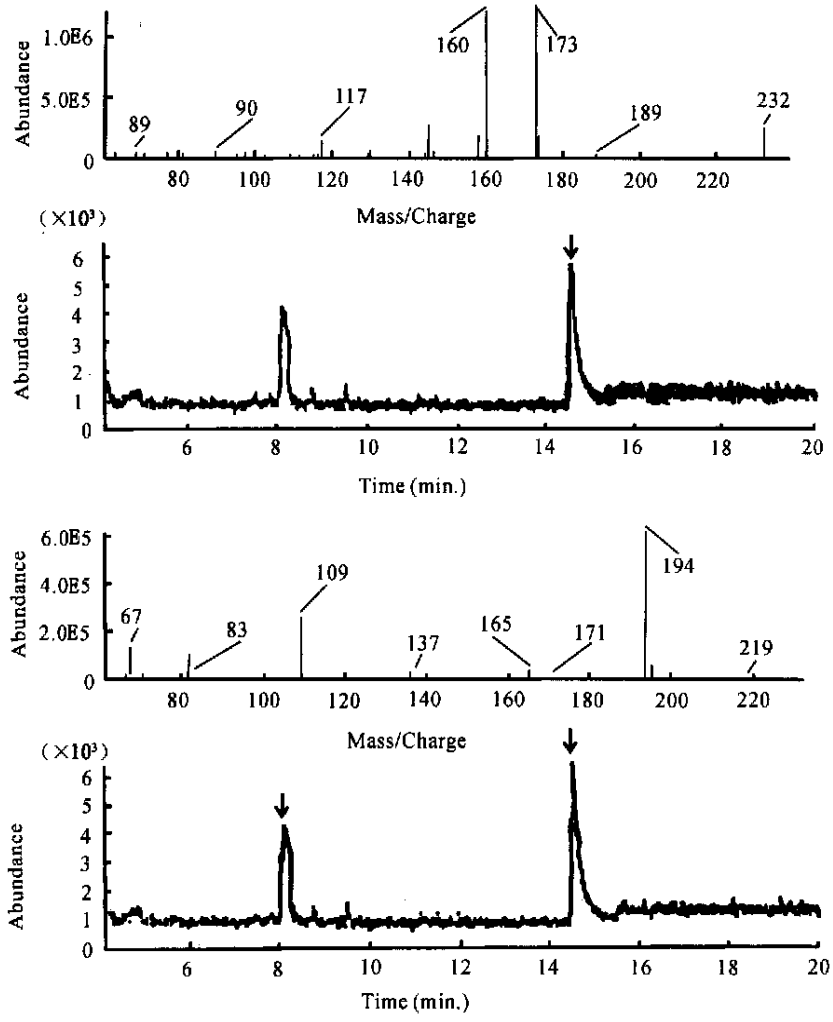

Fig 1 Total ion chromatogram-mass spectrometry of Melatonin and caffeine in plasma by GC/MS

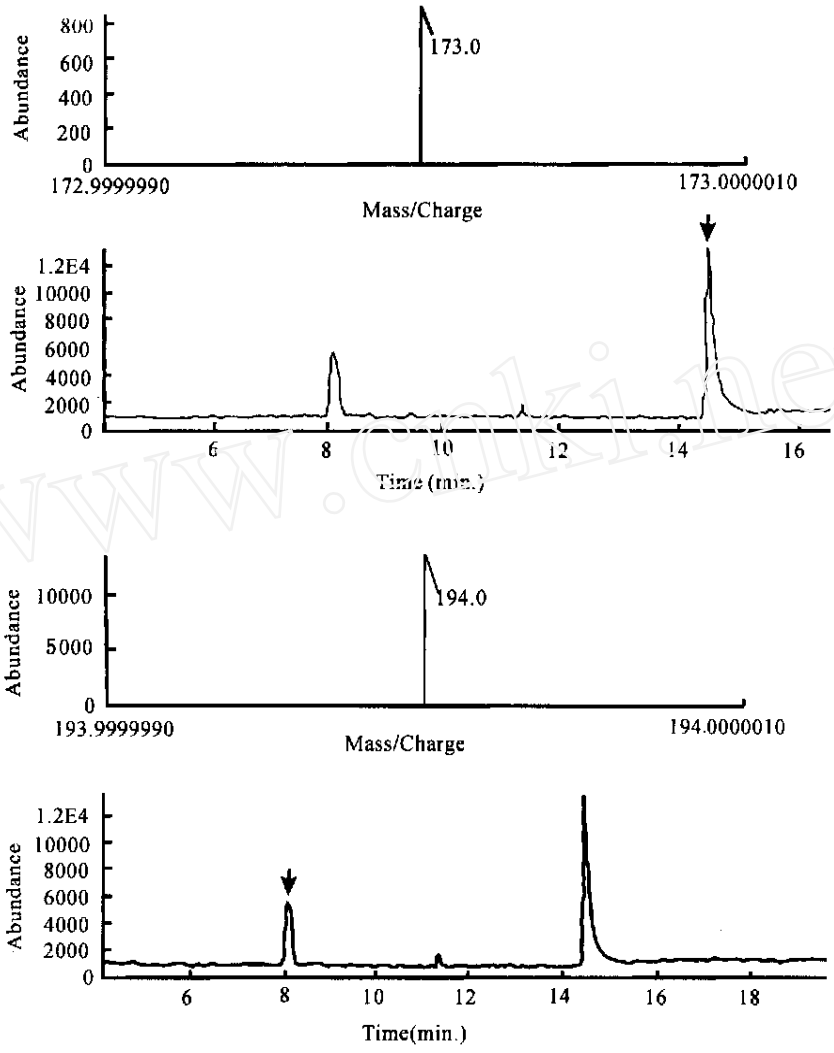

Fig 2　Single ion chromatogram-mass spectrometry of Melatonin and caffeine in plasma by GC/MS

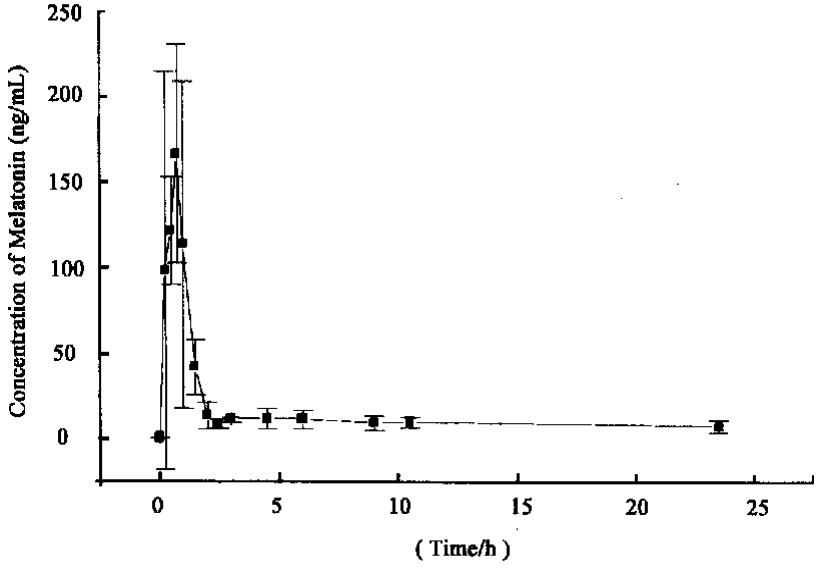

Fig 3　The concentration-time curve of Melatonin in plasma in 4 rabbits

3 DISCUSSION

Melatonin is a new sleep-promoting agent. We developed a sensitive and simple method by GC/MS for pharmacokinetic studies of melatonin. The sample preparation is simple.

The absorption half-life ($T_{1/2\alpha}$), and time to peak (T_{max}) are very short (0.31 h, 0.34 h, respectively); and the terminal half-life ($T_{1/2\beta}$) is only 8.58 h. The results show melatonin absorbed and metabolized very rapidly and its effects disappeare very fast in rabbits.

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新型睡眠促进剂褪黑激素(Melatonin)的药代动力学研究

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摘要: 测定新型睡眠促进剂Melatonin 的药代动力学参数的方法是以咖啡因为内标、以 GC/MS 的 SM (选择性离子监测)为检测手段, 定量测定家兔体内Melatonin 的含量及药代动力学参数。其结果为家兔灌胃给予Melatonin 30 mg·kg^{-1}后, Melatonin 在家兔体内呈二室分布, 药代动力学参数为 $t_{1/2\alpha}$= 0.31 h, $t_{1/2\beta}$= 8.58 h, $t_{1/2Ka}$= 0.34 h, T_{max}= 0.63±0.47 h, C_{max}= 322.23±255.40 ng/mL, K_{12} = 0.81 h^{-1}, K_{21}= 2.80 h^{-1}, K_{10}= 2.10 h^{-1}, AUC= 697.49 (ng/mL) * h, CL (s)= 0.097±0.054 (mg/kg)/h/(ng/mL). 这种测量方法灵敏度高、特异性强、准确性好, 为测定Melatonin 药代动力学参数提供了有效实用的分析方法。Melatonin 在家兔体内分布及消除都很快, 不会在体内蓄积。

关键词: 褪黑激素; 药代动力学; 气相色谱-质谱; 选择性离子监测模式