Pharmacokinetics of aristolochic acid A in *Radix Aristolochiae* and Guanxinsuhe Capsule

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Abstract: Objective To study the pharmacokinetics of aristolochic acid A in *Radix Aristolochiae* and the compound preparation of Guanxinsuhe Capsule in mice *in vivo* after single-dose oral administration and observe the difference of aristolochic acid A absorption and distribution. **Methods** Aristolochic acid A assay was performed by RP-HPLC on a Waters apparatus with a Diamonsil™ C_{18} column (250 mm×4.6 mm, 5 μm), a mobil phase: a mixture of methanol-water-acetic acid (72 : 27 : 1), flow rate: 1.0 mL/min, detection wavelength: 315 nm, and column temperature: 20 ℃. **Results** Mice were given *Radix Aristolochiae* and Guanxinsuhe Capsule by ig at the same level of 2.5 mg/kg of aristolochic acid A, respectively, which were suspended in 0.3% CMC-Na solution. Plasma concentrations were determined by RP-HPLC. After single-dose ig administration of *Radix Aristolochiae* or Guanxinsuhe Capsule to mice, the mean plasma concentration-time courses of aristolochic acid A obtained fitted the one-compartment model. The main pharmacokinetic parameters of aristolochic acid A in *Radix Aristolochiae*, $t_{1/2\,ka}$, $t_{1/2\,ke}$, t_{max}, AUC, C_{max} are 5.103 min, 43.63 min, 17.89 min, 80.45 (μg·min)/mL, and 0.916 8 μg/mL; the relative pharmacokinetic parameters in Guanxinsuhe Capsule are 5.294 min, 43.50 min, 18.32 min, 33.08 (μg·min)/mL, and 0.381 8 μg/mL. **Conclusion** The C_{max} of aristolochic acid A in Guanxinsuhe Capsule is significantly less than that in *Radix Aristolochiae*, which indicates that the compound compability could decrease the absorption of aristolochiae acid A.

Key words: aristolochic acid A; pharmacokinetics; *Radix Aristolochiae*; Guanxinsuhe Capsule

青木香和冠心苏合胶囊中马兜铃酸 A 的药动学研究

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摘 要: 目的 研究青木香及复方制剂冠心苏合胶囊中马兜铃酸 A 在小鼠体内的药动学特点及小鼠在 ig 给予含相同量马兜铃酸 A 的青木香和冠心苏合胶囊后, 马兜铃酸 A 的吸收、分布规律的差异。**方法** 采用 RP-HPLC 法测定血浆中马兜铃酸 A 的量。色谱条件: 色谱柱为 Diamonsil™ C_{18} 柱 (250 mm×4.6 mm, 5 μm), 流动相为甲醇-水-冰醋酸 (72 : 27 : 1), 体积流量为 1.0 mL/min, 检测波长为 315 nm, 柱温为 20 ℃。**结果** 药动学实验结果显示小鼠分别 ig 给予青木香和冠心苏合胶囊 (相当于 2.5 mg/kg 马兜铃酸 A) 后, 其体内的药动学房室模型均符合一室模型, 青木香中马兜铃酸 A 主要药动学参数: $t_{1/2\,ka}$、$t_{1/2\,ke}$、t_{max}、AUC、C_{max} 分别为 5.103 min、43.63 min、17.89 min、80.45 (μg·min)/mL、0.916 8 μg/mL; 冠心苏合胶囊中相应的参数分别为 5.294 min、43.50 min、18.32 min、33.08 (μg·min)/mL、0.381 8 μg/mL。**结论** 小鼠给予含马兜铃酸 A 相同剂量的青木香和冠心苏合胶囊后, 冠心苏合胶囊中马兜铃酸 A 的 C_{max} 明显低于青木香中的 C_{max}, 说明复方配伍作用可减少马兜铃酸 A 的吸收。

关键词: 马兜铃酸 A; 药动学; 青木香; 冠心苏合胶囊

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Aristolochic acid A, a chemical constituent existing in the species of *Aristolochia* L. and some

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Asarum species herbs, was the impetus behind the FDA import alert in May, 2000. This alert called for the "detainment without physical inspection, of herbs suspected to be composed, in whole or part, of any species of *Aristolochia* L., or other herbs that may be substituted for it"[1]. Aristolochic acid A is commonly found in medicinal plants such as *Aristolochia debilis* Sieb. et Zucc. which has been reported to cause renal failure.

Guanxinsuhe Capsule is composed of *Styrax* (*Liquidambar orientalis* Mill.), *Lignum Santali Albi* (*Santalum album* L.), *Olibanum* (*Boswellia carterii* Birdw.), *Borneolum Syntheticum* (borneol) and *Radix Aristolochiae* (*Aristolochia debilis* Sieb. et Zucc.) which contains aristolochic acid A. It is a traditional Chinese compound medicine and has been used as a cardiovascular medicine for several hundred years[2]. The aim of this study was to investigate the pharmacokinetics of aristolochic acid A in *Radix Aristolochiae* and Guanxinsuhe Capsule after oral administration.

1 Materials

1.1 Chemicals and reagents: Aristolochic acid A reference substance was bought from National Institute for the Control of Pharmaceutical and Biological Products, Lot No.: 746-9002. *Radix Aristolochiae* was purchased from market and identified by Professor Nie Feng-zhi. Guanxinsuhe Capsule was purchased from market (Manufacturer: The Fifth Pharmacy of Chinese Traditional Medicine in Tianjin, Lot No.: 000633). Methanol used was HPLC grade. Acetic acid used was analytical grade. Water used for HPLC was ultrapure water.

1.2 Animals: Kunming mice (♀ and ♂, 25—30 g) were purchased from Experimental Animal Center of Hebei Medical University. All mice were kept in an environmentally controlled breeding room [temperature: (24±1) ℃, humidity: (60±5)%] for one week, and they drank freely but were not fed 15 h before the experiment.

1.3 Apparatus: The high performance liquid chromatographic system was equipped with the pump (Waters 515, USA) and an ultraviolet detector (Waters model 2487, Milford, Massachusetts, USA) and a C_{18} column (250 mm×4.6 mm, 5 μm). An equilibrating model recorder N—2000 was the product of Zhejiang University, China.

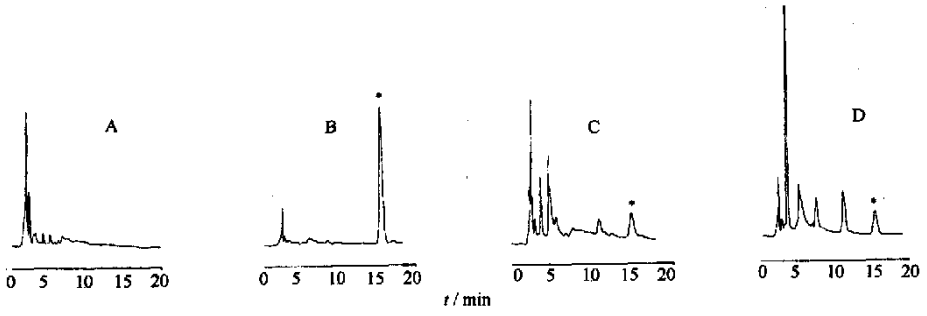
2 Methods and results

2.1 Standard solution: An accurately weighed quantity of aristolochic acid A was transferred to a 10 mL volumetric flask containing methanol to obtain a final concentration of 0.183 mg/mL solution. This prepared solution was stored at about 4 ℃.

2.2 Chromatographic condition for aristolochic acid A: A few (20 μL) of the plasma samples were injected into chromatograph and the peak response value of aristolochic acid A was measured. There was no detectable endogenous material in the plasma interfering with the determination of aristolochic acid A. Mobile phase: methanol-water-acetic acid (74 : 27 : 1), flow rate: 1.0 mL/min, column temperature: 20 ℃, and detection wavelength: 315 nm with 0.5 absorbance units full scale (AUFS). Chromatograph replicated injections of the standard solutions and recorded the peak responses as directed under procedure: the relative standard deviation was less than 4.0% and the tailing factor for aristolochic acid A peak is less than 1.20. The retention time of aristolochic acid A was approximately 16 min (Fig. 1).

2.3 Calibration: The standard solution was diluted with methanol to make a series of solutions between 0.109 8 and 36.6 μg/mL. Dilutions (0.6 mL) was transferred to 0.2 mL blank plasma. After 10 s vortex and 10 min centrifugation at 3 000×g, the supernatant was filtrated with filter (0.45 μm) and then 20 μL of the filtered sampe was injected for HPLC analysis. The standard curve showed a good linearity over a range of 0.109 8—36.6 μg/mL for aristolochic acid A (Y= 13.723 X+0.169, r=0.999 6, where Y and X indicate the aristolochic acid A chromatographic peak area and plasma concentration, respectively).

2.4 Recovery: The mean recovery of aristolochic acid A from mice was 97.0%, 99.4%, and 101.4% (n=5) at high, medium, and low concentrations (1.830, 0.366, and 0.109 8 μg/mL) with



* -aristolochic acid A

A-blank plasma B-blank plasma sample spiked with standard aristolochic acid A

C-mice' s plasma sample of *Radix Aristolochicae* D-mice' s plasma sample of Guanxinsuhe Capsule

Fig. 1 Chromatograms of aristolochic acid A

the RSD at 1.4%, 0.8%, and 2.4% (n=5).

2.5 Determination of method precision: The coefficient of variation for analysis in plasma was 1%—5% with regard to both intra-day analysis of any concentration on the standard curve and inter-day comparison of standard curves (Table 1).

Table 1 Relative standard deviations of intra-day and inter-day precisions for method to determine concentrations of aristolochic acid A in plasma (n=5)

Concentration/ ($\mu g \cdot mL^{-1}$)	Coefficient of variation/%	
	Intra-day	Inter-day
1.732	1.5	3.3
0.366	1.0	3.4
0.109 8	2.2	4.7

2.6 Limit of detection: The minimal detectable concentration of aristolochic acid A was 0.018 3 μg/mL ($S/N \geqslant 3$).

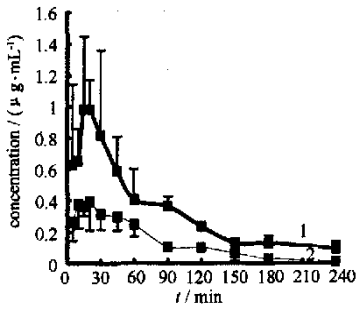
2.7 Medication and sampling[3-6]: Suitable quantities of *Radix Aristolochicae* and Guanxinsuhe Capsule were transferred and extracted with methanol by Soxhlet-extraction for 8 h. The extract solutions were concentrated to dry and the residues were dissolved in volumetric flasks respectively with methanol. An aliquot volume of the extract was filtered through a 0.45 μm filter and injected into HPLC. The results showed the concentrations of aristolochic acid A in the extract of *Radix Aristolochicae* and Guanxinsuhe Capsule were 0.028 57% and 0.028 76%, respectively.

Radix Aristolochicae powder and the contents of Guanxinsuhe Capsule were suspended with 0.3% CMC-Na as the test solutions for future use. The concentrations of aristolochic acid A in the suspensions of *Radix Aristolochicae* and Guanxinsuhe Capsule were 95.41 μg/mL and 97.42 μg/mL, respectively.

Mice were given *Radix Aristolochicae* or Guanxinsuhe Capsule suspension by ig at dose of 2.5 mg/kg of aristolochic acid A. Plasma concentrations were determined by HPLC. Six mice were studied at each time point. Blood was collected from arteries of the eyepit into heparinized glass tubes. Samples were obtained at 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, and 240 min after ig administration, respectively. The blood samples were centrifuged (3 000×g for 10 min) to obtain plasma and analyzed immediately. A methanol solution (0.6 mL) was added to 0.2 mL aliquots of plasma sample. After 10 s vortex and 10 min centrifugation at 3 000×g, the solution was filtrated with filter (0.45 μm) and injected for HPLC analysis.

2.8 Pharmacokinetic analysis: Time courses of plasma concentrations of aristolochic acid A in *Radix Aristolochicae* and Guanxinsuhe Capsule were analyzed by 3P97 software using weighted least-squares estimation[7]. The concentration-time curves of aristolochic acid A were fitted to one-compartment model according to the minimum Akaike' e Information Criterion (AIC), the minimum square sum (SUM), and the maximum relative coefficient (Fig. 2).

The Concentration-time curves of aristolochic acid A were fitted to one-compartment model. Each mouse was given the same dose level of



1-*Radix Aristolochicae* 2-Guanxinsuhe Capsule

Fig. 2 Mean concentration-time curves of aristolochic acid A in mice after *Radix Aristolochicae* and Guanxinsuhe Capsule administration

aristolochic acid A in *Radix Aristolochicae* or in Guanxinsuhe Capsule, there was a significant difference in terms of the maximum plasma concentration of aristolochic acid A (C_{max}) and the area under the concentration-time curve (AUC). The response to Guanxinsuhe Capsule demonstrated a significant decrease in C_{max} and AUC. There was no significant di-fference between *Radix Aristolochicae* and Guanxinsuhe Capsule in terms of plasma half-life of aristolochic acid A and the time peak (t_{max}) of plasma aristolochic acid A (Table 2).

Table 2 Pharmacokinetic parameters of aristolochic acid A in mice after oral administration

Parameters	Unit	Aristolochic acid A	
		Radix Aristolochicae	Guanxinsuhe Capsule
A	$\mu g \cdot min^{-1}$	1.447	0.582 0
k_e	min^{-1}	0.015 89	0.015 35
k_a	min^{-1}	0.135 8	0.130 9
Lag time	min	0.565 6	0.333 3
$t_{1/2ka}$	min	5.103	5.294
$t_{1/2ke}$	min	43.63	43.50
t_{max}	min	17.89	18.32
AUC	$(\mu g \cdot mL^{-1}) \cdot min$	80.45	33.08
C_{max}	$\mu g \cdot mL^{-1}$	0.916 8	0.381 8
CL/F(s)	$mg \cdot kg^{-1} \cdot min^{-1}/(\mu g \cdot mL^{-1})$	0.031 08	0.077 94
V/F(c)	$mg \cdot kg^{-1}/(\mu g \cdot mL^{-1})$	1.956	4.891

3 Discussion

This study presented the evidence that Guanxinsuhe Capsule was able to affect the absorption of aristolochic acid A first. The present pharmacokinetic study shows that pharmacokinetics of aristolochic acid A from *Radix Aristolochicae* and Guanxinsuhe Capsule after oral adminitration is different. For example, the values of A, lag time, AUC, and C_{max} for Guanxinsuhe Capsule are less than those for *Radix Aristolochicae*. This indicates that the amount of aristolochic acid A in Guanxinsuhe Capsule absorbed by mice was less. At the same content level of aristolochic acid A administered, the absorption of aristolochic acid A in *Radix Aristolochicae* supplemented with other ingredients in Guanxinsuhe Capsule was significantly reduced compared with that in *Radix Aristolochicae* alone. Thus, the results suggest that the renal toxicity of Guanxinsuhe Capsule would be less significant than that of *Radix Aristolochicae* alone. In this paper, a method used to determine the plasma concentration of aristolochic acid A in mice was successfully established by RP-HPLC. The method has better characteristics with selectivity, linearity, and sensitivity and is suitable for the evaluation in pharmacokinetic studies.

Up to now, no article has demonstrated that Guanxinsuhe Capsule could cause the aristolochic acid nephropathy (AAN). However, as known, aristolochic acid can cause the progressive interstitial fibrosis of the kidney rapidly. As Guanxinsuhe Capsule does contain aristolochic acid A, it should be used with caution.

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