Pharmacokinetic and distribution of arctiin in rats

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ABSTRACT: OBJECTIVE To study the pharmacokinetic and distribution of arctiin in rats. **METHODS** Each rat was given a single dose at random by oral administration. The arctiin in serum and organs were determined by use of RP-HPLC. All pharmacokinetic parameters were calculated with a 3P87 program. **RESULTS** After oral administration of arctiin at the dose of 300 mg·kg^{-1}, Arctiin plasma C-T curve conform to open two-compartment model. The Pharmacokinetic parameters were as follow: A = (37.374 5 ± 8.964 7) μg·mL^{-1}; B = (6.210 6 ± 1.489 3) μg·mL^{-1}; α = (0.004 3 ± 0.000 9) min^{-1}; β = (0.000 4 ± 0.000 2) min^{-1}; K_{α} = (0.420 2 ± 0.167 5) min^{-1}; $t_{1/2\alpha}$ = (115.192 6 ± 14.382 4) min; $t_{1/2\beta}$ = (1 485.578 1 ± 161.173 3) min; K_{10} = (0.001 0 ± 0.000 4) min^{-1}; K_{21} = (0.001 4 ± 0.000 6) min^{-1}; K_{12} = (0.002 3 ± 0.001 3) min^{-1}; C_{max} = (41.786 3 ± 7.521 7) μg·mL^{-1}; T_{max} = (9.891 9 ± 4.341 4) min; AUC = (22 503.272 7 ± 4 120.182 8) μg·min·mL^{-1}. Liver had the highest concentration of arctiin after oral administration. **CONCLUSION** RP-HPLC method is rapid, sensitive and specific for the research of arctiin pharmacokinetic and its distribution in rats. Arctiin is distributed and eliminated quickly in rats.

KEY WORDS: arctiin; pharmacokinetic; distribution

牛蒡子苷代谢动力学与分布研究

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摘要:目的　研究牛蒡子苷代谢动力学与分布。方法　单剂量随机灌胃,高效液相色谱法测定大鼠体内牛蒡子苷浓度及其在脏器中的分布,代谢动力学分析采用 3P87软件。结果　口服牛蒡子苷 300 mg/kg在大鼠体内呈二室模型分布,其主要动力学参数为 A = (37.374 5 ±8.964 7) μg·mL^{-1}; B = (6.210 6 ±1.489 3) μg·mL^{-1}; α = (0.004 3 ±0.000 9) min^{-1}; β = (0.000 4 ±0.000 2) min^{-1}; K_{α} = (0.420 2 ±0.167 5) min^{-1}; $t_{1/2\alpha}$ = (115.192 6 ±14.382 4) min; $t_{1/2\beta}$ = (1 485.578 1 ±161.173 3) min; K_{10} = (0.001 0 ±0.000 4) min^{-1}; K_{21} = (0.001 4 ±0.000 6) min^{-1}; K_{12} = (0.002 3 ±0.001 3) min^{-1}; C_{max} = (41.786 3 ±7.521 7) μg·mL^{-1}; T_{max} = (9.891 9 ±4.341 4) min; AUC = (22 503.272 7 ±4 120.182 8) μg·min·mL^{-1}。牛蒡子苷在心、肝、肾、脑等脏器也有分布,以肝脏中最高。结论　高效液相色谱法测定牛蒡子苷在动物体内代谢变化,快速、受杂质干扰小,且稳定性和重现性较好,适合牛蒡子苷代谢产物含量测定。牛蒡子苷在体内吸收很快,消除也快。

关键词:牛蒡子苷;药代动力学;分布

中图分类号:R945.1　　文献标识码:A　　文章编号:1007-7693(2006)04-0265-03

Arctium lappa L., a biennal herb plant of Compositae, distributes all over China[1]. Its seeds has been used as Chinese traditional medicine for treatment of the cold, cough and throat sore[1]. Arctiin is one of the most important lignans extracted from seeds of *Arctium lappa*. Biological activity of arctiin on the treatment of the acute progress nephritis, chronic glomerulonephritis and nephritis was reported[2]. Arctiin also has the function of the enhancement of immunological activity[3], PAF antagonist[4], anti-inflammatory[3], Ca^{2+} antagonist and antihypertension[5-6]. In this present investigation, we reported the pharmacokinetic and tissue distribution of arctiin in rats.

1　Materials and methods

1.1　Animals

Wister rats (180 ~ 200 g weight, male, certificate No. SCXK(YU) 20020004) were supplied by Laboratory Animal Center of Chongqing Academy of Chinese Materia Medica.

1.2　Chemicals and drugs

The arctiin reference standard compound was purchased

基金项目:Supported by Visiting Scholar Foundation of Key Lab. In University(No: 021015)

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from National Institute for the Control of Pharmaceutical and Biological Products of China, No. 0819-200203. HPLC grade acetonitril was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Water for the HPLC mobile phase was purified in a milli-Q system (Milipore, Bedfold, MA). All other chemicals were of AR grade. Arctiin was supplied by Department of Pharmacy, Bioengineer College, Chongqing Institute of Technology. The purity was above 99.8%, which was determined by the supplier using HPLC methods.

1.3 Instruments and chromatographic conditions

HPLC system consisted of Shimadzu LC-10A pumps, SCL-10AVP controller, photodiode array detector, and a computerized data station equipped with Shimadzu Class-VP6.10 software. Phenomenex Luna C_{18} column (150 mm × 4.6mm I.D., 5μm particle size) was used and operated at ambient temperature. Column was equipped with a 2cm length C_{18} guard column (Organic Chemistry Institute, Dalian, P.R. China). The mobile phase was acetonitrile and 0.05% H_3PO_4 (36∶64) at a flow rate of 0.6 mL·min^{-1} for an isocratic elution at 210 nm.

1.4 Medication and sampling

On the day of dosing, rats received a dose of 300 mg·kg^{-1} by oral administration. Doses were based on individual animal body weight. Animals were sacrificed by decapitation (five per time-point) at 5, 15, 30min, 1, 2, 3, 4, 6, 8, 9, 12, 24 h after oral administration. Blood was collected into heparinized glass tubes and immediately centrifuged at 3000 r·min^{-1} for 10 min to obtain plasma. The liver, kidney, heart and brain of rats above-sacrificed were removed, ground into slurry and weighed prior to freezing. The serum and organ samples were stored at −20℃ until assay.

1.5 Samples preparation

1.5.1 Serum samples 2mL of methanol was added to 0.2mL of plasma. Then the mixture was vigrously vibrate for 30 s and centrifuged at 3000 r·min^{-1} for 5 min. The supernatant was transferred to another tuber and evaporated to dryness at 60℃. The residue was dissolved by the addtion of 0.2mL of methanol and sonicated for 5 min, and passed through a 0.45μm nylon membrane filter. An aliquto of 10μL of filtration was injected into the HPLC system.

1.5.2 Organ samples 5 mL of methanol was added to 0.5 g organ slurry. Then the mixture was vigrously sonicated for 10 min, then centrifuged at 3000 r·min^{-1} for 5 min. The supernatant was transferred to another tuber and evaporated to dryness at 60℃. The residue was dissolved by the addtion of 0.2 mL of methanol and sonicated for 5 min, and passed through a 0.45 μm nylon membrane filter. An aliquto of 20μL of filtration was injected into the HPLC system.

1.6 Pharmacokinetic analysis

Arctiin concentration-time data and its pharmacokinetic parameters were analyzed with 3P87 program.

2 Results

2.1 Method validation

Under the selected chromatographic conditions, a baseline separation of arctiin and arctigenin (metabolic products of arctiin) was obtained in serum and organs. Their typical retention time for arctiin and arctigenin (peak 2) was 9.2 min and 11.5 min respectively. No interference from endogenous serum and organs components at the retention time were never found during the analysis. The limit of detection for arctiin was 1.0 μg·L^{-1}. Chromatographs of arctiin in rat serum and organs were illustrated in Fig 1.

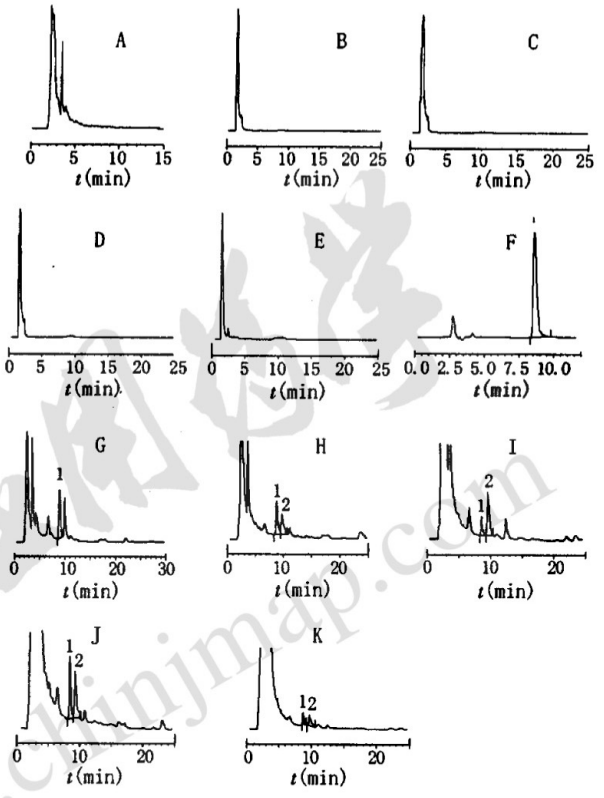

Fig 1 HPLC chromatograms of actiin in rat serum

图 1 大鼠血浆中牛蒡子苷色谱图

A. blank serum sample; B. blank liver sample; C. blank heart sample; D. blank kiney sample; E. blank brain sample; F. serum sample spiked with arctiin standard compound 0.2 mg·L^{-1}; G. serum sample 30 min after a po dose of 300 mg·kg^{-1} arctiin; H. liver sample 2 h after a po dose of 300 mg·kg^{-1} arctiin; I heart sample 2h after a po dose of 300 mg·kg^{-1} arctiin; J kiney sample 2 h after a po dose of 300 mg·kg^{-1} arctiin; K. brain sample 2h after a po dose of 300 mg·kg^{-1} arctiin; peak 1 refer to arctiin; peak 2 refer to arctigenin.

A. 空白血浆色谱图; B. 空白肝脏色谱图; C. 空白心脏色谱图; D. 空白肾脏色谱图; E. 空白脑色谱图; F. 含 0.2 mg·L^{-1}牛蒡子苷标准品血浆样品色谱图; G. 口服 300 mg·kg^{-1}牛蒡子苷后 30 min血浆样品色谱图; H. 口服 300 mg·kg^{-1}牛蒡子苷后 2 h肝脏样品色谱图; I 口服 300 mg·kg^{-1}牛蒡子苷后 2h心脏样品色谱图; J 口服 300 mg·kg^{-1}牛蒡子苷后 2 h肾脏样品色谱图; K. 口服 300 mg·kg^{-1}牛蒡子苷后 2 h脑组织样品色谱图; 1号峰为牛蒡子苷; 2号峰为牛蒡子苷元

Calibration curves exhibited excellent linearity over a range of 3.3~ 660μg for arctiin. Linear equation: $Y = 4\,042\,200X + 129\,718$ ($r = 0.999\,6$) where Y is peak area, and X is the con-

centration of arctiin. The mean recovery was 104.32% for arctiin. RSD of Intra and inter day precision for determination of arctiin was 2.86% and 3.24% respectively. The stability of this analysis method was fine, and its RSD was 2.81%.

2.2 Pharmacokinetics in rats

The mean serum concentration-time profiles of arctiin after oral administration were shown in Fig 2. Serum concentration versus time data was analyzed and the pharmacokinetic parameters were shown in Tab1. Model discrimination was assessed by the analysis of the data, and actually most concentration-time profiles were best fitted to the open two-compartment model.

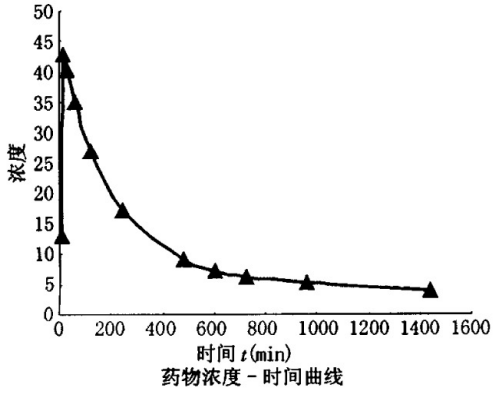

Fig 2 Profile of mean plasma concentration versus time after oral administration of arctiin in rats (po 300 mg·kg^{-1})

图 2 大鼠口服 300 mg·kg^{-1}牛蒡子苷后血药浓度-时间曲线

Tab 2 Contents of arctiin in the organs (μg·g^{-1})

表 2 脏器中牛蒡子苷的含量 (μg·g^{-1})

Organs	Time						
	5min	15min	30min	1h	2h	4h	6h
Heart	1.97±0.34	2.08±0.24	1.61±0.52	21.80±3.4	5.49±1.34	1.04±0.37	0.93±0.39
Liver	13.12±2.16	29.68±4.96	15.58±2.34	34.60±6.12	120.11±15.69	0.83±0.31	0.80±0.24
Kidney	18.92±3.68	13.08±4.13	4.32±1.23	33.38±6.14	17.94±3.93	1.60±0.54	0.13±0.28
Brain	0.27±0.14	0.65±0.34	1.10±0.46	20.11±6.31	1.97±0.72	0.33±0.19	0.16±0.01

2.3 Organs distribution in rats

Arctiin content in rats organs was determined by the use of HPLC after oral administration (in Tab 2). The top peak of arctiin in heart, kidney and brain appeared respectively after one hour of oral administration. Arctiin distributed in heart, kidney and brain is much lower. The results indicated that it was excreted less through kidney. The top peak of arctiin in liver appeared after two hours of oral administration, then immediately tobogganed.

Tab 1 Main pharmacokinetic parameters after a po dose of 300 mg·kg^{-1} arctiin

表 1 牛蒡子苷的主要药代动力学参数 (po 300 mg·kg^{-1})

Parameters	Unit	Data
A	μg·mL^{-1}	37.374 5±8.964 7
B	μg·mL^{-1}	6.210 6±1.489 3
α	min^{-1}	0.004 3±0.000 9
β	min^{-1}	0.000 4±0.000 2
$t_{1/2\alpha}$	min	115.192 6±14.382 4
$t_{1/2\beta}$	min	1485.578 1±161.173 3
K_a	min^{-1}	0.420 2±0.167 5
K_{10}	min^{-1}	0.001 0±0.000 4
K_{21}	min^{-1}	0.001 4±0.000 6
K_{12}	min^{-1}	0.002 3±0.001 3
Cmax	μg·mL^{-1}	41.786 3±7.521 7
Tmax	min	9.891 9±4.341 4
AUC	μg·min·mL^{-1}	22 503.272 7±4 120.182 8

3 Discussion

In the present paper, HPLC method was developed for quantitative determination of arctiin in rat serum and organs after oral administration. The method has been well validated and applied to measure serum concentration of biological sample of arctiin. This HPLC method was stable, rapid, sensitive, specific and producible.

Pharmacokinetic researches were shown that arctiin was absorbed rapidly after oral administration. Arctiin was detected at 5 min after oral administration. The top peak was appeared at 15-30 min, then immediately tobogganed. Arctiin content distributed in rat liver was the highest in all organs.

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收稿日期: 2005-01-20