Pharmacokinetics and Tissue Distribution of Orientin from *Trollius chinensis* in Rabbits

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Abstract: **Objective** To study the pharmacokinetics (PK) and tissue distribution of orientin in rabbits. **Methods** The high-performance liquid chromatography method was developed and validated for the determination of orientin in rabbit plasma and tissues. The PK characteristics of orientin in rabbits were compared by three kinds of administration, iv, im, and ip injection, using 3P97 pharmacokinetic software to examine the PK parameters. **Results** The elimination half-life ($t_{1/2\beta}$) of three kinds of administration was higher than the distribution half-life ($t_{1/2\alpha}$). The PK of orientin eliminating in rabbit fitted the two compartment model. Orientin was mainly eliminated in rabbit. The distribution of orientin in tissue after iv injection was investigated, and the order of orientin AUC in each group was kidney > liver > lung > spleen > heart > brain. **Conclusion** Orientin is distributed extensively in tissues such as kidney, liver, and lung. It is difficult for orientin to cross the blood brain barrier.

Key words: high-performance liquid chromatography; orientin; pharmacokinetics; tissue distribution; *Trollius chinensis*

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Introduction

Trollius chinensis Bunge, the dry flowers and flower buds in the plants of Ranunculaceae, is commonly used for folk medicine, with the effect of heat-clearing and detoxifying, detumescence, and improving eyesight. It was widely used in clinical treatment, such as upper respiratory tract infection, tonsillitis, and so on (Li, 1995). The flowers of *T. chinensis* contain flavonoids, organic acids, and polysaccharides. Orientin is a C-glycoside compound of flavonoids. Previous research shows that there is a high content of orientin in the flowers of *T. chinensis* (Huang, Wang, and Duan, 2000; Qu *et al*, 2010; Yang *et al*, 2011). Orientin has a wide variety of biological properties such as anti-oxidation (Yang *et al*, 2011; Yang, 2011), inhibition of human esophageal carcinoma cells EC-109, and inducing apoptosis of tumor cells (Zhu, 2011). Orientin plays a vital role in the protection on cells of myocardium injured by hypoxia/ reoxygenation (Fu *et al*, 2007). The pharmacokinetics (PK) of orientin by different methods for the administration to rabbits have not been reported, the aim of this study is to clarify the PK of orientin in rabbits by iv, im, and ip injection, and to characterize the distribution of orientin in tissues of rabbits by iv administration, so as to provide a scientific basis for the rational medication in clinic.

Materials and methods

Experimental material

Trollius chinensis Bunge was collected from Guyuan, Hebei province and identified by Prof. MA Shu-lan, Hebei North University. The reference substance of orientin (purity of 99% by HPLC) was purchased from the National Institute for Food and Drug Control (Beijing, China). Orientin (purity > 98.8% by HPLC) was obtained from the flowers of *T. chinensis* in our laboratory. The reagents used for the mobile phase were of chromatographic grade and other reagents were of analytical grade. Double distilled water was used for all the preparations.

The healthy New Zealand white rabbits, weighing (2.5 ± 0.3) kg, were provided by the Center of Laboratory Animal, Hebei North University. All the

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animals were individually housed and fed with water and standard rabbit feed. Rabbits were fasted overnight before the experiments.

Agilent 1100 HPLC (USA), Agilent Preparative HPLC (USA); High-speed Freezing Centrifuge (Sigma); Vacuum Freeze Drying Machine (Thermo, USA); GR—202 Analytical Balance (Japan); HQ—60 Vortex Agitator (China).

Chromatographic conditions

The HPLC was carried out on Zorbax SB-C_{18} column (250 mm × 21.2 mm, 7 μm) (Yan *et al*, 2011), the mobile phase consisted of 1% acetic acid-acetonitrile (85:15) at a flow rate of 20.0 mL/min, the injection volume was 6 mL, the orientin was baseline resolved, and the detection was performed at a wavelength of 340 nm at 25 ℃. The structure of orientin was identified by ^{1}H-NMR. The purity of orientin was about 98.8%, detected by HPLC.

The HPLC separation of orientin was achieved on a Hypersil BDS-C_{18} column (150 mm × 4.6 mm, 5 μm); The mobile phase was 1% acetic acid-acetonitrile (85:15); The detection wavelength was 340 nm; The column temperature was 30 ℃, the flow rate was 1.0 mL/min, and the injection volume was 20 μL.

Preparation of standard curve

Stock solution of orientin was prepared in methanol at the concentration of 10 mg/mL and stored at 4 ℃. The calibration curves were prepared by adding stock solution of orientin to blank plasma and various tissues, and the samples were analysed by HPLC and fitted to the standard curve equation.

Method validation

Precision Precision of orientin was assessed by determining quality control (QC) samples at three concentration levels (plasma samples 5.00, 0.50, and 0.250 μg/mL, tissue samples 40.0, 10.0, and 0.10 μg/mL) on consecutive 3 d. Precision was expressed as relative standard deviation (RSD).

Recovery rate The recovery rates of orientin were determined in low-, mid-, and high-concentration (plasma samples 0.25, 0.50, and 5.00 μg/mL, tissue samples 0.10, 10.0, and 40.0 μg/mL).

Stability The stability of orientin was evaluated after storage at room temperature (15—25 ℃) for 0, 2, 4, 6, 8, 10, 12, and 24 h. The stability of orientin was all below 3.3%.

PK application

Healthy New Zealand white rabbits, (2.5 ± 0.3) kg, were divided into three groups (n = 6 in each group). In the PK study, animals were iv, ip, and im injected with orientin (15 mg/kg), respectively. Blood samples (0.5 mL) were gathered in heparin tube at 2.5, 10, 15, 20, 25, 30, 35, 45, 55, 65, 80, 95, 110, and 125 min after iv injection. Blood samples (0.5 mL) were gathered in heparin tube at 5, 10, 15, 20, 25, 30, 35, 45, 55, 65, 80, 95, 110, 120, 160, 220, and 280 min after im injection, and blood samples (0.5 mL) were gathered in heparin tube at 5, 10, 15, 20, 25, 30, 35, 45, 55, 65, 80, 95, 110, and 120 min after ip injection. Plasma samples (0.5 mL) were centrifuged at 8000 r/min for 10 min, and the 0.1 mL supernatant, involved protein precipitation with 0.2 mL acetonitrile, was vortexed for 1 min, centrifuged, and taken on the supernatant. The supernatant was collected by millipore filter (0.45 μm) and stored at −20 ℃.

Tissue distribution application

Healthy New Zealand white rabbits, (2.5 ± 0.3) kg, were divided into twelve groups (n = 6 in each group). Animals were iv administered via the marginal vein of the ear with orientin (20 mg/kg), after 5, 10, 15, 30, 45, 60, 75, 90, 120, and 180 min, all the rabbits were euthanatized and the kidney, liver, lung, spleen, heart, and brain were harvested. The tissues were cleaned out with normal saline and weighed. The tissue samples were made of 50% homogenate, the 0.1 mL homogenate, involved protein precipitation with 0.2 mL acetonitrile, was vortexed for 1 min. The homogenate was centrifuged at 11 000 r/min for 10 min, and the supernatant was passed through 0.45 μm filter and stored at −20 ℃.

Data processing method

The PK parameters of different administration were calculated by 3P97 software. With a minimum value of Akaike information criterion (AIC), data were simulated and executed by compartmental model. PK parameters were obtained from statistical moment calculation. In data analysis, SPSS 16.0 statistical package was employed to perform the test of analysis of variance (ANOVA).

Results

Specificity of determination method

Orientin was separated well from other ingredients

in samples by HPLC. The endogenous substance in plasma and tissues did not interfere with the determination of the samples. The theoretical plates of orientin exceeded 5000; The retention time of orientin was 4.90 min; The resolution was larger than 3 (Fig. 1).

Method validation

Linearity and lower limit of quantitation Linear calibration curves for orientin were obtained through the concentration range of 0.0125—50.00 μg/mL. The standard curves of orientin in plasma and tissues were shown in Table 1. The lower limit of quantification for orientin was 0.0125 μg/mL.

Precision The data of intra- and inter-day precision for orientin showed that the intra- and inter-day RSD values were less than 10.5% (n = 6), indicating the acceptable precision of the present method.

Extraction recovery The mean extraction recoveries of orientin from rabbit plasma and tissues were shown in Table 2. The recovery rates were more than 85% (n = 6).

Stability The results showed that the samples maintained their stability in 24 h.

Concentration-time curves and PK parameters

Concentration-time curves of rabbits with different administration methods were shown in Figs. 2—4. The main PK parameters were shown in Table 3. The tissue distribution of rabbits by iv injection (20 mg/kg) and the area under curve (AUC) of tissues were calculated with statistical moment theory, the result was kidney > liver > lung > spleen > heart > brain. It showed that the orientin was widely distributed in tissues of rabbits. In data analysis, SPSS 10.0 statistical package was employed to perform the normality, ANOVA, t tests, etc, after logarithmic transformation was applied in AUC of tissues. The results showed that there were significant differences among groups (P < 0.05) (Fig. 5).

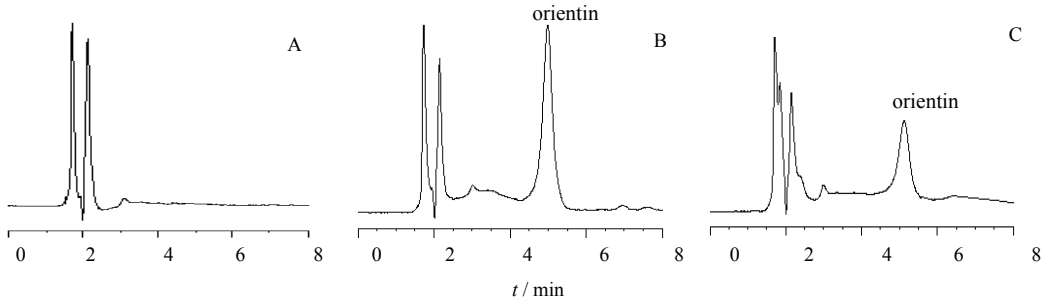

Fig. 1 Chromatograms of orientin in blank (A), reference substances (B), and sample (C) plasma

Table 1 Linear range, linear equation, and correlation coefficients in plasma and various tissues (n = 6)

Tissues and plasma	Linear ranges / (μg·mL^{-1})	Standard curve equation	r
heart	0.050 —50.00	Y = 37.796 X + 1.832	0.9987
liver	0.050 —50.00	Y = 33.091 X − 3.578	0.9992
brain	0.050 —50.00	Y = 38.415 X − 2.376	0.9995
kidney	0.050 —50.00	Y = 40.831 X − 3.189	0.9986
lung	0.050 —50.00	Y = 41.357 X − 4.365	0.9993
spleen	0.050 —50.00	Y = 35.247 X − 2.845	0.9987
plasma	0.0125—10.00	Y = 31.911 X − 6.636	0.9992

Table 2 Absolute recovery rates for analysis on orientin in plasma and various tissues ($\bar{x} \pm s$, n = 6)

Tissues and plasma	Recovery rates / %		
	High-concentration	Mid-concentration	Low-concentration
heart	99.68 ± 0.3	100.20 ± 0.1	103.30 ± 0.2
liver	101.80 ± 0.6	93.04 ± 2.9	95.68 ± 3.1
brain	100.30 ± 0.2	101.30 ± 0.2	98.30 ± 0.2
kidney	100.20 ± 1.4	99.22 ± 3.1	103.50 ± 1.8
lung	98.65 ± 1.6	99.32 ± 0.3	101.50 ± 0.1
spleen	93.35 ± 2.9	93.35 ± 2.9	95.08 ± 0.2
plasma	99.50 ± 9.2	101.80 ± 10.1	100.40 ± 7.7

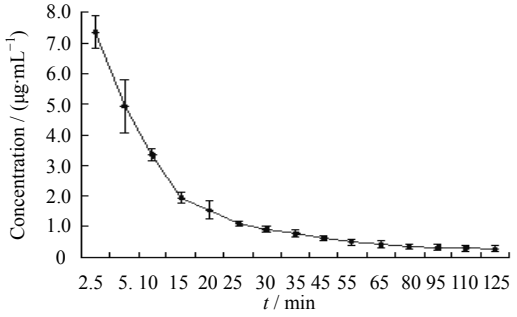

Fig. 2 Plasma concentration-time curves after iv injection

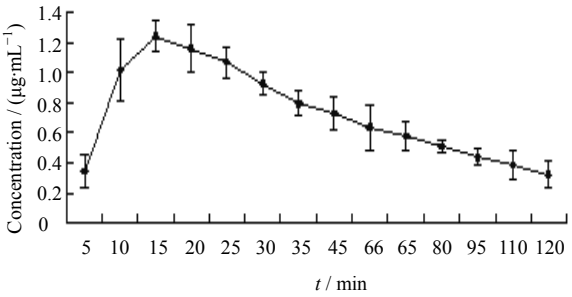

Fig. 4 Plasma concentration-time curves after ip injection

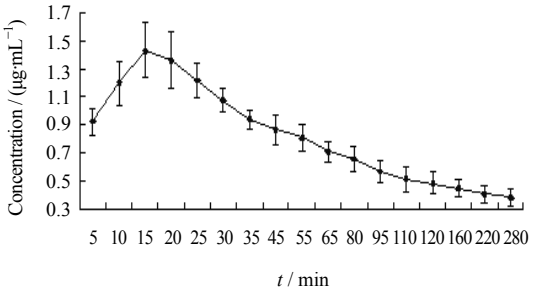

Fig. 3 Plasma concentration-time curves after im injection

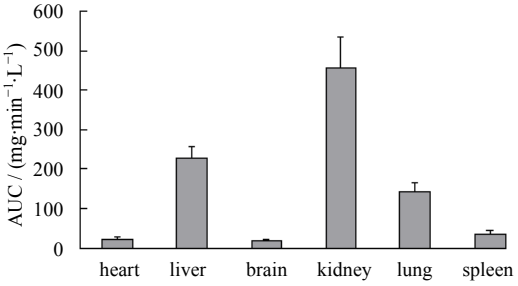

Fig. 5 AUC of orientin in various tissues

Table 3 PK parameters of rabbit after iv, ip, and im administration of orientin ($\bar{x} \pm s$, $n = 6$)

Parameters	Unit	iv	ip	im
A	$mg \cdot L^{-1}$	8.48 ± 0.910	3.73 ± 0.890	9.43 ± 2.36
α	$L \cdot min^{-1}$	0.182 ± 0.069	0.086 ± 0.014	0.121 ± 0.045
B	$mg \cdot L^{-1}$	1.65 ± 0.461	1.04 ± 0.120	0.897 ± 0.142
β	$L \cdot min^{-1}$	0.045 ± 0.039	0.007 ± 0.002	0.013 ± 0.008
$t_{½\alpha}$	min	4.61 ± 0.532	8.19 ± 1.94	6.39 ± 1.98
$t_{½\beta}$	min	40.8 ± 3.89	109 ± 16.3	59.5 ± 16.5
K_{21}	$L \cdot min^{-1}$	0.034 ± 0.014	0.041 ± 0.016	0.042 ± 0.013
K_{10}	$L \cdot min^{-1}$	0.071 ± 0.019	0.014 ± 0.006	0.028 ± 0.011
K_{12}	$L \cdot min^{-1}$	0.061 ± 0.019	0.040 ± 0.017	0.048 ± 0.012
Vc	$\mu g \cdot mL^{-1}$	1.54 ± 0.130	8.66 ± 1.44	7.33 ± 1.28
AUC	$mg \cdot min^{-1} \cdot L^{-1}$	264 ± 31.8	93.7 ± 18.6	67.9 ± 15.4
CL_S	$L \cdot min^{-1} \cdot kg^{-1}$	0.106 ± 0.003	0.119 ± 0.015	0.223 ± 0.121
AUMC	$mg \cdot L^{-1}$	3557.09 ± 337.68	5143.07 ± 302.34	3336.19 ± 314.91
MRT	min	28.5 ± 7.35	54.3 ± 24.3	48.4 ± 15.4
Ka	$L \cdot min^{-1}$	−	0.132 ± 0.037	0.135 ± 0.033
$T_{1/2}$Ka	min	−	5.44 ± 1.26	5.16 ± 1.88
T_{peak}	min	−	13.2 ± 3.79	18.8 ± 5.96
C_{max}	$\mu g \cdot mL^{-1}$	−	1.28 ± 0.392	1.22 ± 0.350

Discussion

We have succeeded in quantitative determination of orientin in rabbit plasma and tissues by three different administration methods. The method is validated for linearity, specificity, precision, and recovery, and good results are obtained. This method has been successfully applied to analyses of orientin in rabbit plasma and tissues after different administration methods.

The results of present study indicate that the concentration-time curve of orientin in plasma fits the two compartment model after iv, ip, and im injection. $t_{1/2\beta}$ is much higher than $t_{1/2\alpha}$, showing that orientin is mainly eliminated in rabbits. The absolute bioavailability by im injection (25.7%) is less than that by ip injection (35.3%), and it may be correlated with

abundant blood vessels and large blood flow of abdominal cavity. The pharmacological action is related to the tissue distribution characteristics. The results show that the tissues distribution of orientin is kidney > liver > lung > spleen > heart > brain after iv injection. It suggests that the higher uptake rates of orientin in kidney are found. The PK result and tissue distribution are useful for the further study of the clinical applications of orientin.

Conclusion

We developed a simple HPLC approach for rapid and selective analysis of orientin in plasma and tissues in rabbits. The method is suitable as an alternative to the previous assays for the pharmacokinetic study of the drug in rabbits.

The PK of orientin in rabbits fits the two compartment model by iv, im, and ip injection. Orientin is absorbed in rabbit. Orientin is distributed extensively in tissues such as kidney, liver, and lung. It is difficult for orientin to cross the blood brain barrier.

References

Fu XC, Wang X, Zheng H, Ma LP, 2007. Protective effects of orientin on myocardial ischemia and hypoxia in animal models. *J Southern Med Univ* 27(8): 1173-1175.

Huang WZ, Wang L, Duan JA, 2000. Studies on the chemical constituents of *Trollius ledebouri*. *Chin Tradit Herb Drugs* 31(10): 731-732.

Li LQ, 1995. The geographical distribution of SUBFAM. Helleboroideae (Ranunculaceae). *Acta Phytotaxon Sin* 33(6): 535-537.

Li XQ, Xiong ZL, Ying XX, Cui LC, Zhu WL, Li FM, 2006. A rapid ultra-performance liquid chromatography-electrospray ionization tandem massspectrometric method for the qualitative and quantitative analysis of the constituents of the flower of *Trollius ledibouri* Reichb. *Anal Chim Acta* 580(2): 170-180.

Qu CX, Yan J, Tian JM, Wang SH, An F, 2010. Determination of three flavonoids in *Trollius chinensis* Bunge by HPLC. *Chin Tradit Pat Med* 32(3): 500-502.

Yan J, Qu CX, Tian JM, Wang SH, An F, 2011. Isolation and purification of orientin and vitexin in *Trollius chinensis* Bunge by PHPLC. *Chin Tradit Pat Med* 33(4): 655-658.

Yang GD, 2011. Studies on antioxidation of orientinand vitexin from *Trollius chinensis* to mice aged by *D*-galactose. Hebei North University: Zhangjiakou.

Yang GD, Rao N, Tian JM, An F, Wang SH, 2011. Study on antioxidation activity of orientin and vitexin in *Trollius chinensis* Bunge. *Lishizhen Med Mat Med Res* 22(9): 2172-2173.

Zhu DX, 2011. Preliminary study on purification of orientin and vitexin in *Trollius chinensis* Bunge and its antitumor effect. Hebei Medical University: Shijiazhuang.