

Full-length article

Pharmacokinetic behaviors and oral bioavailability of oridonin in rat plasma¹Wen XU², Jin SUN², Ting-ting ZHANG², Bo MA², Sheng-miao CUI³, Da-wei CHEN², Zhong-gui HE^{2,4}²Department of Pharmaceutics, School of Pharmacy, Shenyang Pharmaceutical University, Shenyang 110016, China; ³Department of Pharmacy, Guangdong Pharmaceutical University, Guangzhou 510006, China**Key words**

oridonin; pharmacokinetics; intravenous; oral bioavailability; intraperitoneal injections; rats

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Abstract

Aim: To study the intravenous and oral pharmacokinetic behavior of oridonin and its extent of absolute oral bioavailability in rats. **Methods:** Oridonin was administered to rats via iv (5, 10 and 15 mg/kg), *po* (20, 40 and 80 mg/kg) or ip administration (10 mg/kg). The concentrations of oridonin in rat plasma were determined by a high performance liquid chromatography with electrospray ionization mass spectrometric detection (HPLC/ESI-MS) method and the pharmacokinetic parameters were determined by non-compartmental analysis. **Results:** The plasma concentration of oridonin after intravenous administration decreased polyexponentially, and the pharmacokinetic parameters of oridonin were dose-independent within the examined range. Oridonin was absorbed rapidly after oral gavage with a t_{max} of less than 15 min; the extent of absolute bioavailability of oridonin following oral administration was 4.32%, 4.58% and 10.8%. The extent of absolute bioavailability of oridonin following intraperitoneal administration was 12.6%. **Conclusion:** First order rate pharmacokinetics were observed for oridonin within the range of iv doses, while the extent of absolute oral bioavailability was rather low and dose-dependent. The low and dose-dependent extent of oral bioavailability may be due to the saturation of first-pass effects.

Introduction

Rabdosia rubescences (Chinese name “Donglingcao”), a herbal medicine, is traditionally used in China for the treatment of tonsillitis and a variety of cancers. Oridonin, a diterpenoid extracted from *Rabdosia rubescences*, is the marker compound and one of the major antitumor components of this herb^[1,2]. Oridonin injection is used alone or in combination with other drugs to treat human cancers, especially for the treatment of liver cancer^[3,4], esophageal carcinoma and carcinoma of gastric cardia^[5]. Oridonin injection and *Rabdosia rubescences* can also extend the lives of advanced cancer patients as well as improve their living quality. Due to the low toxicity of oridonin and *Rabdosia rubescences*, they can be used at a high dose for a long period of time, and only a few patients suffer abdominal discomfort^[5]. Because of the abundance of *Rabdosia rubescences* in China, recently, oridonin and *Rabdosia rubescences* have attracted special attention.

Although *Rabdosia rubescences* and oridonin have been used clinically for a long time, there is little information in published literature on the pharmacokinetics of oridonin. To our knowledge, only the pharmacokinetics of oridonin after iv administration in rabbits and iv and ip administration in mice have been reported^[6,7], and there is no published information about the pharmacokinetics of oridonin following oral administration. In clinical trials, *Rabdosia rubescences* is usually administered orally, so a better understanding of the pharmacokinetics and oral bioavailability of oridonin is very important for explaining the therapeutical outcomes produced by the drug in clinical trials and to help establish a rational dosage regimen.

In the present paper, we describe the pharmacokinetics behavior of oridonin after intravenous, oral and intraperitoneal administrations to rats.

Materials and methods

Chemicals and reagents Oridonin (98.9%) was extracted

from the aerial parts of *Rabdosia rubescences* and refined in our laboratory (identified by $^1\text{H-NMR}$, UV and MS). The internal standard, ethyl hydroxybenzoate (99.5%), was supplied by Shenyang Dongxing Reagent Factory (Shenyang, China). HPLC-grade methanol was obtained from Concord Tech Co (Tianjin, China) while HPLC-grade ethyl acetate and *n*-butanol were from purchased from Tianjin Kermel Chemical Reagents Development Centre (Tianjin, China). All other reagents were of analytical grade. Distilled water, prepared from deionized water, was used throughout the study.

Oridonin solution For intravenous, oral and intraperitoneal administration, oridonin (5 mg/mL) solution was prepared in 0.9% (*w/v*) saline containing 30% ethanol (*v/v*).

Animals and surgical procedures Male Wistar rats (230–250 g) were supplied by the Lab Animal Center of Shenyang Pharmaceutical University (Shenyang, China). All experimental procedures were carried out in accordance with the guidelines of the Experimental Animal Care and Use Committee of Shenyang Pharmaceutical University. The animals were maintained under standard laboratory conditions on a 12 h light/dark cycle and were fed standard rat chow and water *ad libitum*. The rats fasted overnight before the experiments and food was returned 2 h after dosing. Water was available *ad libitum* throughout the experiments.

Drug administration and sample collection Three groups of rats (each group contained 6 rats) were given oridonin solution as a single dose of 5, 10 and 15 mg/kg via the femoral vein (slightly anaesthetized by aether). The infusion time was about 10 s. Blood samples (250 μL) were collected in heparinized tubes from orbit veins with an heparinized glass tube at 0.083, 0.167, 0.5, 1, 2, 4, 6, 9, 12, 24, 36, and 48 h after administration; another 3 groups of rats (each group contained 6 rats) received a single dose of 20, 40, and 80 mg/kg by oral gavage. Blood samples (250 μL) were collected in the heparinized tubes from each rat at 0.05, 0.1, 0.167, 0.25, 0.5, 1, 2, 4, 6, 9, 12, 24, 36, and 48 h after administration. Six rats were given oridonin solution as single dose of 10 mg/kg intraperitoneally and blood samples (250 μL) were collected in the heparinized tubes from each rat at 0.083, 0.167, 0.5, 1, 2, 4, 6, 9, 12, 24, and 36 h after administration. Blood samples were immediately centrifuged and stored at $-20\text{ }^\circ\text{C}$ until analysis.

Sample preparation and analysis The concentrations of oridonin in rat plasma were determined by an HPLC/electrospray ionization mass spectrometric detection (HPLC/ESI-MS) method developed and validated in our laboratory^[8]. Briefly, to 100 μL plasma in glass centrifuge tubes 50 μL ethyl hydroxybenzoate (internal standard, 80 ng/mL) and 50 μL mobile phase were added. Samples were then vortex-mixed for 30 s and extracted with 3 mL ethyl acetate-*n*-butyl

alcohol (100:2, *v/v*). After vortex-mixing for 1 min and shaking for 10 min, the organic and aqueous phases were separated by centrifugation at $2000\times g$ for 10 min, then the upper organic layer was transferred to another tube and evaporated to dryness at $40\text{ }^\circ\text{C}$ under a gentle stream of nitrogen. The residue was reconstituted in 100 μL mobile phase followed by vortex-mixing and centrifugation at $2000\times g$ for 10 min. Then, 20 μL of an aliquot of the supernatant was injected onto the HPLC/ESI-MS system.

The high-performance liquid chromatography was performed using a Waters 1525 Binary pump (Framingham, Massachusetts, USA), which was controlled by Masslynx 4.0 Software (Waters Corp, Framingham, Massachusetts, USA). The mobile phase consisted of methanol-water (80:20, *v/v*) at a flow rate of 1.0 mL/min and the injection volume was 20 μL . The analytical column used was a DiamonsilTMC₁₈ column (200mm \times 4.6mm id, 5 μm) from Dikma Tech (Beijing, China) at a column temperature of $25\text{ }^\circ\text{C}$.

A ZQ2000 micromass spectrometer (Waters Corp, USA) fitted with a Z-Spray ion interface was used for all analyses. Ionization was achieved by using electrospray in the negative mode. The following parameters were optimized for the analysis of oridonin: capillary voltage, 3.0 kV; cone voltage, 25 V; source temperature, $105\text{ }^\circ\text{C}$; and desolvation gas (nitrogen) heated to $350\text{ }^\circ\text{C}$ and delivered at a flow rate of 350 L/h. Quantification was performed using the selected ion recording of *m/z* 363 for oridonin and *m/z* 165 for ethyl hydroxybenzoate. The lower limit of quantification of the method was 10 ng/mL and the quantitation range was 10–4000 ng/mL. For samples containing oridonin at a concentration higher than the upper limit of the range in the standard curve, an aliquot of the sample was first diluted with blank rat plasma and then 100 μL of the diluted sample was treated as described. The intraday and interday accuracy and precision of the assay were less than 9%.

Pharmacokinetic analysis All pharmacokinetic parameters were determined by non-compartmental analysis. The peak plasma level (C_{max}) and the time to reach the peak plasma concentration (t_{max}) were obtained directly from the concentration-time data. The elimination rate constant (K_e) was calculated from the slope of the logarithm of the plasma concentration versus time using the final 4 points. The apparent elimination half life ($t_{1/2}$) was calculated as $0.693/K_e$. The area under the plasma concentration-time curve (AUC) and the area under the first moment curve (AUMC) were calculated by the trapezoidal rule. Total body clearance was calculated as X_0/AUC . The extent of absolute bioavailability was estimated from the dose-normalized ratios of $(\text{AUC}_{0-\infty})_{\text{po}}$ to $(\text{AUC}_{0-\infty})_{\text{iv}}$ (based on iv 10 mg/kg). The mean residence times

after intravenous administration (MRT_{iv}) and oral administration (MRT_{po}) were calculated by dividing the AUMC by the AUC. The mean absorption time (MAT) was calculated by subtracting MRT_{iv} from MRT_{po} . The values were calculated by Microsoft Excel (Microsoft, Seattle, Washington, USA) and each value was expressed as mean±SD.

Results

Pharmacokinetic analysis of plasma concentrations after intravenous administration After intravenous administration, the plasma concentration of oridonin first decreased rapidly and then more slowly, that is to say, the plasma concentration of oridonin after intravenous administration decreased polyexponentially and the terminal elimination half life was relatively long (about 10 h). After intravenous administration, the pharmacokinetic parameters of oridonin were dose-independent at 3 doses: 5, 10, and 15 mg/kg. These results show that oridonin exhibits linear kinetics following intravenous administration over the dose range studied (Table 1, Figure 1).

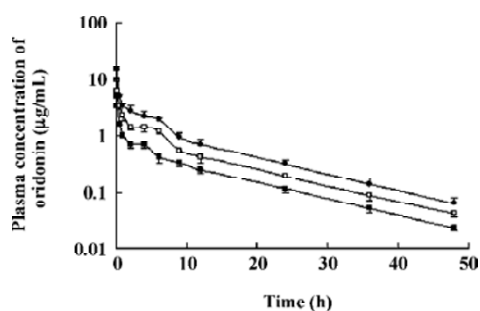


Figure 1. Plasma concentration-time curves of oridonin after intravenous administration to rats. Oridonin were given as a dose of 5 (■), 10 (□), or 15 mg/kg (●). *n*=6. Each value is expressed as mean±SD.

Pharmacokinetic analysis of plasma concentrations after oral administration The mean plasma concentration versus time curve of oridonin increased rapidly after oral administration reaching the maximum level less than 15 min after administration. Starting 9 h after administration, the profiles of oral and intravenous administration declined in parallel. The mean plasma concentration versus time curves after intravenous administration was much higher than that after oral administration at a higher dose. The extent of absolute bioavailability was rather low and appeared to be dose dependent. At a high dose (80 mg/kg), the extent of absolute oral bioavailability was about 2 times higher than those of low doses (20 and 40 mg/kg) (Table 1, Figure 2).

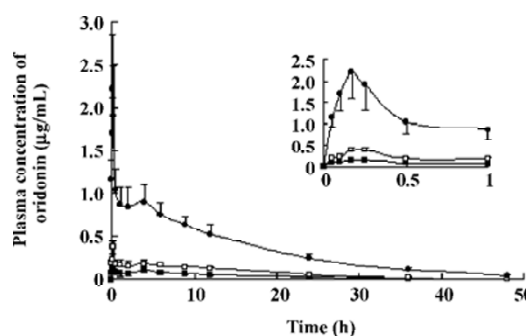


Figure 2. Plasma concentration-time curves of oridonin after oral administration to rats. Oridonin were given as a dose of 20 (■), 40 (□), or 80 mg/kg (●). *n*=6. Each value is expressed as mean±SD.

Pharmacokinetic analysis of plasma concentrations after intraperitoneal administration After intraperitoneal administration, the plasma concentration-time curve was similar to that after intravenous administration, except that the plateau was absent. Furthermore, the extent of absolute

Table 1. Pharmacokinetic parameters of oridonin after intravenous, oral and intraperitoneal administration. Values are expressed as mean±SD. *n*=6.

Parameter	iv			po			ip
	Dose (mg/kg)	Dose (mg/kg)	Dose (mg/kg)	Dose (mg/kg)	Dose (mg/kg)	Dose (mg/kg)	
	5	10	15	20	40	80	10
$t_{1/2}$ (h)	10.6±0.34	10.9±0.43	10.4±0.14	10.7±0.25	10.4±0.20	10.6±0.21	11.0±0.55
AUC _{0-∞} (mg·h·L ⁻¹)	11.6±0.91	22.6±1.77	36.5±4.11	1.95±0.21	4.13±0.33	19.6±4.0	2.61±0.79
MRT (h)	11.2±0.99	10.4±0.69	10.0±0.96	16.1±0.49	15.8±0.33	13.7±1.1	8.59±0.79
CL (L·kg ⁻¹ ·h ⁻¹)	0.433±0.037	0.446±0.036	0.425±0.051	—	—	—	—
t_{max} (h)	—	—	—	0.19±0.04	0.18±0.03	0.19±0.04	—
C_{max} (µg/mL)	—	—	—	0.166±0.023	0.372±0.061	2.25±0.60	—
<i>F</i> (%)	—	—	—	4.32±0.47	4.58±0.37	10.8±2.2	12.6±3.9

bioavailability of oridonin was only 12.6% (Figure 3).

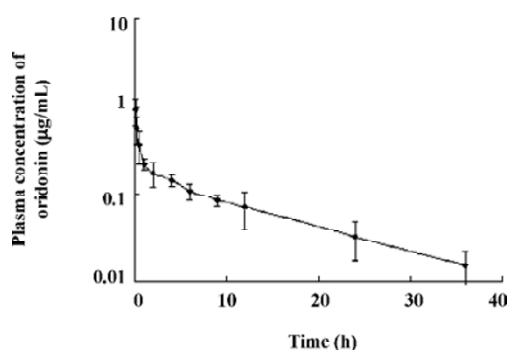


Figure 3. Plasma concentration-time curve of oridonin after intraperitoneal administration to rats as a single dose of 10 mg/kg. *n*=6. Each value is expressed as mean±SD.

Discussion

The oral absolute bioavailability of oridonin was rather low (4.32%–0.8%) and appeared to be dose dependent. Considering the amount of unchanged oridonin recovered from the gastrointestinal tract and feces 48 h after oral administration (the mean value was approximately 6.52%; Figure 4), the low extent of absolute oral bioavailability values are most likely due to hepatic, gastric and/or intestinal first-pass effects. After intraperitoneal administration of oridonin solution to rats at a single dose of 10 mg/kg, the bioavailability of oridonin was 12.6%; Figure 5). This result shows that hepatic first-pass effect may be the main reason for the low oral bioavailability of oridonin. Oridonin is a water-insoluble drug; some researchers have tried to enhance its oral bioavailability by simply increasing the dissolution rate of the drug from its dosage form^[9]. Although *in vivo* behavior of those dosage forms had not been performed, based on the result of our

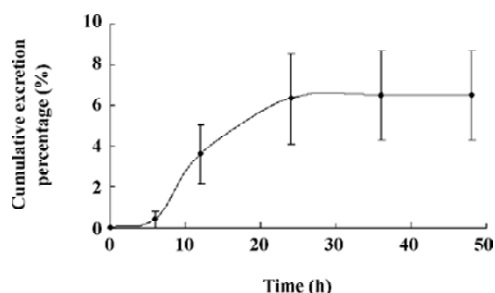


Figure 4. Mean fecal cumulative excretion percentage profiles of oridonin in rats after oral administration of 20 mg/kg oridonin. *n*=5. Each value is expressed as mean±SD.

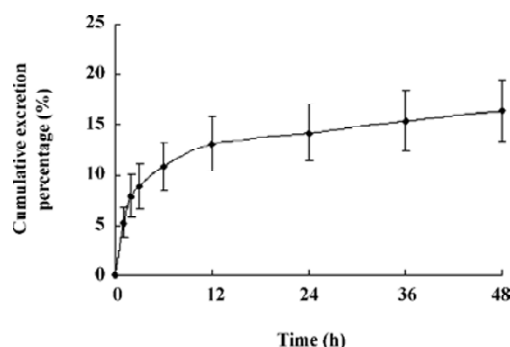


Figure 5. Mean bile cumulative excretion percentage profiles of oridonin in rats after intravenous administration of 10 mg/kg oridonin. *n*=5. Each value is expressed as mean±SD.

paper we can speculate that through those meanings, the enhanced oral bioavailability is limited. Other drug delivery systems which can circumvent the liver first-pass effect may work, such as the M cell drug delivery system^[10] (Table 1, Figure 3).

Many natural products display beneficial anticancer effects *in vitro*, but only a few have been involved in clinical trials. One of the main obstacles which prevent the development of natural anticancer drugs is that the concentration of drugs *in vivo* can not reach the level used *in vitro*. The *in vitro* anticancer effect of oridonin has been studied by several groups^[11–16]. These research show that the anticancer effect of oridonin is time and dose-dependent; at a low µmol/L concentration (about 1 µg/mL), oridonin has weak apoptosis-inducing effects, while at a concentration of 8–10 µmol/L, oridonin has strong apoptosis-inducing effects on most cancer cells^[11,13]. According to the clinical dose of *Rabdosia rubescences*^[17] and the content of oridonin in the herb^[1], a patient receiving about 175 mg oridonin per time orally is equal to the dose of 13.5 mg/kg to rats^[18]. Obviously at this dose the plasma concentration of oridonin is too low to have a beneficial effect. In clinical trials, patients receive *Rabdosia rubescences* solution 3 times per day. If the similar poor and dose-dependent oral bioavailability phenomenon is found in men, a higher dose and a longer dosing interval may produce better curative effects.

In clinical trials, *Rabdosia rubescences* and oridonin are usually used in combination with other drugs to enhance the anticancer effects of chemical drugs^[17,19]. Since the hepatic first-pass effect may be the main reason for the low oral bioavailability of oridonin, potential drug-drug interactions may occur: enhanced oral bioavailability of oridonin and decreased drug metabolism, which may lead to better anticancer effects or more severe side effects.

The pharmacokinetic parameters of oridonin were dose independent at 3 doses, 5, 10, and 15 mg/kg after intravenous administration. The dosage range was set according to the toxicity of the menstruum and the sensitivity of our analytical method, but the dosage range is somewhat narrow to give more pharmacokinetic information. Considering that the length of the plateaus of the 3 curves in Figure 1 look dose dependent, we wonder what will happen with a reduction of the dose. Although an intravenous administration experiment at an even lower dose was not carried out, the intraperitoneal administration experiment may be used as a reference. After intraperitoneal administration of oridonin solution to rats at a single dose of 10 mg/kg, the plateau of plasma concentration-time disappeared. Since the percentages of the intravenous dose of oridonin excreted in bile as unchanged drug was 16.0% in the rats (data not shown), the enterohepatic circulation may contribute to the plateau in the plasma concentration-time curves of Figure 1 and Figure 2. The plateaus of the curves in Figure 1 may also be accounted by saturable tissue uptake since the sharp decrease of the plasma oridonin concentration after intravenous administration was due to tissue uptake.

In conclusion, we systematically investigated the pharmacokinetic behaviors of oridonin after intravenous, oral and intraperitoneal administration in rats. The mean plasma concentration-time curve appeared to be polyexponential, and oridonin exhibited linear kinetics at 3 doses following intravenous administration. The extent of absolute oral bioavailability was rather low and dose-dependent.

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