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Research Article

PHARMACOKINETICS OF INJECTABLE BETA-CYCLODETRIN INCLUSION COMPLEX IN WISTAR RATS

BAO-QIU LI[a*], NA LI[b], SHU-JUAN WANG[c] JI-YOU GAO[b], SHI-HONG FANG[b]

[a]Department of Pharmacology, School of Pharmaceutical Sciences, Shandong University, Jinan, Shandong 250012, China, [b]Shandong Hongli Laboratory Animal Experiment Co., Ltd., Jinan, Shandong 250101, China, [c]A-Think Pharmaceutical Co., Ltd. Changchun, Jilin 130616, China. Email: liqiu@163169.net

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ABSTRACT

Aim: Oridonin is a new anti-tumor drug candidate with promising broad spectrum antitumor activity. The aim of the present study is to investigate the single- and repeated-dose pharmacokinetics of injectable beta-cyclodextrin-oridonin inclusion complex in rats. Methods: Rats were given single- or multiple-dose (7 days) of injectable beta-cyclodextrin-oridonin inclusion complex (single-dose: 33 mg/m^2, 99 mg/m^2, 296 mg/m^2; repeatede-dose: 99 mg/m^2 calculated as oridonin) by intravenous injection. Plasma oridonin was analyzed by LC–ESI-MS. The main pharmacokinetic parameters were calculated and compared. Results: The PK data of single-dose injectable beta-cyclodextrin-oridonin inclusion complex in rats were best fit by a three-compartment model.The terminal elimination half-life ($t_{1/2z}$) of oridonin ranged from 8.72±1.14 to 10.87±2.03h. AUC $_{(0-t)}$ and C_{max} for oridonin showed statistically significant differences ($p < 0.05$) between singledose and repeated dose. Conclusion: Our study has led to the view that injectable beta-cyclodextrin-oridonin inclusion complex can increase the bioavailability of oridonin in rats and is suitable for once a day dosing. However, caution should be taken with when injectable beta-cyclodextrin-oridonin inclusion complex is given by i.v. repeatedly.

Keywords: Pharmacokinetics; Oridonin; Beta-cyclodextrin; Rats.

INTRODUCTION

Chinese herbal medicine is widely used in China and plays an important role in the prevention and treatment of various kinds of human diseases. Some chemical compounds extracted from Chinese medicinal herbs show promising biological and pharmacological activities, such as artemisinin, paclitaxel and salvianolic acid [1-5]. For example, artemisinin have been used worldwide as an antimalarial agent and paclitaxel has been successfully used in the clinic as an antitumor agent.

Rabdosia rubescens is a Chinese medicinal herb which has been approved by State Food and Drug Administration of China for the treatment of inflammation such as acute tonsillitis, sphagitis, astomatitis and gingivitis [6]. It has also been used in folk medicine in China for the treatment of esophageal and cardia cancer.

Over the past thirty years, considerable in vitro and animal studies have been performed to determine the active ingredient of Rabdosia rubescens and the precise mechanism responsible for its antitumor activity.

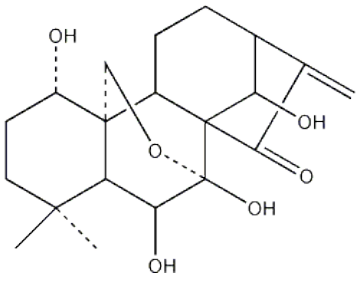

Fig. 1: It shows the structural formula of oridonin

It is found that oridonin (Fig 1), an ent-kaurane diterpenoid derived from the herbal Rabdosia rubescens inhibit the proliferation of human nasopharyngeal carcinoma CNE2 cells [7], human gastric cancer MKN45 cells [8], U937 cells [9], HeLa cells [10], murine fibrosarcoma L929 cells [11], human laryngeal carcinoma HEp-2 cells [12], HT1080 cells [13], human hepatocelluar carcinoma BEL-7402 cells [14], human melanoma A375-S2 cells [15], leukemia K562 cells [16] and some other tumor cells. Preliminary animal data demonstrate that oridonin can significantly reduce the volume of sarcoma-180 solid tumors in mice [11] and prolong survival of leukemia mice models [18].

All these findings indicate that oridonin is a new anti-tumor drug candidate with promising broad spectrum antitumor activity. However, oridonin is insoluble in water and has a very bitter taste. The absolute bioavailability of oridonin following oral administration and intraperitoneal administration were rather low [19].

Drug delivery is the method or process of administering a pharmaceutical compound to achieve a therapeutic effect in humans. It has been widely used to modify drug release profile, drug absorption, drug distribution and drug elimination[20-22]. Injectable beta-cyclodextrin-oridonin inclusion complex (freeze dried powder) is a new formulation of oridonin prepared by A-Think Pharmaceutical Co., Ltd.It is designed to increase the bioavailability of oridonin. The aim of the present study is to investigate the pharmacokinetics of injectable beta-cyclodextrin-oridonin inclusion complex in rats following intravenous injection.

MATERIALS AND METHODS

Chemicals and Reagents

Injectable beta-cyclodextrin-oridonin inclusion complex(freeze dried powder; entrapment efficiency: 86.01±4.33%; average particle size:392 nm) was supplied by A-Think Pharmaceutical Co., Ltd. (Changchun, China). Oridonin was purchased from Chinese national institute for the control of pharmaceutical and biological products (Beijing, China). Ethyl p-Hydroxybenzoat (internal standard) was purchased from Sinopharm chemical reagent Beijing Co., Ltd. Normal saline (0.9% NaCl) was purchased from Shandong hualu pharmaceutical Co., Ltd (Liaocheng, Chian). Heparin was purchased from Changzhou Qianhong Bio-pharm Co., Ltd. (Changzhou, China).

Animals

Two hundred and twenty-two specified-pathogens free Wistar rats (111 male, 111 female) weighted 180-220g, were obtained from the Experimental Animal Center of Shandong University (Jinan, China) and quarantined for 1 week. The rats were housed in stainless steel wire-mesh cages under specific pathogen free conditions (12h day/night cycle, temperature 22–24℃, humidity 52-58%).

The study protocol was approved by the Animal Ethics Committee of Shandong Hongli Laboratory Animal Experiment Co., Ltd. All aspects of the study involving animal procurement, care, housing, use, disposal and welfare were performed in compliance with Chinese regulations for the care and use of experimental animals.

LC–ESI-MS

Oridonin levels in plasma were analyzed by LC–ESI-MS. The HPLC was equipped with a SPD-10A ultraviolet detector, a Shim-Pack VP-ODS C18column (150 mm × 2.0 mm I.D., 5 μm particle size) and a Shim-pack GVP-ODS C18 guard column (5 mm × 2.0 mm I.D., 5μm particle size) purchased from Shimadzu (Tokyo, Japan). The column temperature was kept at 40℃ The mobile phase was a mixture of acetonitrile and water (50:50, V/V) and pumped at a flow rate of 0.2 ml/min.

The mass spectrometer was operated in negative ESI model with the following conditions: CDL temperature of 250°C, CDL voltage of 15V, block heater temperature of 200°C, nebulizing gas (N_2) flow rate of 1.5L/min, dying gas pressure of 0.1MPa and probe voltage of 1.6kV. Monitor ions for SIM analysis were m/z 363 for oridonin ($M\text{-}H^-$) and m/z 165 for ethyl p-Hydroxybenzoat ($M\text{-}H^-$).

Preparation of biological samples

Blood samples were collected, anticoagulated with heparin, centrifuged at 3000rpm for 10 min to obtain plasma and stored at -80°C (Forma Scientific Bio-freezer, Marietta, Ohio, USA) until analysis. For quantitative analysis, the plasma (50μl) was spiked with ethyl p-Hydroxybenzoat (2μg/ml, internal standard), deproteinizated with methanol, vortexed for 15 s using a vortex mixer (IKA, Staufen, Germany) and centrifuged at 1000rpm for 10 min. Then 5 μl of the supernatant was injected into the LC–ESI-MS system.

Single-dose PK study

Two hundred and sixteen Wistar rats (108 male, 108female) were randomly divided into 3 groups after an overnight fast and given three different dose of injectable beta-cyclodextrin-oridonin inclusion complex (296 mg/m², 99 mg/m² and 33 mg/m² respectively, calculated as oridonin) by intravenous injection.

Blood samples were collected 5min, 10 min, 30 min, 1h, 2h, 4h, 8h, 12h, 16h, 24h, 36h and 48h postdose and subsequently treated and analyzed by the method above.

Repeated-dose PK study

Six rats were given injectable beta-cyclodextrin-oridonin inclusion complex (99 mg/m², calculated as oridonin) by intravenous injection once a day for 7 days. Blood samples of rats were collected at prodose (day 4, day 5, day 6 and day 7), 0.5, 1, 2, 8 and 24h after the last injection.

Curve fitting and PK parameters calculation were performed using the DAS version 2.0 pharmacokinetic program (Chinese Pharmacology Society, Beijing, China). The peak concentration (C_{max}) and time to peak concentration (T_{max}) for oridonin were determined from the actual measurements. The area under the concentration-time curve (AUC) was calculated by using the linear trapezoidal rule.

Statistics analysis

Main PK parameters were calculated by DAS 2.1.1. Values were expressed as mean ±SD. Statistical significance in inter-group comparison was analyzed by Student's t-test.

RESULTS

Single-dose PK of injectable beta-cyclodextrin-oridonin inclusion complex in rats

The mean concentration-time profiles and PK parameters of single-dose injectable beta-cyclodextrin-oridonin inclusion complex in rats were shown in Fig2 and Table 1 respectively. The PK data of single-dose injectable beta-cyclodextrin-oridonin inclusion complex in rats were best fit by a three-compartment model. AUC and C_{max} of oridonin increased proportionally when the dose of oridonin was escalated. The terminal elimination half-life ($t_{1/2z}$) of oridonin ranged from 8.72±1.14 to 10.87±2.03h.

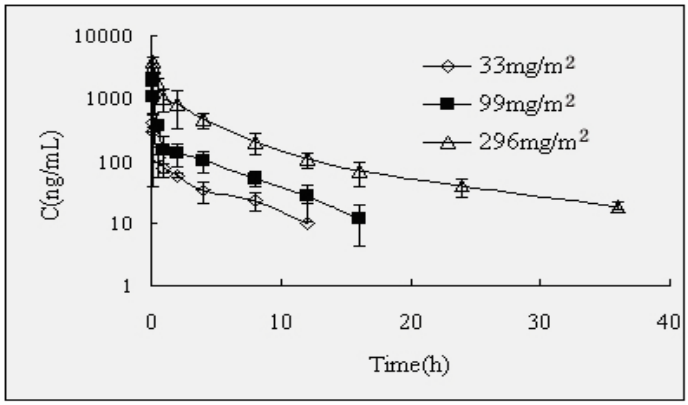

Fig. 2: It shows the concentration-time profile of single dose injectable beta-cyclodextrin-oridonin inclusion complex in rats following intravenous injection

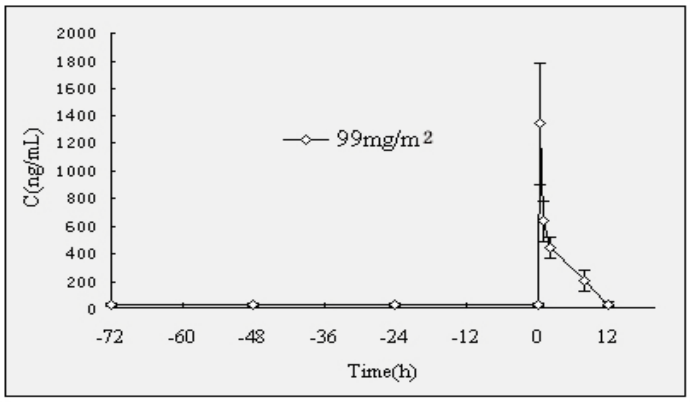

Fig. 3: It shows the concentration-time profile of repeated-dose (99mg/m²) injectable beta-cyclodextrin-oridonin inclusion complex in rats following intravenous injection

Table 1: PK parameters of single- and repeated-dose of injectable beta-cyclodextrin-oridonin inclusion complex following intravenous injection

PK parametes	Single-dose			Repeated-dose
	33 mg/m²	99 mg/m²	296 mg/m²	99 mg/m²
$AUC_{(0-t)}$（ng/ml·h）	511.49±27.03	1507.57±50.52	7002.75±421.67	5175.81±85.40*
$AUC_{(0-\infty)}$（ng/ml·h）	563.54±12.64	1586.12±65.08	7280.37±240.0	5364.07±49.57*
$MRT_{(0-t)}$（h）	3.21±0.40	4.25±1.62	5.88±1.03	5.20±0.48
$MRT_{(0-\infty)}$（h）	4.52±0.51	5.15±0.19	7.60±2.30	6.12±2.54
$t_{1/2z}$（h）	8.72±1.14	8.45±0.98	10.87±2.03	10.27±1.69
T_{max}（h）	0.17±0.05	0.17±0.02	0.17±0.06	0.50±0.01
V_z（l/m²）	314.63±12.15	400.66±3.94	614.44±23.06	140.25±6.49
CL_z（l/h/m²）	58.56±11.27	62.42±9.68	40.66±10.11	18.46±6.88
C_{max}（ng/ml）	420.87±3.10	1097.43±14.67	3032.40±89.20	1337.50±102.54

Six rats were used in the repeated-dose PK study and were given injectable beta-cyclodextrin-oridonin inclusion complex (99 mg/m², calculated as oridonin) by intravenous injection once a day for 7 days.

Mean ±SD;*$p < 0.05$ vs. first injection (day 1); Single-dose: n=6; Repeated-dose: n=6

Repeated-dose PK of injectable beta-cyclodextrin-oridonin inclusion complex in rats

The mean concentration-time profiles and PK parameters of repeated-dose injectable beta-cyclodextrin-oridonin inclusion complex in rats following intravenous injection were shown in Fig 3 and Table 1 respectively. $AUC_{(0-t)}$ and C_{max} for oridonin showed statistically significant differences ($p < 0.05$) between singledose and repeated dose.

DISCUSSION

Many natural products display beneficial anticancer effects in vitro. But only a few have been used in clinical practice.

Oridonin is extracted and purified from Rabdosia rubescens, one of the most important traditional Chinese herbs commonly used in China nowadays. It has been studied exclusively by several groups in China and has been shown to possess anti-tumor activity [23, 24]. However, limited data are available concerning the toxicokinetics of oridonin.

In the present study, we investigated the PK of injectable beta-cyclodextrin-oridonin inclusion complex (freeze dried powder), a new preparation of oridonin, in rats.

The AUC and C_{max} of single- and repeated-dose of injectable beta-cyclodextrin-oridonin inclusion complex were significantly higher than that of common oridonin injection [25]. It indicates that the beta-cyclodextrin-oridonin inclusion complex can increase the solubility and bioavailability of oridonin. Based on the ED_{50} of oridonin [26], high levels oridonin in rats observed in the present study is helpful to exert its anticancer activity in vivo.

The terminal elimination half-life ($t_{1/2z}$) of three doses of injectable beta-cyclodextrin-oridonin inclusion complex showed no significantly difference when compared with oridonin tablet and common oridonin injection [25]. It is long enough for once a day dosing. $AUC_{(0-t)}$ and C_{max} for oridonin showed significant differences between singledose and repeated dose. Thus, caution should be taken with when njectable beta-cyclodextrin-oridonin inclusion complex is given by i.v. repeatedly.

In summary, we conclude that injectable beta-cyclodextrin-oridonin inclusion complex can increase the solubility and bioavailability of oridonin in rats and is suitable for once a day dosing. However, caution should be taken with when njectable beta-cyclodextrin-oridonin inclusion complex is given by i.v. repeatedly.

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