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Studies on pharmacokinetics and tissue distribution of oridonin nanosuspensions

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Abstract

The purpose of the present study was to investigate the effects of particle size on the pharmacokinetics and tissue distribution of oridonin nanosuspensions after intravenous administration. Two oridonin nanosuspensions with markedly different size were prepared by high pressure homogenization method. The particle size of nanosuspension A is 103.3 ± 1.5 nm, while B is 897.2 ± 14.2 nm. Dissolution studies showed that complete dissolution could be obtained within 10 min for nanosuspension A, however, nanosuspension B showed a slower dissolution, only 85.2% dissolved by 2 h. The pharmacokinetics and tissue distribution of oridonin nanosuspensions A and B were studied after intravenous administration using New Zealand rabbits and Kunming mice as experimental animals, respectively. An Oridonin control solution was studied parallelly. The results showed that oridonin nanosuspension A exhibited pharmacokinetic and biodistribution properties similar to solution due to its rapid dissolution in blood circulation. Oridonin nanosuspension B, however, showed a high uptake in RES organs, meanwhile exhibited a markedly different pharmacokinetic property compared to nanosuspension A. These differences could be attributed to the different particle size of the two nanosuspensions considering their zeta potential had no significant difference. In conclusion, particle size showed obvious effects on pharmacokinetics and tissue distribution of nanosuspensions.

Keywords: Oridonin; Nanosuspensions; High pressure homogenization; Pharmacokinetics; Tissue distribution

1. Introduction

The research on colloidal drug delivery systems may be the hottest field in pharmaceutics in the last several decades. Among these colloidal systems, drug nanosuspensions have been paid more and more attentions as a promising approach due to its pharmaceutical advantage and pharmacoeconomics value (Muller and Peters, 1998; Muller et al., 2001). Nanosuspensions, a carrier-free colloidal drug delivery system, consist essentially of pure drug nanoparticles (100–1000 nm) and a minimum amount of surface active agent required for stabilization. At present, several production techniques have been applied to produce drug nanosuspensions, such as precipitation (Siostrom et al., 1993), pearl milling (Liversidge et al., 2003) and high pressure homogenization (Krause and Muller, 2001; Hecq et al., 2005; Zhang et al., 2007a,b; Muller and Jacobs, 2002). The development of nanosuspensions is so rapid due to its unique advantage that many nanosuspensions products have been launched into market or put into clinical experiments within 20 years from the rise of this conception (Muller and Kecka, 2006).

An important advantage of the drug nanosuspensions is that they can be applied to various administration routes, such as oral (Liversidge and Cundy, 1995), parenteral (Peters et al., 2000), ocular (Rosario et al., 2002) and pulmonary delivery (Jacobs and Muller, 2002), and have shown great superiority over the counterparts of the traditional formulation products in every administration routes.

For nanosuspensions, according to Noyes–Whitney and Ostwald–Freundlich equations, particle size in nanometer range can lead to increased dissolution velocity and saturation solubility (Hintz and Johnson, 1989; Bernhard et al., 1999), so

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it can more efficiently improve the oral absorption of poorly soluble drugs and achieve a higher bioavailability compared to the traditional formulation (Liversidge and Conzentino, 1995; Liversidge and Cundy, 1995). Apart from these, due to its sufficiently small size and safe composition, nanosuspensions can be injected intravenously, and 100% bioavailability can be obtained (Muller and Kecka, 2004). Sometimes nanosuspesions can show passive targeting property like colloidal drug carriers (Peters et al., 2000) through intravenous administration. Following modification on the surface with special stabilizers it can also exhibit active targeting property in vivo (Muller and Kecka, 2004).

Compared with other carrier-based solid nanoparticles, such as solid lipid nanoparticles, polymer-based nanoparticles and liposomes, nanosuspensions show a much faster particle size reduction during the process of dissolution, which may influence the in vivo fate of nanosuspension after intravenous administration. In this study, the influence of particle size on in vivo fate of nanosuspensions was investigated using oridonin as the model drug.

Oridonin (ORI, Fig. 1), poorly soluble in water, is an active diterpenoid compound extracted from the Chinese traditional medicine *Rabdosia rubescens* (Zhang et al., 2003; Osawa et al., 1994). Pharmacological experiments showed that ORI exhibited efficient anti-cancer activities, and clinical data indicated that it could effectively inhibited the proliferation of many human cancer cells including liver, prostate, breast and cervices cancer cells, non-small cell lung cancer cells, acute promyelocytic leukemia and glioblastoma multiforme (Zhang et al., 1999; Ikezoe et al., 2003; Chen et al., 2005). It has been successfully used for the treatment against live cancer, esophageal carcinoma in the clinical for decades (Zhang and Ren, 2003; Fujita et al., 1988). However, its application in the clinic have been limited due to its poor solubility and a low bioavailability (Zhang et al., 2007a,b).

In this study, two ORI nanosuspensions with different particle size were prepared by high pressure homogenization method, and dissolution behavior were examined. Pharmacokinetics and tissue distribution of ORI nanosuspensions of different size were studied after intravenous administration. It is the first report on the influence of particle size on the pharmacokinetics and tissue distribution of drug nanosuspensions after intravenous administration.

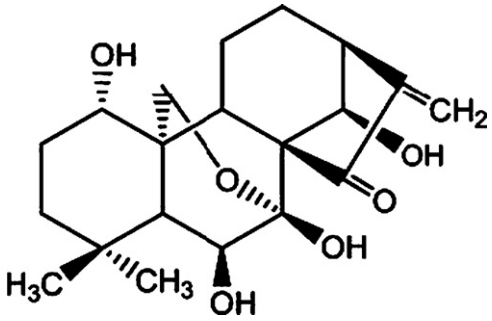

Fig. 1. Chemical structure of ORI.

2. Materials and methods

2.1. Materials

ORI (99%) was purchased from Shanxi Huike Plants Exploitation Co., Ltd.; Pluronic F68 and Cremophor EL were purchased from Sigma (USA); Lecithin was purchased from Beijing Shuangxuan Microbic Medium Co., Ltd. (China); Mannitol, sodium hydroxide, potassium dihydrogen phosphate and 1,2-propylene glycol were purchased from Shanghai Chemical Agent Co., Ltd. (China). Nitrogen gas was purchase from Gas Company of Jinan (China). Water used in the experiment was deionized. The methanol (Fisher Corporation, USA) was of HPLC grade, and other materials were of analytical reagent.

2.2. Preparation of ORI nanosuspensions of different size

The Pluronic F68 and Lecithin (0.5%, w/v; molar ratio, 3:1) were completely dissolved or dispersed in 200 ml water. Then ORI powder (5% ORI, w/v) was poured in the surfactant solution, the resultant suspensions were sufficiently homogenized using Heidolph homogenizer (DIAX 900, Germany) at 10000 rpm for 1 min. Then the nanosuspensions were prepared using a piston-gap high pressure homogenizer (NS1001L, Italy). At first, two cycles at 150 bar and five cycles at 500 bar were conducted as a kind of pre-milling, then a high pressure homogenization step was applied on the suspensions at 1500 bar or 1300 bar for 20 cycles, by which ORI nanosuspension A and B were obtained, respectively.

2.3. Water removal

To further study the dissolution behavior, the nanosuspensions were freeze-dried immediately after preparation. First the suspensions were filled into glass flasks and pre-freezed using an ultra cold freezer (MDF-382E, SANYO, Japan) at −80 °C for 24 h, later the samples were freeze-dried using a lyophilizer (LGJ 0.5, Beijing, China) at temperature −40 °C and pressure 0.10 mbar for 48 h to yield dry powder. Mannitol (1%, w/v) was added into nanosuspensions as cryoprotectant prior to freezing.

2.4. Measurement of size and zeta potential of ORI nanosuspensions

The size and zeta potential of nanoparticles were measured by a Zetasizer (3000SH, Malvern Instruments Ltd., UK) following their resuspension in water. Each sample was analyzed in triplicate.

2.5. Dissolution studies

Dissolution behavior of ORI nanosuspensions dried-powder was estimated using a dissolution appparatus (RC-3B, Tianjin, China) with the paddle method. Samples containing an equivalent of ORI (10 mg) were placed into 900 ml phosphate buffered solution (pH 7.4) (containing 5% ethanol) which was used as

release medium to simulate the in vivo environment, the temperature and rate were set to 37 °C and 100 min^{-1}, respectively. At predetermined intervals 5 ml medium was taken and placed in Ultrafree tube with a cutoff of 10,000 Da (Ultrafree, MC Millipore, Bedford, USA) and centrifuged at 20,000 × *g* for 30 min at 4 °C (Biofuge stratos, Kendro Laboratory Products, Osterode, Germany). Equal blank medium was compensated immediately to sustain the sink condition following each sampling. The amount of drug in supernatant was determined at 238.0 nm using an ultraviolet detector (UV-2102, Unico, USA). Each sample was analyzed in triplicate.

2.6. Animals

New Zealand white rabbits (2.5 ± 0.2 kg) and Kunming strain mice (25 ± 2 g), supplied by the Experimental Animal Center of Shandong University (Jinan, China), were used for pharmacokinetic and biodistribution studies, respectively. At first, the animals were acclimatized at a temperature of 25 ± 2 °C and a relative humidity of 70 ± 5% under natural light/dark conditions for 1 week and were fed with food and water ad libitum. Prior to experiment the animals were kept under fasting overnight. All experimental procedures abided by the ethics and regulations of animal experiments of Pharmaceutical Sciences, Shandong University, China.

2.7. Pharmacokinetics studies

Nine New Zealand white rabbits were randomly and equally divided into three groups. Three formulations, ORI solution, ORI nanosuspension A and ORI nanosuspension B, were administered to the three groups respectively at a 15 mg kg^{-1} dose level via the left auricular vein after dilation with ethanol solution (75%). ORI solution, acting as a control solution, was prepared by dissolving 250 mg ORI in mixture of Cremophor EL (2%) and 1,2-propylene glycol (1.56%) followed by dilution with isotonic sodium chloride solution (0.9%) to 50 ml. All the ORI solution mentiond below was the same preparation. Time taken for administration was 30 s. Blood samples were drawn via the right auricular vein using vacuum blood collection tubes (3 ml, XUEGE, Weihai, China) at predetermined time points (for ORI solution and nanosuspension A at 15, 30 min; 1, 3, 6, and 12 h post i.v. dose, and for nanosuspension B at 15, 30 min; 1, 3, 6, 12, 24, 36 and 48 h post i.v. dose). The samples were centrifuged (5000 × *g*, 15 min) and serum was collected and stored at −20 °C until analysis.

2.8. Tissue distribution studies

Kunming strain mice were divided into three groups and administered with three types of formulations, ORI solution, ORI nanosuspension A and ORI nanosuspension B at a dose level of 20 mg kg^{-1} via tail vein using an 1 ml insulin syringe equipped with 28 $G^{1/2}$ needle after dilation with ethanol solution (75%). At predetermined time points (for ORI solution and nanosuspension A at 15, 30 min; 1, 3, 6, 12 h and for nanosuspension B at 15, 30 min, 1, 3, 6, 12, 24, 36, 48 h) post i. v. administration three animals from each group were given anaesthesia and blood was collected from postorbital vein and was centrifugated to get the serum. Tissues of interest (heart, liver, spleen, lung, and kidney) were collected immediately after lightly rinsed with normal saline and dried with tissue paper. Serum and tissue samples were frozen at −20 °C until analysis.

2.9. Serum and tissue sample analysis

Five hundred micro litres of serum were extracted and homogenized with 5 ml acetic ether on a vortex mixer (XW-80, Shanghai, China) for 5 min. After centrifugalization at 3500 × *g* for 15 min the organic layer was transferred into another clean test tube and evaporated under nitrogen gas flow. Then the residue was redissolved using 500 μl mobile phase and filtered through a 0.22 μm filter; 20 μl of the filtrate was injected into HPLC column. The blood concentration can be calculated through the drug concentration-peak areas standard curve.

Tissue sample was weighed accurately and homogenized using a glass tissue homogenizer after addition of 1 ml physiologic saline. Tissue homogenates were processed similarly as 500 μl serum samples and analyzed by HPLC.

The serum and tissue samples were all analyzed by an Agilent 1100 high performance liquid chromatography (HPLC) analysis system (Agilent, USA), detection conditions were set as follows: Phenomenex-ODS column (150 × 4.60 mm, 5 μm) mobile phase: methanol: water (55:45); flow rate of the mobile phase: 1.0 mL min^{-1}; measured wavelength: 242 nm. The detection limit was 0.05 mg kg^{-1} for each tissue.

2.10. Statistics

Statistical differences were estimated using Student's *t*-test. Pharmacokinetic parameters were obtained using the practical pharmacokinetic program-version 87 (Committee of Mathematic Pharmacology of the Chinese Society of Pharmacology, China).

3. Results and discussion

3.1. Measurement of size and zeta potential

Average particle size and size distribution of ORI nanosuspensions were shown in Fig. 2 and Table 1. The mean size of nanosuspension A (prepared at 1500 bar) was significantly smaller than nanosuspension B (prepared at 1300 bar), which was unnecessary to be confirmed by statistical calculation. This

Table 1
Particle size and zeta potential (means ± S.D., $n = 3$) of ORI nanosuspensions produced at 1500 bar (A) and 1300 bar (B)

Formulation	Size (nm)	Polydispersity index	Zeta potential (mV)
ORI nanosuspension A	103.3 ± 1.5	0.254 ± 0.002	−20.3 ± 0.4
ORI nanosuspension B	897.2 ± 14.2	0.326 ± 0.057	−21.8 ± 0.8[a]

[a] No significant difference with ORI nanosuspension A

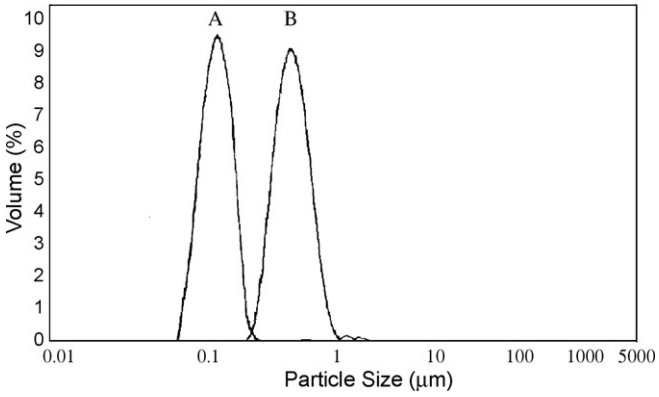

Fig. 2. Particle size distribution of ORI nanosuspension A and B.

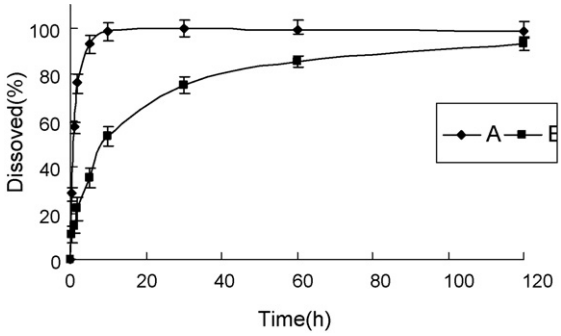

Fig. 3. Dissolution profiles for ORI nanosuspension A (A) and ORI nanosuspension B (B).

indicated that a higher pressure could provide more energy to comminute particles leading to the attainment of a smaller size. Muller and Peters (1998) reported that the particle size of nanosuspension producted by high pressure homogenization was a function of pressure and cycle number, and different particle size could be achieved by adjusting the two procedure parameters. The results in Table 1 showed that there was no statistical significance ($P > 0.05$) in zeta potential between nanosuspension A and B.

3.2. Dissolution studies

Fig. 3. showed the dissolution profiles of freeze-dried powder of ORI nanosuspension A (A) and B (B). It indicated that dissolution velocity of ORI increased with the decreased particle radius. The dissolution rate of nanosuspension A was so fast that 93.2% had dissolved at 5 min and 99.9% had dissolved within 10 min. However, nanosuspension B, approximately eight times larger than nanosuspension A in radius, only dissolved 35.4% at 5 min and 75.2% at 30 min. It could be explained by Noyes–Whitney equation, the increase of surface area (A) and saturation solubility (Cs) due to the reduction of radius could enhance dissolution velocity of poorly soluble compounds (Hintz and Johnson, 1989; Bernhard et al., 1999).

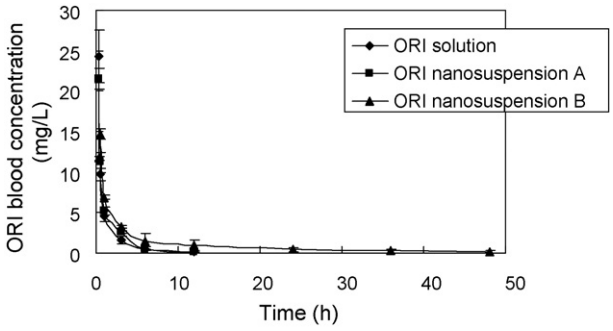

Fig. 4. The mean blood concentration–time curve of ORI in rabbits following i.v. administration of ORI solution, ORI nanosuspension A and B at a dose of 15 mg kg^{-1}. (Data were given as mean ± S.D., $n = 4$).

After every centrifugalization, nanoparticles layer in the nanosuspension B samples withdrawn at predetermined time was resuspended with dissolution medium (saturated by ORI) of appropriate volume and was given a size measurement, the results was showed in Table 2.

3.3. Pharmacokinetic parameters

The ORI blood concentration–time curves after intravenous administration of different formulations in rabbits were shown in Fig. 4. The results indicated that ORI nanosuspension A possessed a drug concentration–time curve similar to ORI solution which meant that ORI was quickly removed from the circulating system. On the contrary, ORI nanosuspension B exhibited a markedly delayed blood clearance. This result showed the influence of particle size on the pharmacokinetic property of ORI nanosuspensions. ORI nanosuspension A, with a mean particle size 103.3 ± 15 nm, might show a rapid dissolution behavior in blood circulation just like the in vitro dissolution curve in Fig. 3. Therefore, the completion of dissolution of ORI nanosuspension A in blood might precede the phagocytosis of reticuloendothelial system (RES). For nanosuspension B, however, the dissolution of nanoparticles was rather slower than A (the results in Table 2 showed that the mean particle size of nanosuspension B after in vitro dissolution for 2 h was about 300 nm), so ORI nanoparticles in nanosuspension B could be recognized and phagocytized by RES in blood circulation. It was reported the uptake of nanoparticles by RES organs following intravenous injection might taken from a few minutes to hours, depending on particle size and composition (Manjunath and Venkateswarlu, 2005). The ORI nanoparticles, uptaken by RES, might dissolve slowly in phagocytic cell and release slowly into blood circula-

Table 2
Particle size and size distribution (means ± S.D., $n = 3$) of ORI nanosuspension B after dissolution in PBS (7.4) for different predetermined times

Time (min)	0.5	1	2	5	10	30	60	120
Size (nm)	852.3 ± 21.7	821.6 ± 31.2	794.3 ± 19.6	731.7 ± 20.3	701.2 ± 25.4	520.8 ± 10.2	475.3 ± 13.8	295.5 ± 18.7
Polydispersity index	0.42 ± 0.084	0.35 ± 0.073	0.32 ± 0.009	0.41 ± 0.003	0.36 ± 0.012	0.41 ± 0.065	0.37 ± 0.007	0.40 ± 0.012

Table 3
Mean pharmacokinetic parameters of ORI after administration of ORI solution, ORI nanosuspension A and B

	AUC (mg L^{-1} h)	MRT (h)	CL (ml h^{-1})	Vc (ml kg^{-1})	$t_{1/2}\alpha$ (h)	$t_{1/2}\beta$ (h)
ORI solution	28.47	1.89	0.54	0.19	0.12	1.74
ORI nanosuspension A	28.77**	2.09**	0.52**	0.29*	0.16*	1.83**
ORI nanosuspension B	50.75	11.78	0.29	0.97	0.91	12.5

Each data was from four rabbits.
* $P < 0.05$, statistical significance compared with ORI solution group.
** $P > 0.05$, no statistical significance compared with ORI solution group.

tion, and remain a longer blood level compared to ORI solution and ORI nanosuspension A. The ORI concentration–time curves for ORI solution, ORI nanosuspension A, and B were fitted with the two-compartment model, and pharmacokinetic parameters were summarized in Table 3. ORI in nanosuspension B was eliminated rather slowly with a larger $t_{1/2}\beta$ (12.5 h) compared with ORI solution and ORI nanosuspension A. The $t_{1/2}\beta$ of ORI nanosuspension A (1.83 h) showed a little longer than that of ORI solution (1.74 h), but $t_{1/2}\beta$ of ORI solution and ORI nanosuspension A did not show significant difference ($P > 0.05$). The AUC of nanosuspension B was significantly larger than ORI solution and ORI nanosuspension A, but there was no significant difference between AUC of ORI solution and ORI nanosuspension A ($P > 0.05$).

3.4. Biodistribution studies

The standard curves having ORI concentrations ranging from 0.1 to 40 μg ml^{-1} exhibited good linearity and correlation coefficients over this concentration range were 0.9972–0.9997 for all measured organs. The serum and tissue ORI concentrations versus time after intravenous administration of ORI solution,

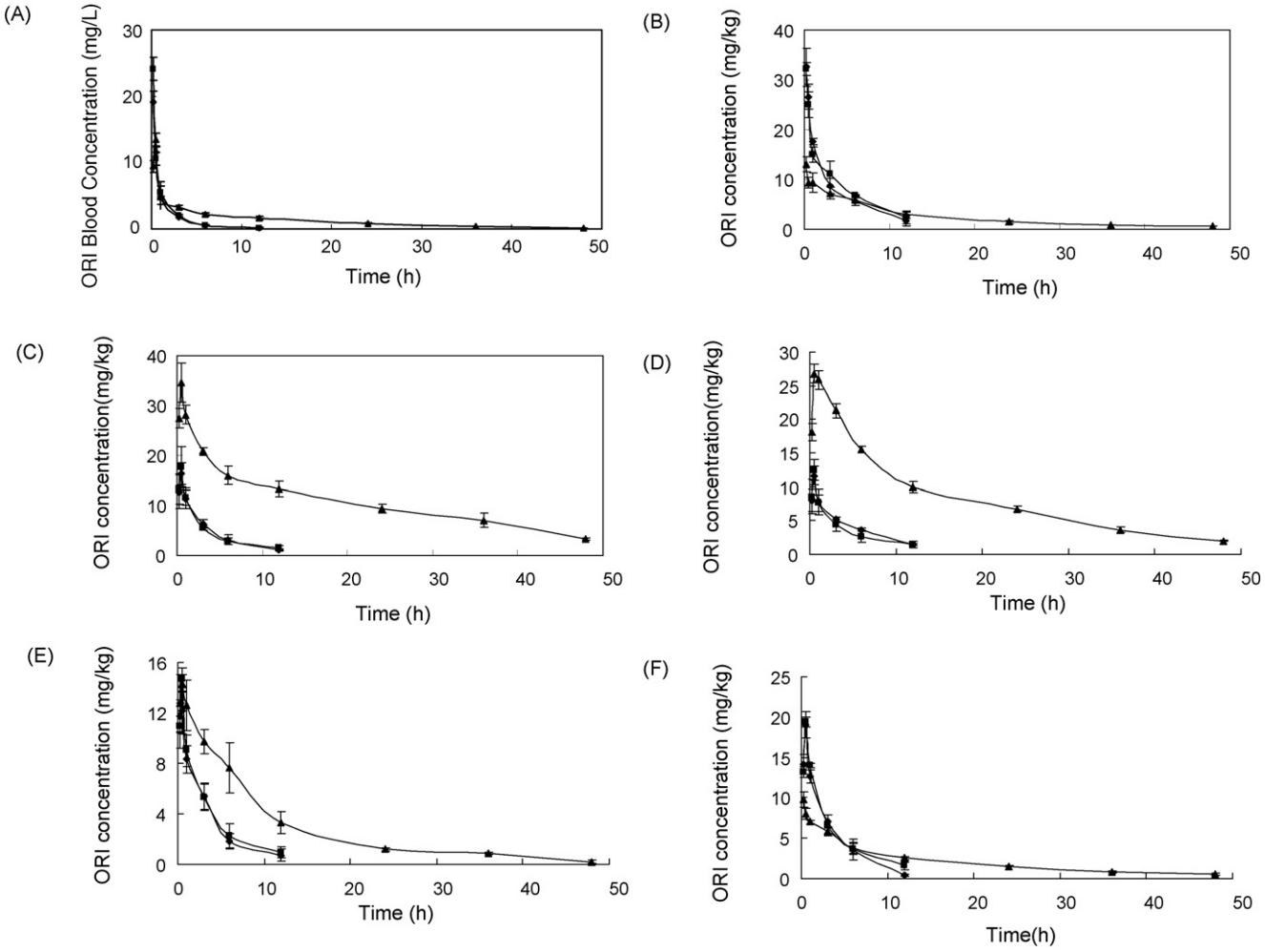

Fig. 5. Mean ORI concentration–time profiles in organs after intravenous administration of ORI solution, ORI nanosuspension A and ORI nanosuspension B to mice with dose 20 mg kg^{-1} (Data were given as mean ± S.D., $n = 4$).: (A) serum; (B) heart; (C) liver; (D) spleen; (E) lung and (F) kidney (♦ ORI solution; ■ ORI nanosuspension A ▲ORI nanosuspension B).

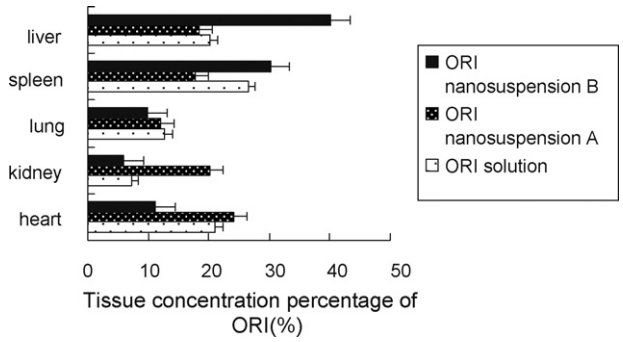

Fig. 6. ORI tissue concentration percentage of liver, spleen, lung, kidney and heart at 12 h following i.v. administration of three formulations.

ORI nanosuspension A and B were shown in Fig. 5 A–F. The results indicated that ORI solution and nanosuspenison A had similar serum kinetic curve which was markedly different from that of nanosuspension B, it is similar to kinetics in rabbits. The level of ORI concentrations of ORI solution and nanosuspension A were markedly lower than that of nanosuspension B in all tissues except the heart and kidney. The higher serum concentration of ORI nanosuspension B in mice might be due to the slower dissolution in blood circulation and slow release of drug from the RES, just like in rabbits. For suspension B, not like suspension A, the ORI nanoparticles did not dissolve rapidly, that was the drug nanoparticles could circulate in the blood circulation as submicron particles for a certain time period. Then the nanoparticles might be recognized as foreign matters and rapidly cleared by phagocytic cells of mononuclear phagocyte system (MPS) which abounded in special tissues and organs, such as liver, lung and spleen. Therefore for nanosuspension B the ORI had a markedly higher concentration compared with ORI solution and nanosuspension A in these organs, meanwhile the drug concentration in heart and kidney decreased. Similar results were reported by Peters et al. (2000). Fig. 6. showed ORI tissue concentration percentage of liver, spleen, lung, kidney and heart at 12 h following i.v. administration of three formulations. For nanosuspension B, 40.12% and 30.22% ORI were distributed in liver and spleen, which is higher than nanosuspension A and solution. $AUC_{(0–12)}$, and $MRT_{(0–12)}$ values of tested organs for three formulations were given in Table 4. $AUC_{(0–12)}$, and $MRT_{(0–12)}$ values of nanosuspension B for all the tissues were found higher than that of solution and nanosuspension A.

Table 4
Average pharmacokinetic parameters ($n=4$) of ORI after intravenous administration of ORI solution, ORI nanosuspension A and B to mice with dose of 20 mg kg^{-1}

Organ	$AUC_{(0–12)}$ (mg L^{-1} h)	$MRT_{(0–12)}$ (h)
ORI solution		
Serum	26.01	1.66
Heart	97.78	3.20
Liver	58.66	3.26
Spleen	51.06	3.98
Lung	43.3	2.99
Kidney	63.5	2.89
ORI nanosuspension A		
Serum	27.2	1.8
Heart	106.3	3.5
Liver	57.01	3.2
Spleen	45.01	3.75
Lung	47.3	3.16
Kidney	66.9	3.45
ORI nanosuspension B		
Serum	63.45	11.46
Heart	122.55	12.3
Liver	517.35	16.8
Spleen	393.2	14.4
Lung	139.9	10.2
Kidney	100.74	13.7

AUC: Area under tissue concentration–time curve; MRT: Mean residence time.

4. Conclusion

It is conspicuous that particle size has marked effects on the pharmacokinetics and tissue distribution of ORI nanosuspension after intravenous administration. ORI nanosuspension A, having a mean size of 103 nm, exhibited a pharmacokinetic property similar to solution due to its rapid dissolution in blood circulation. While ORI nanosuspension B showed high uptake in RES organs after intravenous administration. Therefore, it is important to screen a most appropriate particle size for nanosuspension for intravenous administration in terms of therapeutic purpose. Of course, particle size is just one of factors affecting in vivo fate of nanosuspension. We will investigate other important factors such as surface properties of nanosuspension in the future work.

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