

# Preparation, Pharmacokinetics, and Tissue Distribution Properties of Icariin-Loaded Stealth Solid Lipid Nanoparticles in Mice

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**Abstract: Objective** To evaluate the difference of the pharmacokinetic (PK) and tissue distribution properties in mice administrated with lyophilized icariin stealth solid lipid nanoparticles (Ica-SSLN) modified by polyethylene glycol and icariin control solution (Ica-Sol). Meanwhile, to establish a sensitive, specific, and stable HPLC method for the determination of Ica in mice plasma and various tissues. **Methods** Ica-SSLN was prepared by high temperature melt-cool solidification method. Particle size and Zeta potentials were measured by a ZetaPlus. After iv administration of Ica-SSLN and Ica-Sol at a single dose of 7.46 mg/kg, the blood and tissues including brain, liver, spleen, lung, heart, and kidney were collected at different time points. The obtained concentration from HPLC analysis was statistically treated to determine the PK model and the relevant PK parameters using DAS1.0. Tissue distribution studies of Ica-SSLN were carried out in Kunming mice after iv administration and compared to Ica-Sol. **Results** The characteristic data showed that the mean particle size of Ica-SSLN was  $(50.03 \pm 0.90)$  nm, entrapment efficiency was  $(71.67 \pm 1.09)\%$ , and the particles carried negative charge, Zeta potential was  $(-22.77 \pm 1.89)$  mV. The concentration-time profiles of Ica in mice after iv administrated with Ica-SSLN and Ica-Sol were shown to fit a two-compartment open model. Compared with Ica-Sol, the  $t_{1/2\beta}$  of Ica-SSLN was prolonged by seven times and the AUC was increased by four times. The levels of Ica concentration in the kidney tissues were significantly increased. In addition, compared with Ica-Sol, the relative target efficiency to kidney tissue was 79% and the relative tissue exposure was 16.95. **Conclusion** It demonstrates that Ica-SSLN has selective targeting to kidney tissue and the kidney targeted Ica-SSLN seems to have significant advantages and good development value.

**Key words:** icariin; PEG; pharmacokinetics; stealth solid lipid nanoparticles; tissue distribution

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## Introduction

Icariin (Ica), 2-(4'-methoxyl-phenyl)-3-rhamnosido-5-hydroxyl-7-glucosido-8-(3'-methyl-2-butylenyl)-4-chromanone, is a major constituent of flavonoids isolated from *Epimedium brevicornum* Maxim (Berberidaceae), which is used in traditional Chinese medicine (TCM) to nourish the kidney and reinforce Yang. Ica has many pharmacological effects (Li and Wang, 2008; Liu 2009), such as promoting cerebral blood flow and blood circulation, regulating immunity, antitumor, promoting proliferation and development of osteoblasts, antilipid peroxidation, enhancing the estrogenic activity, protecting neurons from ischemia/

reperfusion damage, preventing and treating atherosclerosis, and so on. It is a promising monomer of Chinese materia medica (CMM). However, Ica has poor oral bioavailability and obvious first-pass effect (Ye, Chen, and Liu, 1999; Qiu, Chen, and Kano, 1999) since its solubility is poor which results in lower intestinal absorption and more difficult to prepare.

Solid lipid nanoparticles (SLN) often have targeting and sustained-releasing effect. The rapid removal of parenterally administered liposomes from circulation by cells of the mononuclear phagocytic system (MPS) significantly impairs the ability of liposome-associated drugs targeting to non-reticuloendothelial

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system (RES) tissues and prevents the liposomes from becoming a good sustained-release system. The lipids derivatized with the hydrophilic polymer polyethylene glycol (PEG) could prolong the circulation of liposomes greatly. Because the reduced recognition and capture by cells of MPS were achieved by steric stabilization of these lipids, this liposome was called sterically stabilized liposome. In this report, we studied the pharmacokinetic (PK) and tissue distribution properties of icariin stealth solid lipid nanoparticle (Ica-SSLN) to investigate the renal targeting characteristics.

## Materials and methods

### Materials

Icariin (110737-200414) and Omeprazole reference substances (100367-200702) were purchased from Tianjin Institute for Drug Control (China). Icariin sample was purchased from Shanxi Huike Science & Technology Development Co. (HK20091018, purity > 98%, China). Sephadex G-50 (50–150  $\mu\text{m}$ , Sigma, USA). Methanol and acetonitrile (chromatographic grade) was purchased from Tianjin Concord Technology Co., Ltd. (China).

### Animals

Kunming mice (SPF grade), weight ( $20 \pm 2$ ) g, were obtained from Tianjin Shanchuanhong Experimental Animal Technology Co., Ltd. (China).

### Preparation of Ica-SSLN

Ica-SSLN was prepared by high temperature melt-cool solidification method. An optimized result of orthogonal design test based on the effect of single factors was described as follows. Ica (4.7 mg), cholesterol (0.1 g), PEG2000 (0.06 g), and lecithin (0.6 g) were dissolved in 10 mL absolute ethanol. The mixture was heated to 70 °C to produce a nearly clear melt lipid phase. An aqueous phase was prepared by dissolving Tween 80 (0.35 g) in 65 mL distilled water. A pre-dispersion of the melt lipid in the aqueous phase, which was previously heated to approximately the same temperature as the lipid melt, was prepared by magnetic stirring. The pre-mixture was continually heated until 5 mL remained due to the evaporation of ethanol and water. Then the mixture was quickly dispersed into 10 mL distilled water at the temperature of 0 °C and continually stirred for 2 h under the temperature (Zeng,

Zhao, and Han, 2009; Date, Joshi, and Patravale, 2007; Yang, Sun, and Liu, 2008; Duan, Yu, and Zhang, 2002; Zhang, Qin, and Li, 2008).

Icariin control solution (Ica-Sol) was prepared by dissolving Ica (20 mg) in a 50 mL mixture of PEG400 and normal saline (35:65).

### Characterization of Ica-SSLN

**Particle size and Zeta potential analyses** Size and Zeta potentials of Ica-SSLN suspension were measured by a Zeta Potential & Size Analyzer (ZetaPlus, Brookhaven, USA). In brief, Ica-SSLN suspension was prepared by diluting 10 times and put into the specific sample cell. Then the size and Zeta potentials were tested. Zeta potential was estimated on the basis of electrophoretic mobility under an electric field.

**Determination of encapsulation efficiency** The encapsulation efficiency of the system was determined by measuring the concentration of free drug separated by a Sephadex column chromatography. The Sephadex column chromatography was filled with Sephadex gel G-50 and swelled overnight using distilled water. The prepared Ica-SSLN (1.0 mL) was splashed into the column and eluted with distilled water, keeping at a flow rate of 1.0 mL/min. The eluent (55–150 mL) contained the free drug and the concentration of Ica was analyzed by HPLC on a Dikma-C<sub>18</sub> column (250 mm  $\times$  4.6 mm, 5  $\mu\text{m}$ ) at the wavelength of 270 nm, using acetonitrile-water (30:70) as mobile phase.

### PK study and tissue distribution

**PK study** Before experiment, mice were randomly assigned to 12 groups with seven mice in 1–6 groups and five mice in 7–12 groups. Mice in 1–6 groups were administrated with Ica-SSLN (7.46 mg/kg) by injection through the lateral tail vein, while the other groups were administrated with Ica-Sol. Then the mice blood of Ica-SSLN groups was collected at specified time intervals of 0.25, 0.5, 1, 2, 4, 8, and 12 h through the orbit vein, while the other groups at the specified time intervals of 0.08, 0.25, 0.67, 1, and 1.5 h. Plasma samples were collected by the centrifugation at 4000 r/min for 15 min and stored at –20 °C until analysis. The plasma samples were extracted with methanol prior to analysis by HPLC. Briefly, plasma (50  $\mu\text{L}$ ) was pipetted into a 1.5 mL centrifuge tube, and 30  $\mu\text{L}$  of Omeprazole (1.3  $\mu\text{g/mL}$ ), internal standard (IS), and

100  $\mu$ L of acetonitrile were added and mixed well. Then the mixture was vortexed for 3 min followed by centrifugation at 10 000 r/min for 10 min. The supernatant was transferred to a new centrifuge tube and dried in vacuum oven. The residue was reconstituted in 100  $\mu$ L of methanol and 20  $\mu$ L were injected into HPLC column. The ratios of peak areas of drug to IS were calculated (Duan, Yu, and Zhang, 2002; Sun, Zhou, and Liu, 2007; Zhang, Qin, and Li, 2008).

**Tissue distribution** After collecting the blood from orbit vein, mice were lightly anesthetized and perfused with normal saline (NS) through heart. The tissues of interest were removed and weighed accurately. Afterwards, the tissue was taken for homogenization, for every 0.2 g of tissue, 1 mL of NS, with the help of a tissue homogenizer. Then 200  $\mu$ L of tissue homogenates were taken to be processed similarly as that of plasma samples and analyzed by HPLC (Ye, Chen, and Liu, 1999; Sun, Zhou, and Liu, 2007; Yang, Feng, and Zhu, 2007; Bi, Huang, and Zhang, 2007; Tong, Huang, and Yao, 2002).

#### HPLC analysis on Ica in plasma and tissue

Concentration of Ica in plasma and tissue samples was measured by HPLC. The analytical column was Dikma-C<sub>18</sub> column (200 mm  $\times$  4.6 mm, 5  $\mu$ m). Ica was monitored at the wavelength of 270 nm. The mobile phase was acetonitrile-water (27:73) at a flow rate of 1.0 mL/min with 20  $\mu$ L for HPLC analysis. The detection limit of Ica was 5.3 ng/mL. Intra- and inter-day variations were less than 10%. Mean recovery rates of each tissue exceeded 70%, RSD < 10%.

#### Data analyses

Concentration of Ica in plasma and tissues obtained through HPLC analysis was averaged for mean values. These results were used to determine the PK model and the relevant PK parameters using Drug and Statistics Program (DAS, 1.0). Part of the obtained PK parameters was analyzed statistically by Student *t*-test, using statistical package for social sciences SPSS 11.5. Data of tissue concentration were calculated to evaluate the target ability following the interrelated formulas and parameters given below.

## Results

### Characterization of Ica-SSLN

The characteristic data showed that the mean

particle size of prepared Ica-SSLN was (50.03  $\pm$  0.90) nm, entrapment efficiency was (71.67  $\pm$  1.09)%, Zeta potential was (-22.77  $\pm$  1.89) mV, and the particles carried negative charge. When the Ica-SSLN was prepared to lyophilized powder with 3% mannitol as a protection, the mean particle size of prepared Ica-SSLN was (359.53  $\pm$  6.71) nm, entrapment efficiency was (74.00  $\pm$  1.45)%, and the Zeta potential was (33.79  $\pm$  2.96) mV.

### PK study

In the early studies, evidence became available indicating that hydrophobic particles were removed from the circulation rapidly, while more hydrophilic particles remained in the bloodstream for longer period of time, which was followed by suggesting that particles with hydrophobic surface were rapidly opsonized by certain plasma proteins as soon as they were introduced into the bloodstream (Storm, Belliot, and Daemenb, 1995).

In this study, the plasma concentration observed (at 15 min) is lower for Ica-Sol than Ica-SSLN following iv administration, which possibly results from the poor solubility and subsequent distribution and elimination due to its particular hydrophilic structure. In case of Ica-SSLN, the covering colloidal particles with hydrophilic, nonionic polymers, and PEG coatings, are used to obtain the reduced affinity for MPS.

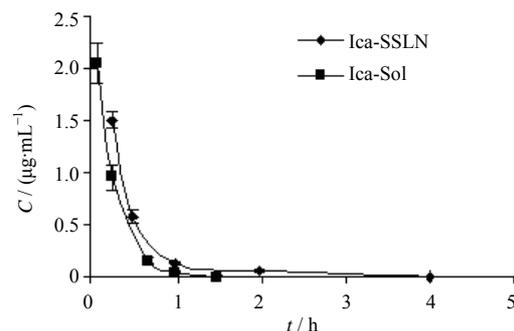
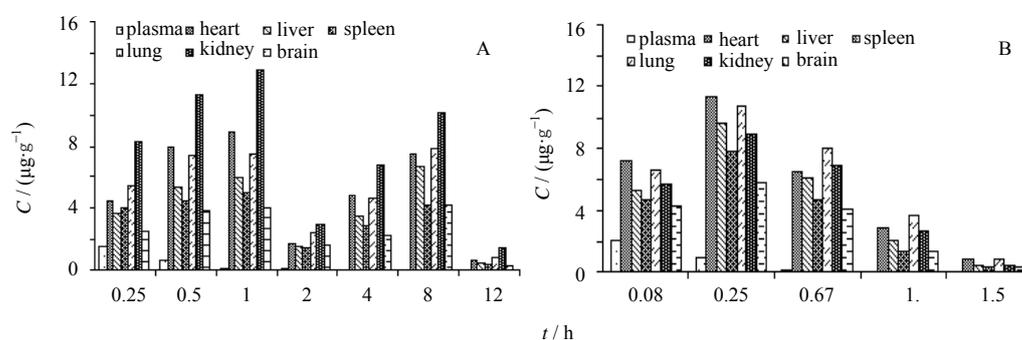
The comparative average PK parameters are reported in Table 1 and the mean concentration-time curves of Ica-SSLN and Ica-Sol are shown in Fig. 1. The PK results indicated that the Ica-SSLN and Ica-Sol followed the open two-compartment model after iv administration. AUC<sub>0- $\infty$</sub>  and *t*<sub>1/2 $\beta$</sub>  of Ica-SSLN were higher than those of Ica-Sol (*P* < 0.05).

### Tissue distribution

A comparative biodistribution study with Ica-SSLN and Ica-Sol was performed to investigate the distribution profile of the drug when administrated in the liposomal formulation. The concentration of Ica in selected tissues at several time points after iv administration is shown in Fig. 2. It was observed that after iv administration of Ica-Sol, the concentration of Ica in blood was lower than that in other tissues, implying that Ica could be quickly purged from blood and distributed extensively into tissues. However, the concentration of Ica in blood of SSLN formulation

**Table 1** Comparative plasma PK parameters after iv administration of Ica-SSLN and Ica-Sol ( $n = 6$ )

Parameters	Unit	Ica-SSLN	Ica-Sol
$\alpha$	$\text{h}^{-1}$	4.39	13.56
$\beta$	$\text{h}^{-1}$	0.53	3.50
$k_{10}$	$\text{h}^{-1}$	3.51*	5.88
$k_{12}$	$\text{h}^{-1}$	0.75*	4.30
$k_{21}$	$\text{h}^{-1}$	0.67	6.87
$t_{1/2\alpha}$	h	0.16*	0.11
$t_{1/2\beta}$	h	1.40*	0.21
CL	$\text{L}\cdot\text{h}^{-1}$	6.07*	10.69
$\text{AUC}_{0-\infty}$	$\text{mg}\cdot\text{L}^{-1}\cdot\text{h}$	3.34*	0.82
$\text{MRT}_{0-\infty}$	h	0.68	0.26

\* $P < 0.05$  vs Ica-Sol**Fig. 1** Mean concentration-time curves of Ica in plasma after iv administration of Ica-SSLN and Ica-Sol ( $n = 6$ )**Fig. 2** Concentration of Ica-SSLN (A) and Ica-Sol (B) distributed into each tissue at several time points

was higher than that of the solution formulation. This result showed that the Ica-SSLN was able to prolong the circulation time of imaging agent in blood. The concentration of Ica of SSLN formulation in kidney was significantly higher than that in other tissues. The results demonstrated that SSLN formulation has excellent ability targeting the kidney.

#### Evaluation of target ability

Target ability of drug used in targeting drug delivery system was an important standard. Therefore, relative tissue exposure ( $Re$ ), target efficiency ( $Te$ ), target index ( $TI$ ), and relative target efficiency ( $Rte$ ) were put forward to evaluating the target ability of Ica-SSLN (Sun, Zhou, and Liu, 2007; Yang, Feng, and Zhu, 2007; Bi, Huang, and Zhang, 2007; Tong, Huang, and Yao, 2002).

#### $Re$

$Re$  was described by the following equation:  $Re = \text{AUC}_n / \text{AUC}_s$ . Where  $n$  expresses SSLN and  $s$  expresses solution. AUC means the area under the concentration-time curve of each tissue.

If the value of  $Re$  exceeds 1, the tissue is exposed to the drug at a greater extent targeted by the liposomes.

In our study, the value of  $Re$  for kidney was 16.95, which was obviously higher than 1, demonstrating that the uptake of Ica by kidney was significantly increased by the entrapment in liposomes.

#### $Te$

$Te$  is a good indication for the relative efficiency of two delivery systems to one tissue, but it does not provide any information about the targeting efficiency of a given delivery system to a specific tissue. This information could be obtained by  $Te$ , which was defined as  $Te = (\text{AUC}_{0-\infty})_p / (\text{AUC}_{0-\infty})_s$ . In the formula,  $(\text{AUC}_{0-\infty})_p$  means the AUC of target tissue of the preparation and  $(\text{AUC}_{0-\infty})_s$  means the total AUC of the preparation.  $Te$  demonstrates the selectivity of a delivery system towards the target tissue. A greater  $Te$  represents a higher targeting towards a given tissue.  $Te$  of tested organs was given in Table 2.  $Te$  values of kidney for Ica-Sol were lower than those for Ica-SSLN, indicating the  $Te$  of Ica-Sol was low.

#### $TI$ and $Rte$

$TI$  was described by the following equation:  $TI = \rho_{\text{max},n} / \rho_{\text{max},s}$  where  $\rho_{\text{max}}$  means the maximum serum or tissue concentration.  $Rte$  was described by the following

**Table 2** *Te* of Ica-SSLN and Ica-Sol

Tissues	<i>Te</i>	
	Ica-Sol	Ica-SSLN
plasma	1.80	0.78
heart	19.11	19.86
liver	16.34	14.20
spleen	13.49	11.24
lung	22.61	16.10
kidney	15.80	28.24
brain	10.85	9.58

equation:  $Rte = (Te, n - Te, s) / Te, s$ . *TI* and *Rte* demonstrate the changes of target ability in tested tissues. Greater *TI* and *Rte* value represent more obvious changes of target ability towards a given tissue. *TI* and *Rte* of tested tissues are given in Table 3. These results indicated that Ica-SSLN increased the kidney targeting obviously compared with Ica-Sol.

**Table 3** *TI* and *Rte* of each tissue

Tissues	<i>TI</i>	<i>Rte</i> / %
plasma	0.74	-57
heart	0.78	4
liver	0.69	-13
spleen	0.65	-17
lung	0.73	-29
kidney	1.49	79
brain	0.70	-12

## Discussion

Recent study showed that Ica has a wide range of pharmacological activities. However, its clinical application is limited by its poor solubility and low bioavailability due to first pass metabolism. In this study, we used liposomal vesicles to encapsulate Ica to enhance its water solubility. In addition, PEG was used as the targeting surface modification agent of Ica-SLN to reduce the removal of nanoparticles from circulation by cells of MPS.

The PK properties in the blood were significantly changed after iv administration of the entrapped drug by SLNs, which was featured by the increase of AUC as well as prolongation of half-life of Ica in blood, etc. These results agreed with those in the literature. The characteristics of Ica lead to its rapid blood clearance. The clearance rate of Ica when encapsulated by SSLN decreased likely because the external PEG of SLNs prevented the interaction with opsonins in blood. The mechanism of action was explained by the concept of

steric stabilization (Storm, Belliot, and Daemenb, 1995). The higher concentration of Ica in blood provided more opportunities for Ica to enter into kidney. The kidney uptake of Ica was significantly increased by incorporating PEG into SLNs. This mightily results from adherence to the local capillary endothelium rather than engulfment by cells.

The PK results showed that Ica in the SSLN formulation exhibited the rapid distribution and high kidney uptake, and could be a potential imaging agent for kidney diseases.

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