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Pharmacokinetics, tissue distribution and relative bioavailability of puerarin solid lipid nanoparticles following oral administration

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ABSTRACT

Puerarin has various pharmacological effects; however, poor water-solubility and low oral bioavailability limit its clinical utility. A delivery system of solid lipid nanoparticles could enhance its oral absorption. The objective of this study was to investigate the pharmacokinetics, tissue distribution and relative bioavailability of puerarin in rats after a single dose intragastric administration of puerarin solid lipid nanoparticles (Pue-SLNs). The puerarin concentrations in plasma and tissues were determined by rapid resolution liquid chromatography electrospray ionization–tandem mass spectrometry. The C_{max} value of puerarin after the administration of Pue-SLNs was significantly higher than that obtained with puerarin suspension (0.33 ± 0.05 μg/mL *vs.* 0.16 ± 0.06 μg/mL, $P < 0.01$). The T_{max} value after the administration of the Pue-SLNs was significantly shorter than that after puerarin suspension administration (40 ± 0 min *vs.* 110 ± 15.49 min, $P < 0.01$). The $AUC_{0\to t}$ values of puerarin were 0.80 ± 0.23 mg h/L, and 2.48 ± 0.30 mg h/L after administration of the puerarin suspension and Pue-SLNs, respectively. Following administration of the Pue-SLNs, tissue concentrations of puerarin also increased, especially in the target organs such as the heart and brain. These data suggest that SLNs are a promising delivery system to enhance the oral bioavailability of puerarin.

1. Introduction

Puerarin (7,4′-dihydroxyisoflavone-8β-glucopyranoside) is a major active ingredient in the traditional Chinese medicine, *Puerariae radix*, which comes from the kudzu root (*Pueraria lobota* (Wild) Howe). Puerarin is widely prescribed for patients with cardiocerebrovascular diseases in China. It has been reported that puerarin has therapeutic effects on hypertension (Song et al., 1988), cerebral ischemia (Gao et al., 2009), myocardial ischemia (Zhang et al., 2006), diabetes mellitus (Hsu et al., 2003) and arteriosclerosis (Yan et al., 2006). The molecular mechanism underlying these clinical benefits is believed to involve puerarin's ability to act as a scavenger of reactive oxygen species and antioxidants (Han et al., 2007). In diet-induced hypercholesterolemic rats, puerarin markedly reduces the total cholesterol by the promotion of cholesterol and bile acid excretion in liver (Yan et al., 2006). Puerarin also improves endothelial function by inhibiting cellular factors, such as adhesion molecules (Hu et al., 2010) and C-reactive proteins (Yang et al., 2010), and enhancing the expression of nitric oxide synthase (Yan et al., 2006). In addition, as a phytoestrogen, puerarin exhibits weak estrogenic activity *in vivo* (Malaivijitnond et al., 2010). Although puerarin has protective effects on cardiocerebrovascular diseases, owing to the short elimination half-life of puerarin in human beings, intravenous administration of frequent and high doses may be needed, possibly leading to severe and acute side effects. Oral administration is the most preferred route regarding the conventional drug delivery system, especially for the treatment of chronic diseases. However, puerarin is hardly water-soluble, and its absorption *in vivo* is very poor after oral administration (Quan et al., 2007), which diminishes its therapeutic effects. No oral formulations are currently available, thus, the clinical application of puerarin is greatly restricted, and oral formulation with improved absorption of puerarin is highly desired.

Solid lipid nanoparticles (SLNs), a submicron nanoparticle drug delivery system, have increased attention. The particle diameters range from 10 to 1000 nm. SLNs are prepared from solid (at room and body temperature) physiological lipids by either high pressure homogenization or by a mciro-emulsion technique, resulting in a solid particle matrix that can be loaded with variety of drugs. The advantages of SLNs include better biocompatibility, bioavailability and safety (Souto and Muller, 2010). SLNs can be prepared for multiple routes of administration including oral administration

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(Mehnert and Mader, 2001), and their technology could increase the saturation solubility and dissolution rate of the drugs (Kocbek et al., 2006). Accumulated data indicate that the oral bioavailability of poorly hydrophilic drugs can be enhanced when they are incorporated into SLNs.

Recently, Li et al. (2010) report the preparation of puerarin loaded solid lipid nanoparticles (Pue-SLNs) and its crystallinity state. Similarly, we successfully prepared Pue-SLNs for oral administration. Its average particle size was 160 nm with a zeta potential of −35.43 mV (Li et al., 2007). The aim of our work is to study the pharmacokinetics, tissue distribution and relative bioavailability of puerarin in rats after single dose intragastric administration of Pue-SLNs in comparison with puerarin suspension.

2. Materials and methods

2.1. Chemicals

Puerarin was purchased from Guangdong Greatsun Biochemical Pharmaceutical Co., Ltd, (Guangzhou, China). Lyophilizing Pue-SLNs powder was provided by the Drug Research Center of Guangzhou Medical University. Tectoridin (internal standard) was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol and acetonitrile (Merck, Darmstadt, Germany) were of HPLC grade, and acetic acid was purchased from Acros Organics (Geel, Belgium). Drug-free rat plasma and tissue were collected from healthy Sprague–Dawley rats of both sexes and stored at −20 °C. Ultrapure water was prepared using a Millipore water- purification system (Millipore, MA, USA).

2.2. Preparation of Pue-SLNs

Pue-SLNs were prepared using the solvent injection method (Li et al., 2007). Briefly, puerarin (20 mg), monostearin (150 mg), and soya lecithin (150 mg) were mixed in a 10 mL solvent consisted of methanol (1 mL) and ethanol (9 mL). The mixture was sonicated to obtain the organic phase. And the aqueous phase was 0.5% poloxamer 188 (W/V) in ultrapure water and kept at 75 ± 2 °C. The organic phase was injected into the hot aqueous phase under mechanical agitation and the resulting solution was kept at the same temperature with the same agitation speed to remove the organic solvent. The condensed solvent (approximate 10 mL) was then injected into 0.5% poloxamer 188 (W/V) in ultrapure water at 0–2 °C to form SLNs. The free drug in Pue-SLNs suspension was separated from Pue-SLNs by ultrafiltration. The mean particle size of Pue-SLNs was 160 nm with a zeta potential of −35.43 mV. To prepare the lyophilized Pue-SLNs powder, mannitol (final concentration is 10%, W/V) was added to the Pue-SLNs dispersion, and the solution was filled into glass vials. After frozen at −45 °C for 8 h, they were quickly moved to a freeze-dryer with a temperature of −50 °C and a vacuum of 30 mm Hg; the temperature was increased at rate of 5 °C/h to −25 °C, maintaining for 24 h. Consequently, the temperature was increased at rate of 5 °C/h to 30 °C, maintaining for 24 h. At last, the Pue-SLNs lyophilized powder was obtained. Before administration to rats, the Pue-SLNs lyophilized powder was suspended in ultrapure water. There was no significant difference in morphology, mean particle size, or zeta potential before and after lyophilization.

2.3. Animals

Specific-pathogen-free Sprague–Dawley rats, weighing 180–220 g, were obtained from Guangdong Medical Laboratory Animal Center (Guangzhou, China). The rats were kept in an environmentally controlled room (temperature 25 ± 2 °C, humidity 60 ± 5%, 12/12 h dark/light cycle) for one week prior to experiments. They were fed a soy-free custom diet (Guangdong Medical Laboratory Animal Center, Guangzhou, China) and water *ad libitum*. All rats were fasted overnight before the experiments, and all animal handling and treatments followed the EC Directive 86/609/EEC. The animal use and care protocol was reviewed and approved by the ethics committee of the second affiliated hospital of Guangzhou Medical University.

2.4. Drug administration and sampling

Twelve rats (six females and six males) were divided into the following two groups: the Pue-SLNs group and puerarin suspension group. Animals in the former group were administered a single intragastric dose of lyophilizing Pue-SLNs powder suspended in water (equivalent to 20 mg/kg of puerarin). Those in the latter group were administered 20 mg/kg of puerarin suspended in 0.5% carboxymethyl cellulose sodium (CMC-Na). Serial blood samples (approximately 0.3 mL) were obtained from the jugular vein at 0, 10, 20, 30, and 40 min, and at 1, 2, 3, 4, 6, 8, 12, 18 and 24 h post-dosing under anesthesia with pentobarbital sodium (30 mg/kg). For rats in the puerarin suspension group, serial blood samples were obtained at 0, 20, and 40 min, and at 1, 1.5, 2, 3, 4, 6, 8 and 12 h after dosing. Beginning at 2 h post-dosing, an equal volume of sterile physiological saline (0.3 mL) was injected. We then centrifuged the blood at 13,000 g for 3 min and isolated the plasma. All samples were stored at −20 °C until later analysis.

To study tissue distribution, 48 rats (24 females and 24 males) received the same dosing as described above and six rats for each formulation were sacrificed at 0.5, 1, 2.5 and 6 h post-dosing to collect samples from the heart, lungs, liver, spleen, kidneys and brain. All of the tissue specimens were rinsed with sterile physiological saline and immediately stored at −20 °C until later analysis.

To study the excretion of puerarin after administration, 6 rats (3 females and 3 males) were administered puerarin suspension or Pue-SLNs at an interval of 1 week with same dosing as described above. All urine and feces for each drug formulation were collected separately for the rats that were housed in individual metabolism cages. Urine and feces were collected quantitatively at the following intervals: predose, 0–12 h, 12–24 h. Feces were frozen immediately after drying at the end of each collection interval. The volume of the urine and weight of the fecal samples were recorded and stored at −20 °C until later analysis.

2.5. Plasma sample treatment

10 μL of 35% methanol–water (V/V) solution and 10 μL tectoridin solution were added to 100 μL of rat plasma, respectively. Next, 200 μL of a mixture of methanol and acetonitrile (90:10, V/V) was added to precipitate protein, and the samples were vortexed for 5 min and centrifuged at 13,000 g for 10 min. The supernatant was centrifuged for 5 min at 13,000 g. Samples were analyzed by LC–MS/MS immediately following preparation.

2.6. Tissue sample treatment

Tissue specimens were thawed and 0.2 g of the samples were cut into small pieces, and 10 μL of 35% methanol–water solution and 10 μL of tectoridin solution were added, and homogenized in 1 mL of methanol and acetonitrile (90:10, V/V) mixture. The left-over pieces on the homogenizer were washed with a small volume of methanol after each homogenization and the remaining substance was transferred to the corresponding samples. Calibration standards and quality control samples for puerarin analysis were prepared by adding known amounts of puerarin to normal tissues that were homogenized in the same manner as above. The

homogenates were centrifuged for 10 min at 13,000 g, and the supernatant was evaporated until dry using a stream of N_2. The residue was dissolved in 200 μL of 35% methanol and centrifuged at 13,000 g for 5 min, and then immediately analyzed by LC–MS/MS. Puerarin concentrations in the tissue were calculated based on 0.2 g of tissue. Blank samples of all matrices were also extracted to ensure the absence of endogenous interfering peaks.

2.7. LC–MS/MS analysis

LC–MS/MS analyses of rat plasma samples were performed using an Agilent 1200 series RRLC and 6330 Ion Trap system consisting of a vacuum degasser, a binary pump, an autosampler, a column thermostat and a 6330 Ion Trap XCT Ultra mass spectrometer (Agilent Technologies, CA, USA). Chromatography was recorded on a Zorbax XDB C18 reversed-phase column (4.6 × 100 mm, particle size 1.8 μm) (Agilent Technologies, CA, USA), preceded by a guard column filled with C18 (Zorbax XDB, particles size 1.8 μm). The injection volume was 2 μL, and the column temperature was set at 30 °C. The mobile phase consisted of a mixture of methanol and 0.05% acetic acid in water (35:65, V/V). Elution was performed at flow rate of 0.3 mL/min from 0 to 5.4 min and 0.6 mL/min from 5.41 to 12 min.

Column effluence was monitored by a mass spectrometer operated in ESI positive ionization mode. Nitrogen was used as a nebulizing and drying gas at 350 °C, with a pressure of 40 psi and a flow rate of 10 L/min. The capillary voltage was set at −4500 V, and multiple reaction monitoring (MRM) was used to perform mass spectrometric quantification of puerarin and tectoridin. This was created by monitoring the precursor ion to product ion transition from mass-to-charge (*m/z*) of either 417/399 (puerarin) or 463/301 (tectoridin) at two different segments. The ion trap parameters followed the smart parameter setting, and the number of ions stored in the ion trap was under control. The target numbers were 50,000 for *m/z* 417/399 (puerarin) and 500,000 for *m/z* 463/301(tectoridin), and showed maximum accumulation time of 200 ms. The scanning *m/z* range was from 250 to 470 with a scanning speed of 26,000 *m/z* per second. The RRLC and MS/MS systems were controlled by Chemstation version B.01.03 SR2 and Ion Trap software 6.1, respectively.

2.8. Analytical method validation

Following the bioanalytical method validation (FDA, 2001), the analytical method was validated to demonstrate the specificity, recovery, linearity, accuracy and precision of measurements and stability of samples.

Specificity was established by the lack of interference peaks at the retention time for puerarin and the internal standard.

Recovery was determined by comparing the peak area obtained from either the extracted plasma or tissue samples with the peak area obtained by the direct injection of the corresponding spiked standard solutions in 35% methanol in water. Three different concentrations of puerarin (50, 200, and 1000 ng/mL in plasma and 25, 100, and 500 ng/g in tissue samples) were measured.

Linearity was tested at seven concentration levels covering a range of 10–2000 ng/mL (10, 50, 100, 200, 500, 1000, and 2000 ng/mL) in plasma and 5–1000 ng/g (5, 25, 50, 100, 250, 500, and 1000 ng/g) in tissue samples. The calibration curves were established by plotting the peak area ratio of puerarin to internal standards versus the puerarin concentration. The regression parameters of the slope, intercept and correlation coefficient were calculated by linear least-squares regression.

Accuracy and precision were determined in six replicates of 50, 200 and 1000 ng/mL of puerarin in blank rat plasma and 25, 100, and 500 ng/g in blank tissue samples, which were prepared and analyzed on three consecutive days. Accuracy was determined by comparing the calculated concentration to the added concentration, using calibration curves. The intra- and inter-day precisions were assessed by calculating the relative standard deviation (RSD). The mean value of accuracy should be within 15% of the actual value except when at a lower limit of quantification (LLOQ), where it should not deviate by more than 20%. The RSD determined at each concentration level should not exceed 15% of the coefficient of variation (CV) except for the LLOQ, which should not exceed 20% of the CV.

Puerarin stability in rat plasma was examined at room temperature and −20 °C. The storage stability at −20 °C for two months was also evaluated, and triplicate samples of 50 and 1000 ng/mL of puerarin in plasma and 25 and 500 ng/g in tissue samples were determined, respectively.

2.9. Pharmacokinetic data analysis

Pharmacokinetic parameters of puerarin were calculated using non-compartmental methods (Kinetica 4.4.1, Thermo Fisher Scientific Inc., MA, USA).

2.10. Excreta sample treatment and analysis

A 0.1 g aliquot of each feces sample was thawed, then 1 mL of a mixture of methanol and acetonitrile (90:10, V/V) was added for homogenization. The samples were centrifuged for 10 min at 13,000 rpm. The supernatant was evaporated to dryness under N_2. The residue was then dissolved in 200 μL of the mobile phase and centrifuged at 13,000 rpm for 5 min after vortex mixing for 1 min. The final supernatant was analyzed immediately.

An aliquot of 100 μL of each urine samples was thawed, and 200 μL of a mixture of methanol and acetonitrile (90:10, V/V) was added, vortex mixed for 3 min, and centrifuged (13,000 rpm) for 10 min. The separated supernatant was analyzed immediately.

The prepared excreta samples were analyzed by the method of HPLC with fluorescence detection, which we reported previously (Luo et al., 2009). Briefly, analysis was performed by HPLC using an Agilent 1100 series HPLC system, consisting of a vacuum degasser, a quaternary pump, an autosampler, a column thermostat and a fluorescence detector. Separation was achieved using a Venusil XBP C18 reversed-phase column (250 mm × 4.6 mm, 5 μm particle size). The column temperature was set at 30 °C. The mobile phase consisted of a mixture of methanol, acetonitrile and 50 mM ammonium acetate in water (13:8:79, V/V/V) with a flow rate of 1 mL/min. The fluorescence detector was set at excitation wavelength of 250 nm, emission wavelength of 480 nm and PMT-gain of 11. The injection volume was 10 μL. The HPLC system was controlled by Chemstation version A.10.02. The retention time was 12.3 min for puerarin. The method was demonstrated to be selective and sensitive with a the limit of quantification of 24 ng/mL in urine and 33 ng/mL in fecal sample, and a good linear response was observed over a range of 0.3125–240 μg/mL (or μg/g) (correlation coefficient > 0.999). The accuracy was 94.65–108.9% in urine sample and 99.4–109.8% in fecal sample, and the precision was less than 10%.

2.11. Statistical analysis

The data were presented as mean ± SD, and the Student's *t*-test was used to analyze differences between both groups. A *P*-value < 0.05 or 0.01 was considered significant.

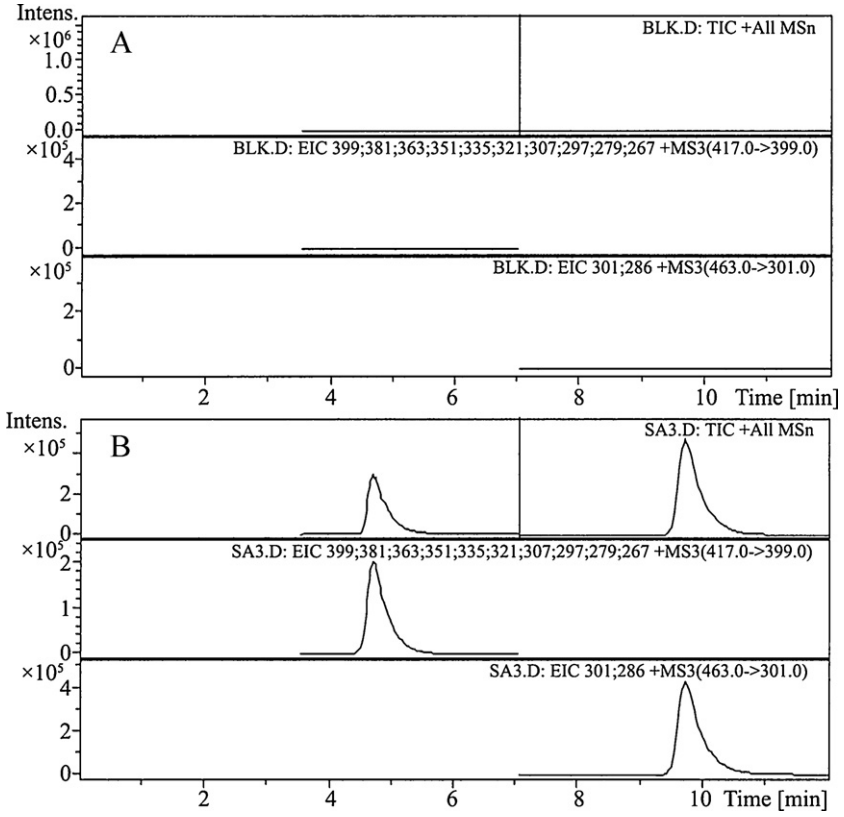

Fig. 1. Representative MRM chromatograms of puerarin (*m/z* 417/399) and internal standard (tectoridin, *m/z* 463/301). A: blank rat plasma; B: rat plasma taken at 30 min after a single intragastric administration of Pue-SLNs (20 mg/kg). Retention time: puerarin = 4.8 min; tectoridin (internal standard) = 9.8 min.

3. Results

3.1. Method validation

The resulting chromatograms were essentially free from endogenous interferences. Representative chromatograms were shown in Fig. 1, including a blank plasma sample (Fig. 1A) and a plasma sample obtained 30 min after the intragastric dosing of 20 mg/kg of Pue-SLNs (Fig. 1B). The typical retention time for puerarin and the internal standard was 4.8 min and 9.8 min, respectively, and the peak shapes were satisfactory and suitable for quantitative analysis.

The recoveries of puerarin from rat plasma and tissue samples were acceptable (Table 1). The results of linear regression analysis are listed in Table 2 and showed that the correlation coefficients of the calibration curves for all sample types were greater than 0.99.

Table 1
Recoveries of puerarin from plasma and tissue samples (mean ± SD, $n = 3$).

	Low concentration (%)	Medium concentration (%)	High concentration (%)
Plasma	102.45 ± 4.56	99.67 ± 2.03	98.72 ± 1.95
Liver	85.23 ± 6.34	93.15 ± 4.32	92.35 ± 8.26
Spleen	86.54 ± 5.67	90.12 ± 7.18	94.51 ± 4.62
Kidney	90.13 ± 5.95	93.18 ± 7.26	91.35 ± 9.24
Heart	82.92 ± 1.58	87.17 ± 4.47	85.46 ± 3.94
Lung	87.23 ± 5.81	91.15 ± 7.53	92.41 ± 7.53
Brain	87.23 ± 4.82	89.33 ± 5.63	91.76 ± 7.57

The low concentration indicates 50 ng/mL of puerarin in plasma or 25 ng/g in tissues; medium concentration indicates 200 ng/mL of puerarin in plasma or 100 ng/g in tissues; high concentration indicates 1000 ng/mL of puerarin in plasma or 500 ng/g in tissues.

The results for accuracy and precision (Table 3) indicated that the method was reliable and reproducible.

Puerarin was stable in methanol and in rat plasma for at least two months when stored at −20 °C. Prepared samples were stable for 24 h at room temperature, and puerarin was stable after three freeze–thaw cycles in rat plasma.

3.2. Pharmacokinetic analysis and bioavailability

The mean plasma concentration–time profiles for the two formulations are presented in Fig. 2. Pharmacokinetic parameters of puerarin were determined using non-compartmental analysis, and the pharmacokinetic parameters are summarized in Table 4.

As shown at all time points in Fig. 2, the puerarin plasma concentrations were higher in rats administered with Pue-SLNs suspension than those administered with puerarin suspension. The C_{max} value of puerarin in the Pue-SLNs was significantly higher than that obtained with puerarin suspension ($P < 0.01$). The T_{max} in animals treated with Pue-SLNs was significantly shorter than

Table 2
Calibration curves of purarin in spiked rat biological matrices.

	Regression equation	r	Linear range	LLOQ
Plasma	$y = 0.0007 + 0.0035x$	0.9996	10–2000 ng/mL	1.0 ng/mL
Liver	$y = -0.0031 + 0.0041x$	0.9989	5–1000 ng/g	0.6 ng/g
Spleen	$y = 0.0024 + 0.0019x$	0.9999	5–1000 ng/g	1.0 ng/g
Kidney	$y = 0.0051 + 0.0011x$	0.9993	5–1000 ng/g	0.5 ng/g
Heart	$y = 0.0047 + 0.0012x$	0.9980	5–1000 ng/g	1.3 ng/g
Lung	$y = 0.0084 + 0.0012x$	0.9977	5–1000 ng/g	0.5 ng/g
Brain	$y = -0.0001 + 0.0022x$	0.9974	5–1000 ng/g	1.5 ng/g

y is the peak area ratio of puerarin and internal standard; x is the concentration of puerarin in rat plasma or tissue; r is the correlation coefficient; LLOQ is the lower limit of quantification.

Table 3
Accuracy and precision of the method used for determination of puerarin in rat plasma and tissue samples ($n = 6$).

		Intra-day Accuracy (%)	Precision (%)	Inter-day Accuracy (%)	Precision (%)
Low concentration	Plasma	99.11	7.52	96.46	7.08
	Liver	105.97	7.14	104.83	6.72
	Spleen	95.56	4.81	93.85	3.91
	Kidney	108.19	4.92	107.61	5.96
	Heart	97.20	5.97	97.73	5.41
	Lung	95.66	14.37	97.46	12.21
	Brain	90.65	5.58	92.30	5.42
Medium concentration	Plasma	97.29	6.22	93.70	5.25
	Liver	107.49	8.84	108.31	5.70
	Spleen	103.00	5.01	103.33	6.27
	Kidney	102.80	4.70	101.86	5.16
	Heart	104.11	5.64	102.73	4.83
	Lung	107.21	12.61	105.99	9.12
	Brain	110.03	7.38	108.11	8.34
High concentration	Plasma	100.05	2.97	101.60	5.48
	Liver	98.35	9.62	98.51	10.24
	Spleen	101.29	2.29	102.16	2.40
	Kidney	95.89	4.89	94.46	4.45
	Heart	103.67	4.32	101.04	5.21
	Lung	109.85	4.67	109.04	2.98
	Brain	98.62	6.41	96.27	4.81

The low concentration indicates 50 ng/mL of puerarin in plasma or 25 ng/g in tissues; medium concentration indicates 200 ng/mL of puerarin in plasma or 100 ng/g in tissues; high concentration indicates 1000 ng/mL of puerarin in plasma or 500 ng/g in tissues.

those treated with puerarin suspension ($P < 0.01$). In addition, a second peak of the puerarin concentration was found 6 h after the administration of the Pue-SLNs. The $AUC_{0\to t}$ values of puerarin post-administration of puerarin suspension and Pue-SLNs were 0.80 ± 0.23 mg h/L and 2.48 ± 0.30 mg h/L. The latter was 3.1 times greater than the former ($P < 0.01$). The bioequivalance study showed that the 90% confidence intervals (CI) for ln ($AUC_{0\to t}$), ln ($AUC_{0\to\infty}$) and ln (C_{max}) were 2.53–3.97, 2.41–3.92 and 1.59–2.49 (reference CI was 0.8–1.2), respectively. The intervals suggest that these two formulations were un-equivalent. Compared with the puerarin suspension, the $t_{1/2}$ and MRT were prolonged ($P < 0.01$) while the Cl, Vz and Vss were decreased with Pue-SLNs ($P < 0.05$). These results showed that the incorporation of puerarin into SLNs resulted in an increased absorption after oral administration.

3.3. Tissue distributions

The mean concentration–time profile of each tissue for the two formulations is presented in Fig. 3. In rats treated by puerarin suspension, the C_{max} in all six collected tissues appeared at 2.5 h post-treatment. In rats treated with Pue-SLNs, the C_{max} appeared at 1 h in the liver, spleen, kidney and lung, and the T_{max} was prolonged in the heart and brain, i.e. 6 h and 2.5 h, respectively. The C_{max} in each tissue of rats administered with Pue-SLNs was higher than that administered with puerarin suspension. The ratios of C_{max} in the liver, spleen, brain, heart, kidneys and lungs for rats administered with Pue-SLNs to rats administered with puerarin suspension were 3.85, 3.60, 2.52, 1.28, 1.15 and 1.94, respectively. The $AUC_{0\to 6\,h}$ values after the administration of puerarin suspension were in the order of kidney > liver > lung > spleen > heart > brain, but the same figures after administration of Pue-SLNs were in the order of liver > kidney > spleen > lung > brain > heart. The ratios of $AUC_{0\to 6\,h}$ in the liver, spleen, brain, heart, kidney and lung of the two formulations were 4.72, 3.78, 2.30, 1.76, 1.18 and 1.35, respectively.

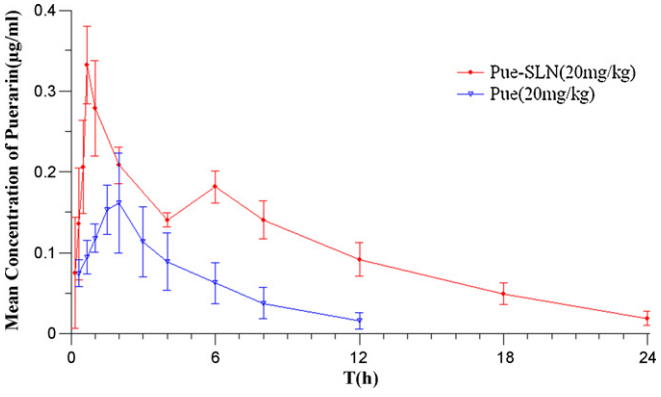

Fig. 2. Mean plasma concentration-time profile of puerarin after single dose intragastric administration of Pue-SLNs and puerarin suspension to rats (20 mg/kg).

3.4. Urinary and fecal excretion

The total excretion of puerarin in feces in 24 h was $34.17 \pm 4.28\%$ and $41.56 \pm 7.27\%$ of the dose after administration of Pue-SLNs and puerarin suspension, respectively, and the total excretion of puerarin in urine in 24 h was only 1.79% and 0.64% of the

Table 4
Pharmacokinetic parameters for puerarin in rats after intragastric administration of Pue-SLNs and puerarin suspension at an equivalent puerarin dose of 20 mg/kg (mean ± SD, $n = 6$).

Pharmacokinetic parameters	Pue-SLNs	Puerarin suspension
$AUC_{0\to t}$ (mg h/L)	$2.48 \pm 0.30^{*}$	0.80 ± 0.23
$AUC_{0\to\infty}$ (mg h/L)	$2.64 \pm 0.39^{*}$	0.88 ± 0.28
C_{max} (μg/mL)	$0.33 \pm 0.05^{*}$	0.16 ± 0.06
T_{max} (min)	$40 \pm 0^{*}$	110 ± 15.49
$t_{1/2}$ (h)	$5.60 \pm 1.00^{*}$	3.27 ± 0.87
MRT (h)	$9.34 \pm 1.40^{*}$	5.22 ± 1.06
Cl (L/h)	$1.49 \pm 0.24^{\#}$	4.79 ± 1.50
Vz (L)	$11.83 \pm 1.02^{\#}$	22.64 ± 9.96
Vss (L)	$13.71 \pm 1.29^{\#}$	24.51 ± 7.88

$AUC_{0\to t}$, area under the concentration–time curve from time zero to the last sampling time point; $AUC_{0\to\infty}$, area under the concentration–time curve from time zero to the infinity; C_{max}, maximum plasma concentration of puerarin; Cl, total clearance; MRT, mean residence time; $T_{1/2\beta}$, elimination half-life; T_{max}, time to maximum plasma concentration of puerarin; Vss, volume of distribution at steady-state; Vz, volume of distribution at terminal state.

* Comparison between the two groups, $P < 0.01$.

$^{\#}$ Comparison between the two groups, $P < 0.05$.

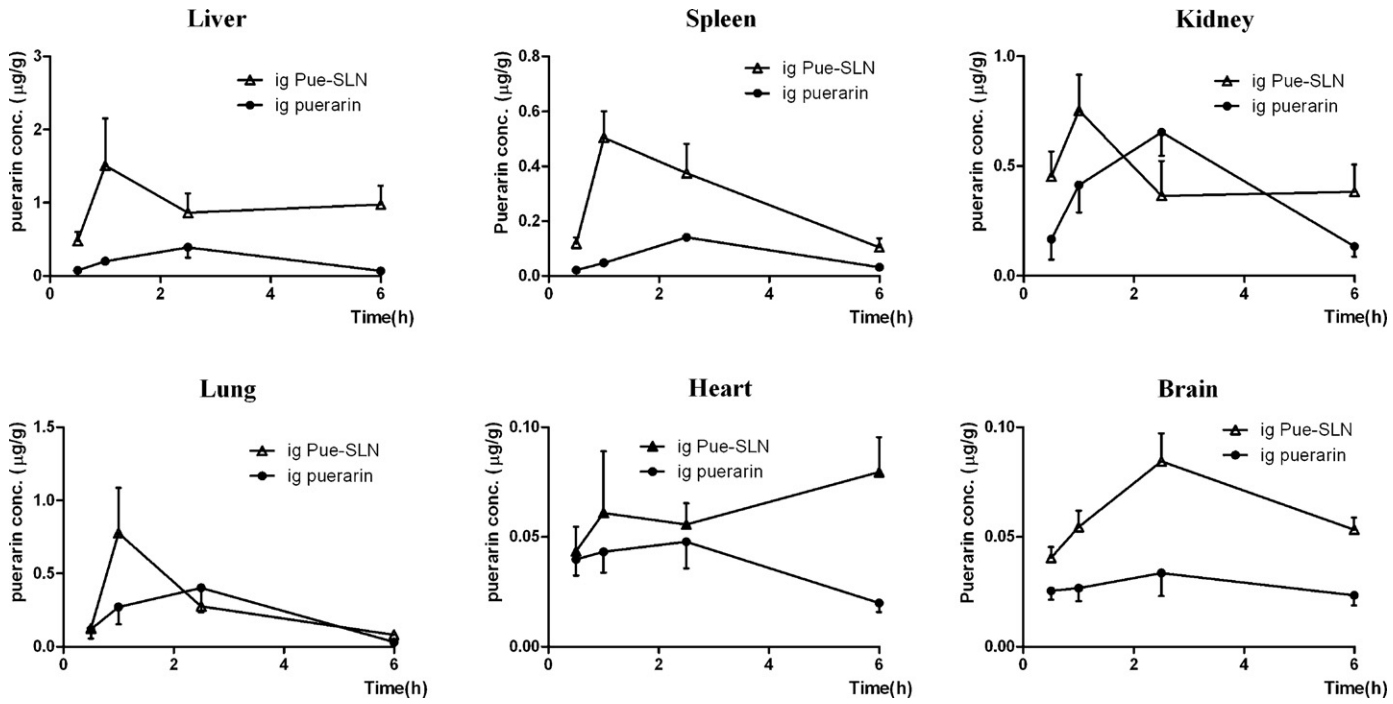

Fig. 3. Mean puerarin concentration- time profiles in tissues following intragastric administration of Pue-SLNs and puerarin suspension to rats (20 mg/kg).

dose, respectively. The percentage of unchanged puerarin excreted in feces was high in the first 12 h after administration of Pue-SLNs and puerarin suspension, accounting for 95.72 ± 1.82% and 99.33 ± 0.86% ($P < 0.01$) of the total excretion in feces, respectively, and it was 4.28% and 0.67% ($P < 0.01$) during 12–24 h after dosing, respectively.

4. Discussion

Many techniques have been employed to improve the oral absorption of puerarin, a drug with poor water-solubility. For example, the bioavailability of puerarin formulated as a phospholipid complex is 1.46-fold higher than that with puerarin suspended in water (Li et al., 2006). We successfully prepared Pue-SLNs for oral administration. When puerarin was incorporated into SLNs, the relative bioavailability of puerarin was 310% in the present study. The C_{max} and MRT of Pue-SLNs suspension were 2.06 and 1.79 times of that of puerarin suspension, respectively, while Cl significantly decreased. These results indicated that the incorporation into SLNs enhanced the absorption of puerarin after oral administration. The decreased excretion of puerarin in feces and the increased excretion of puerarin in urine also suggested the improvement of absorption of puerarin after entrapped into nanoparticles. In the present study, the percentage of unchanged puerarin excreted in feces in the first 12 h in rats administered Pue-SLNs was significantly lower than that of puerarin suspension, and it excreted during 12–24 h was significantly higher. It indicated that the excretion of puerarin was delayed when incorporated into nanoparticles. It has been reported that SLNs may exhibit bioadhesion to the gastrointestinal tract wall, increasing their residual time in the gastrointestinal tract. In addition, lectins, an ingredient used in the formulation of Pue-SLNs, can increase the adherence of microparticles to the intestinal epithelium and enhance penetration of drugs (Vasir et al., 2003).

Following the intragastric administration of puerarin suspension, the mean peak plasma concentration of puerarin was 0.16 ± 0.06 μg/mL at 110 ± 15.49 min after oral dosing, whereas, in the case of Pue-SLNs, a significant higher peak plasma level (0.33 ± 0.05 μg/mL, $P < 0.01$) appeared earlier with the T_{max} being at 40 min ($P < 0.01$, Fig. 2). A similar phenomenon was observed after the intraduodenal administration of lovastatin-SLNs and lovastatin suspension in rats (Suresh et al., 2007). Intestinal lymphatic transportation of drugs incorporated into SLNs is supported by Paliwal et al. (2009). The cellular lining of the gastrointestinal tract is composed of absorptive enterocytes interspersed with membranous epithelial (M) cells. M cells that cover lymphoid aggregates, known as Peyer's patches, take up nanoparticles by a combination of either endocytosis or transcytosis (Andrianov and Payne, 1998). The lectin in SLNs formulation enhances lymph formation and simultaneously increases lymph flow rate. This might be the reason for the shorter T_{max} for Pue-SLNs than for the suspension. The SLNs taken up by Peyer's patches in the intestine bypass the liver through first-pass metabolism, subsequently, the plasma puerarin concentration is increased. There was a second peak at 6 h in the pharmacokinetic profile of the Pue-SLNs (Fig. 2), possibly relating to a prolonged absorption of puerarin from the bioadhesion of the nanoparticles to the gut. The similar two-peak drug concentration time curves have also been reported in the pharmacokinetic study of oral administration of camptothecin-SLNs (Yang et al., 1999) and azidothymidine-SLNs (Löbenberg et al., 1997).

High puerarin concentrations in various tissues are essential for its pharmacological roles. In the present study, the ratios of C_{max} in the brain and heart after the administration with Pue-SLNs and the puerarin suspension were 2.52 and 1.28, respectively. The ratios of $AUC_{0\rightarrow 6\,h}$ in the brain and heart were 2.30 and 1.76, respectively. Since tissue samples were collected at only four time points, we could not calculate the pharmacokinetic parameters of puerarin in the tissues. It is well known that the increasing concentrations of puerarin in the heart and brain are in favor of its protective effects on cardiocerebrovascular diseases upon the oral administration of Pue-SLNs. Under normal conditions, drug penetration into the brain is restricted by the blood–brain barrier (BBB), therefore, BBB specific influx transporters may be involved for the brain delivery of puerarin (Prasain et al., 2004). The mechanisms for the transport of SLNs through the BBB are not completely understood. It attributes a central role to endothelial cells in the process of nanoparticle adhesion and subsequent endocytosis, transcytosis, and tight junc-

tion modulation. Among the plasma proteins adsorbed on the SLNs surface, ApoE, Apo C-II, albumin and immunoglobulin G seem to be crucial in the site-specific targeting of the brain (Brioschi et al., 2007). The P-glycoprotein, an ATP-dependent drug transport protein, is present at the apical membranes of different epithelial cells including those forming the BBB. Inhibition of the P-glycoprotein has been proposed as a possible strategy for enhancing drug penetration into the brain. Some of the surfactants employed in SLNs formulations have shown to inhibit the P-glycoprotein (Blasi et al., 2007).

When incorporated into nanoparticles, the properties of the SLNs will determine the *in vivo* disposition of drugs. The mononuclear phagocyte system (MPS) plays a vital role in clearing the nanoparticles from blood circulation (Joshi and Müller, 2009). The ratios of C_{max} and $AUC_{0\rightarrow 6\,h}$ in the liver and spleen of the two formulations were the largest among all of the tissues studied. Results indicated that Pue-SLNs might be taken up in the gastrointestinal tract and translocated to organs containing MPS.

Orally administered Pue-SLNs were rapidly absorbed, as evidenced with a shorter T_{max} for Pue-SLNs than for puerarin suspension. The relative bioavailability of puerarin improved (more than 3 fold) when incorporated into the SLNs. At the same time, the tissue concentrations of puerarin increased after a single-dose oral administration of Pue-SLNs, especially in its target organs, the heart and brain. These data collectively support that SLNs are a promising delivery system for the enhancement of oral absorption of puerarin, a poorly water-soluble drug.

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