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# Formulation, preparation and evaluation of flunarizine-loaded lipid microspheres

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## Abstract

The aim of this study was to investigate the feasibility of preparing flunarizine-loaded lipid microspheres. Lipid microspheres (LMs) are excellent drug carriers for drug delivery systems (DDS) and are relatively stable and easily mass-produced. They have no particular adverse effects. LMs have been widely studied as drug carriers for water-soluble drugs, lipid-soluble drugs and inadequately soluble (in water or in lipid) drugs, in that they have a lipid layer, a water layer and an emulsifier layer. Flunarizine (FZ), a poorly water-soluble drug, was incorporated in lipid microspheres to reduce side effects by avoiding the use of supplementary agents, compared with solution injection. After investigation, the final formulation was as follows: 10% oil phase (long-chain triglyceride (LCT); medium-chain fatty acid (MCT) = 50:50); 1.2% egg lecithin; 0.2% Tween-80; 2.5% glycerin; 0.3% dl-$\alpha$-tocopherol; 0.02% EDTA; 0.03% sodium oleate; 0.1% FZ and double-distilled water to give a total volume of 100 mL. Homogenization was the main method of preparation and the best conditions were a temperature of 40°C, a pressure of 700–800 bar and a suitable cycle frequency of about 10. The particle size distribution, zeta-potential and entrapment efficacy were found to be 198.7 ± 54.0 nm, –26.4 mV and 96.2%, respectively. Its concentration in the preparation was 1.0 mg mL$^{-1}$. The lipid microspheres were stable during storage at 4°C, 25°C and 37°C for 3 months. Pharmacokinetic studies were performed in rats using a dose of 1.0 mg kg$^{-1}$. The pharmacokinetic parameters were as follows: $AUC_{0-t}$ 6.13 $\mu$g·h mL$^{-1}$, t½ 5.32 h and Ke 0.16 L h$^{-1}$. The preparation data fitted a two-compartment model estimated by using 3p87 analysis software. From the observed data, FZ encapsulated in LMs did not significantly alter the pharmacokinetic characteristic compared with the FZ solution injection and did not produce a delayed release effect, when it was released in-vivo in rats. However, the availability of the drug was increased. These results suggested that this LM system is a promising option for the preparation of the liquid form of FZ for intravenous administration.

## Introduction

Flunarizine, (E)-1-[bis(4-fluorophenyl)methyl]-4-(3-phenyl-2-propenyl) piperazine, is a difluorinated derivative of cinnarizine. It is a selective calcium entry blocker, at least as effective as pizotifen in migraine prophylaxis (Andersson & Vinge 1990; Olesen 1991; Windholz 2001), and in a longer term study as effective as cinnarizine for the treatment of vertigo (Holmes et al 1984). At present, there are only oral dosage forms of flunarizine available on the market, such as tablets and capsules. These products, with low bioavailability and slow absorption, are not suitable for treating patients who are dangerously ill. Flunarizine is poorly water-soluble (Marini & Balestrieri 1984), so supplementary agents must be added to dissolve the drug when preparing a flunarizine solution for injection, and this increases the side effects and is not safe for human use. Therefore, there is a need for alternative new intravenous preparations to improve the therapeutic effects and reduce some adverse reactions.

Lipid microspheres (LMs), with an average diameter of 0.2 $\mu$m and consisting of oil and water phases (Figure 1), appear to be safe and excellent drug carriers (Mizushima 1996; Yamaguchi 1996). They are widely used in clinical medicine for parenteral nutrition preparations, such as Intralipid. The LMs themselves are very stable and can be stored for up to two years at room temperature. Due to their ability to incorporate drugs with poor water solubility in the dispersal phase (Müller et al 2004), microspheres avoid direct contact of the drug with the blood vessels, thereby reducing irritation, and also prolong the release of drug

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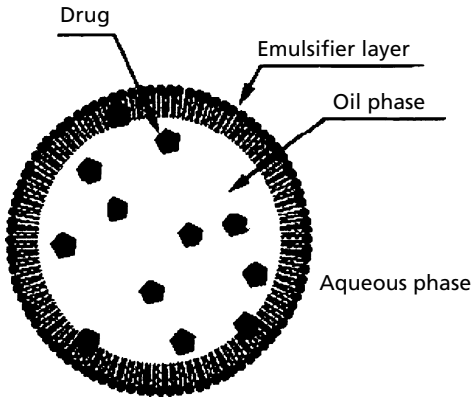


**Figure 1** Schematic model of lipid microspheres (LMs).

in-situ leading to a reduction in side effects. Regarding their distribution in the body, LMs, like liposomes, accumulate easily in the reticulo-endothelial system, which performs as their effective target.

Because of these properties of LMs, the main purpose of this study was to investigate the feasibility of preparing flunarizine-loaded LMs for intravenous injection.

## Materials and Methods

### Materials

Flunarizine hydrochloride was purchased from the Zhengzhou Ruikang Pharmaceutical Limited Co. (China). Flunarizine solution injection (1 mg $mL^{-1}$) was prepared ourselves using 5% PEG 400 and 0.2% Tween-80 as solubilizing agents. Diazepam used as an internal standard was obtained from the Hubei Zhongtian Baike Drug Industry (China). Polyethylene glycol 400 was provided by the Tianjin Concord Reagent Company (China). Egg lecithin (Lipoid E80) and medium-chain fatty acid (MCT) were obtained from the Germany Lipoid Company, while soybean oil, glycerin and Tween-80 for parenteral use were obtained from the Tieling Beiya Medicated Oil Company, Zhejiang Suichang Glycerin Company and the Shanghai Shenyu Medicine Chemical Industry Company, respectively. All other chemicals and reagents were of analytical or chromatographic grade.

### Formulation and preparation of flunarizine-loaded LMs

The basic ingredients in the LMs were a mixture of soybean oil and MCT 10 g, egg lecithin 1.8 g, Tween-80 0.2 g, dl-$\alpha$-tocopherol 0.3 g, sodium oleate 0.03 g, glycerol 2.5 g, EDTA 0.02 g, flunarizine 0.1 g and double-distilled water to 100 mL.

To optimize the formula of LMs, an $L_9$ ($4^3$) orthogonal design experiment was conducted as part of the study. The four factors were: amount of egg lecithin, ratio of soybean oil and MCT, amount of Tween-80 and amount of sodium oleate, the three levels of which were egg lecithin 0.8%, 1.2%, 1.8%; ratio of soybean oil and MCT 20:80, 50:50, 80:20; Tween-80 0%, 0.1%, 0.2%; sodium oleate 0%, 0.03%, 0.1%, respectively.

The preparation of the flunarizine LMs involved four steps. First, preparation of the aqueous phase by dispersing the glycerol, sodium oleate, and EDTA in water using a constant-speed stirrer at 1500 rev $min^{-1}$. Second, preparation of the oil phase by combining a mixture of soybean oil and MCT, lecithin, Tween-80 and dl-$\alpha$-tocopherol at 80°C. When the lecithin dissolved, the drug powder was added. Third, homogenization, in which the oil phase was slowly added to the aqueous phase with continuous stirring. This mixture was pre-emulsified using a high-shear mixer at 8000 rev $min^{-1}$ for 5 min. Then it was passed through a high-pressure homogenizer to obtain the LMs. The homogenization was performed at a temperature of 40°C. Finally, the pH of the lipid microspheres was adjusted to about 8 using 0.1 M hydrochloric acid. The emulsion was sterilized by autoclaving for 15 min at 121°C, 0.095 MP (15 bar).

### Emulsion characterization

*Particle size analysis*

The emulsion mean particle size and distribution were measured by photon correlation spectroscopy (PCS, dynamic light scattering, DLS) using a Nicomp 380 submicron particle sizer (Particle Sizing System, Santa Barbara, USA). PCS is a laser light-scattering technique that uses fluctuations in scattered light intensity to measure the velocity of Brownian diffusion of small particles and, hence, reflects their diameters (Washington 1990). As it is quite sensitive to particles with diameters ranging from 3 nm to 3 $\mu$m, it can detect the majority of small lipid particles (Komatsu et al 1995). The Nicomp and the Gauss distribution of particle size was obtained at the same time with intensity-weighting (z-average), volume-weighting and number-weighting, while the value of the standard deviation instead of the PI showed the width of distribution. The characterization parameters of PCS diameters 50, 90, 95 and 99% were also calculated. For the results obtained here, each emulsion sample was diluted 1:5000 in highly purified water, which had been passed through a 0.2-$\mu$m filter and the measurements were carried out at 25°C. It was verified beforehand that dilution of the samples did not alter the size distributions obtained (Driscoll 2002; Müller et al 2004).

*Zeta-potential measurement*

The zeta-potential of the emulsion particles is of considerable value in evaluating the stability of any colloidal system, and it was determined using the Nicomp 380 by electrophoretic light scattering (ELS). The ELS technique is based on the scattering of light from particles that move in liquid under the influence of an applied electric field. The value of the mean zeta-potential was obtained from the electrophoretic mobility, $\mu$, which was computed from the measured Doppler shift, $\Delta\nu$, for a given applied electric field strength E of 15 V $cm^{-1}$. For the results obtained here, samples were diluted as described for particle sizing measurement, except that the water was adjusted to the desired pH with 0.01 M HCl or NaOH beforehand. The results for each sample are expressed as the mean ± standard deviation of these measurements.

*Stability assessment*

The stability of LMs was studied using two methods.

*Autoclaving.* Using the method of Wang & Cory (1999), emulsions, about 5 mL each sealed in 10-mL glass vials, were sterilized by autoclaving at 121°C for 15 min followed by natural cooling to room temperature. The emulsions were visually examined and sampled to estimate the zeta-potential and carry out particle size analysis.

*Short-time stability.* Following the method of Klang et al (1999), the drug content, pH and droplet size distribution were monitored over short periods of time in the emulsions stored at 4°C, 25°C and 37°C. The degree of creaming and the phase separation were assessed visually at given time intervals. All other visible changes were recorded.

*Entrapment efficiency*

The entrapment efficiency of the system was determined by measuring the concentration of free flunarizine in the dispersion medium. The LMs were ultracentrifuged using a Hitachi Ultracentrifuge at 46 000 rev $min^{-1}$ (approx. 107 000 *g*) for 4 h with presetting to 10°C. The amount of flunarizine in the aqueous phase was estimated using high-performance liquid chromatography.

*High-performance liquid chromatographic analysis*

The high-performance liquid chromatographic (HPLC) analysis of flunarizine was performed as described by the ChP 2005 edition (Chinese Pharmacopeia 2005). The instrument consisted of a C18 (4.6 mm × 200 mm, 5 $\mu$m) analytical column (Diamonsil), Jasco 980 high-precision pump (Jasco, Japan), Jasco L-7200 auto-sampler (Jasco, Japan) and Jasco 975 ultraviolet (UV) detector (Jasco, Japan). The mobile phase consisted of a mixture of a 0.01 M aqueous solution of $KH_2PO_4$ (4 mL triethylamine added and then the pH was adjusted to 3.5 with $H_3PO_4$) and methanol (25:75 v/v); the flow rate was 1 mL $min^{-1}$, and the detection was at a wavelength of 253 nm. No conspicuous peaks of flunarizine degradation products were observed.

### Pharmacokinetic studies

Male Wistar rats, 250 ± 20 g (Animal Center, Shenyang Pharmaceutical University, China), were divided into two groups with 6 rats in each group. On the day of the experiment, the rats received 1.0 mg $kg^{-1}$ of flunarizine solution (prepared using 5% PEG 400 and 0.2% Tween-80 as solubilizing agents) intravenously and flunarizine-loaded LMs, respectively. At 5 min, 15 min, 0.5 h, 1 h, 1.5 h, 2 h, 4 h, 6 h, 8 h, 12 h and 24 h post flunarizine administration, blood samples were collected by retro-orbital puncture and transferred to Eppendorf tubes containing heparin as an anticoagulant and centrifuged immediately at 4000 rev $min^{-1}$ for 10 min to obtain plasma. The samples were stored at −20°C in a refrigerator before analysis.

The disposal of the plasma was carried out by a reported method (Li et al 2001). Five microlitres of diazepam (0.2 $\mu$g $mL^{-1}$) was added to 200 $\mu$L plasma as the internal standard. Then 200 $\mu$L methanol and 20 $\mu$L NaOH solution were added (5 M). Following vortexing for 10 s, the samples were extracted with 1 mL cyclohexane and then vortexed again for 3 min. After centrifugation at 4000 rev $min^{-1}$ for 10 min, the supernatant was transferred to a conical tube. The separated organic phase was then evaporated to dryness, under a gentle stream of nitrogen at 60°C. The residue was reconstituted in 100 $\mu$L methanol and then 10 $\mu$L was injected onto an HPLC system. The retention time was 8.60 min for flunarizine and 7.62 min for the internal standard, diazepam.

The experimental procedures were approved by the institutional animal ethics committee. The study complied with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

### Statistical analysis

In the short-time stability experiment, properties such as the content, entrapment efficiency, particle size, zeta-potential and pH of flunarizine-loaded LMs at different time points and different storage conditions were compared with those at zero time using a one-way analysis of variance. These properties of flunarizine-loaded LMs before and after autoclaving were statistically compared using a paired-samples *t*-test. In Figure 2 the effect of formulation type (flunarizine-loaded LMs and flunarizine solution injection) on the plasma concentration at each time point is shown and in Table 2 the formulation type on the various pharmacokinetic parameters were statistically compared using an independent *t*-test. Significance was assumed at the 0.05 level of probability in all cases. Average values are reported as mean ± standard deviation. All the statistical data were performed using SPSS statistical software.

The plasma concentration–time curve was analysed initially with 3p87 computing program produced by the Mathematics Pharmacological Committee of the Chinese Academy of Pharmacology, which made the most appropriate pharmacokinetic model to describe the experimental data. The model was selected based on the residual sum of squares and the minimum Akaike's information criterion (AIC) value.

The area under the concentration–time curve ($AUC_{0-t}$) from zero to the last time point was calculated by the log-linear trapezoidal method. The area under the cross product of time and plasma concentration–time curve ($AUMC_{0-t}$), mean residence time (MRT), clearance (CL), steady-state apparent volume of distribution (Vss), half-life time (t½) and the elimination rate constant (Ke) of the drug was obtained using the statistical moment method (Yu et al 2006).

## Results and Discussion

### The formulation of flunarizine-loaded LMs

To increase the drug solubility, we studied the entrapment efficiency, and to improve the particle size distribution, stability and zeta-potential of the preparation, we investigated the factors that might influence the character of the LMs, such as the ratio of MCT/LCT in the oil phase, the amount of egg lecithin, the amount of Tween-80 and the effect of sodium oleate.

*Ratio of MCT/LCT*

Long-chain triglycerides (LCTs) as the oil phase of lipid emulsions have been used in clinical settings for over 30 years. However, they have some toxic effects, such as immune dysfunction, accumulation in reticulo-endothelial cells and deposition of adipochrome in liver or lung after long-term use. Alternative lipid emulsion mixtures containing medium-chain triglycerides (MCTs) may reduce the toxicity associated with pure LCT-based lipid emulsions (Smyrniotis et al 2001) and may also provide more stable all-in-one admixtures (Driscoll et al 2000). Thus, LCTs and MCTs were mixed in three different ratios (80:20; 50:50; 20:80). When the ratio was 20:80, oily droplets on the surface of the lipid microsphere were visible after autoclaving. This showed that the emulsion was not stable, so the ratio was not suitable. From the results of the experiment (the entrapment efficiencies of LMs were 86.66% and 93.26% at ratios of 80:20 and 50:50), a 50:50 mixture of LCT and MCT seemed to be the optimal mixture.

*Dosage of egg lecithin*

Egg lecithin was used as the main emulsifier. According to a published report (Sznitowska et al 2001), when there is insufficient emulsifier, the emulsion is unstable. The amount of emulsifier is very important since it protects the system by acting as a solubilizing agent for added drugs or by creating a steric and electrostatic barrier at the interphase (Muchtar et al 1989, 1991). However, if there is too much egg lecithin, small particles are formed by the remaining lecithin and the particle size exhibits a bimodal distribution. Accordingly, the appropriate amount of lecithin was about 1.2 g.

*Dosage of Tween-80*

Tween-80 (polyoxyethylene 80 sorbitan monooleate) as a co-emulsifier was found to increase LM stability, probably because of the formation of a complex interfacial film between Tween-80 and the phospholipid molecules at the oil–water interface. Nevertheless, its toxicity should also be taken into consideration. When the amount used was 187.5 mg $kg^{-1}$, given to cyophoric rabbits, some toxicitiy was seen. However, 80 mg $kg^{-1}$ in monkeys, 56 mg $kg^{-1}$ in rats or 188 mg $kg^{-1}$ in rabbits was found to be safe. In this experiment, the amount used was 0.2 g, which was within the safe limit.

*Effect of sodium oleate*

Sodium oleate is an anionic surfactant and also was a co-emulsifier. It altered the pH of the emulsion system. Also, the most important effect was the change in the electric charge of the surface of the oil drops. Sodium oleate was taken up by, or incorporated into, the interfacial film of the emulsion droplets giving them a negative charge and increasing the force of repulsion. So sodium oleate is one factor that affects the physical stability of LMs. When the sodium oleate content was 0.03%, the zeta-potential was −25.66 mV, and the microspheres appeared satisfactory.

Overall, the optimal formula based on the experimental data was flunarizine 0.1%, oil phase 10% (LCT:MCT = 50:50), Tween-80 0.2%, egg lecithin 1.2%, dl-α-tocopherol 0.3%, EDTA 0.02%, glycerol 2.5%, sodium oleate 0.03% and water to a total volume of 100 mL.

## The preparation of flunarizine-loaded LMs

The efficiency of the emulsification process was investigated by measuring the mean droplet diameter. The effect of factors involved in the preparation of flunarizine-loaded LMs was analysed from three aspects – the temperature, pressure and cycle frequency of homogenization.

Controlling the temperature of the homogenization process was very important, because the viscosity of the oil phase and oil–water interfacial tension were related to temperature and, accordingly, affected the stability of the emulsion. When the temperature was low, the viscosity was higher and the particle size was large and there was a wide distribution. When the temperature was too high, the emulsion could break down. For this experiment, five temperatures (10, 20, 40, 60 and 70°C) were studied. From the results obtained, there were visible oily droplets after sterilizing and some were demulsified, except when the temperature was 40°C.

As is well known, the higher the pressure, the smaller the particle size. When the pressure was no greater than 800 bar, the mean particle diameter was greater than 200 nm, but under 800 bar, the standard deviation of the particle size distribution was the smallest. However, too high a pressure produced an increase in the standard deviation. This observation is similar to those in the studies of Davis et al (1985) and Coulaloglou & Tavlarides (1997). The interpretation of this phenomenon was that when the pressure was greater, the kinetic energy of oily drops increased. Collisions were accelerated, leading to coalescence and the production of larger particles. This phenomenon was called overprocessing of the preparation. Homogenization was performed using 1–20 cycles. The results showed that the particle size and standard deviation were reduced on increasing the number of cycles during the earlier period. However after the tenth cycle, the standard deviation of the particle size increased markedly, which might lead to the instability of the lipid microspheres.

Therefore, the best conditions of homogenization were as follows: a temperature of 40°C, a pressure of 700–800 bar and a cycle frequency of about 10.

## Characterization of LMs

*Particle size and distribution*

The particle size distribution and the number of larger particles were used to assess the physical stability of the emulsions. With regard to possible toxic effects, particular attention was given to the larger particles (Müller & Heinemann 1992). The results obtained showed that the mean particle size was 198.7 ± 54.0 nm and 99% of the distribution was less than 500 nm, with no particles that were larger than 1 μm (n = 3).

*Zeta-potential*

The zeta-potential was about −26.40 ± 0.45 mV (n = 3), and it was measured by a Zeta Potential/Particle Sizer NICOMP 380ZLS. The negative charge is due to the many components of the emulsions that are negatively charged at neutral pH, such as egg lecithin, sodium oleate and drug. The partition of the drug in the oil–water interface of the emulsion particles might affect the zeta-potential of the emulsion particles

(Epps & McCall 1997) because of the protonation of the drug.

Three samples of flunarizine-loaded LMs were prepared, then their particle size and zeta potential were measured (Table 1).

*Drug content and entrapment efficiency*

Three batches of flunarizine-loaded LMs were prepared. Their contents were 99.1%, 101.4% and 100.7%, separately (n = 3), and their entrapment efficiencies were 96.2%, 95.6% and 96.8%, respectively (n = 3), which all complied with the requirements for lipid microspheres.

The water solubility of pure flunarizine was $0.0165 \pm 0.00047\ g\ L^{-1}$ (Marin et al 2002), so only 16.5 mg flunarizine could dissolve in 100 mL water. However, when flunarizine was loaded in the LMs, as much as 100 mg could dissolve, or be encapsulated, in 100 mL LM vehicle.

*Stability*

The stability of the flunarizine-loaded emulsion to autoclaving was evaluated by autoclaving at 121°C for 15 min. The content, entrapment efficiency and particle size of the emulsion were essentially unaffected by autoclaving (n = 5, $P > 0.05$). However, the absolute value of the zeta-potential and pH were reduced slightly (n = 5, $P < 0.05$), presumably because of the hydrolysis of the major emulsifier, egg lecithin, to produce free long-chain fatty acids at the high temperature, and then the formation of new long-chain acid radicals under alkaline conditions, reducing the zeta-potential and pH of the emulsions (Chaturvedi et al 1992; Wang & Cory 1999).

The short-time stability of lipid microspheres during storage at 4, 25 and 37°C was also studied by measuring the properties at different time points over 3 months. The properties of particle size, zeta-potential, content, entrapment efficiency and pH compared with those at zero time were not significantly changed (n = 5, $P > 0.05$). The results showed that the emulsion was stable at 4, 25 or 37°C for 3 months.

## Evaluation of flunarizine-loaded LMs in-vivo

To evaluate the feasibility of a preparation, we should not only study its physical properties in-vitro, but also understand the process of the preparation in-vivo. A pharmacokinetic experiment was designed to compare flunarizine solution injection ($1\ mg\ mL^{-1}$) and flunarizine-loaded LMs ($1\ mg\ mL^{-1}$). In Van Hoeyweghen's study (Van Hoeyweghen et al 1989), three different doses (12.5, 25 and 50 mg) of flunarizine infusions were administered separately to three groups of patients according to a strict dose-range infusion protocol. The pharmacokinetic results indicated that no substantial accumulation of flunarizine occurred and that plasma levels were proportional to the given dose. Therefore, flunarizine pharmacokinetics can be considered as linear for doses up to 50 mg. In our study, the dosage for rats was calculated by skin surface area conversion table according to the humans' dose, 12.5 mg. Considering the injecting volume, a dose of $1.0\ mg\ kg^{-1}$ via the femoral vein was selected.

For this experiment, a comparative pharmacokinetic study was performed by measuring the drug levels in plasma up to 24 h after the administration. The data were analysed by the pharmacokinetic program 3p87. From the results, it was found that the data for both the preparations fitted a two-compartment model (the weight was 1). The main pharmacokinetic parameters were calculated by the statistical

**Table 1** The particle size and zeta-potential of three samples of flunarizine LMs

| Sample | 1 | 2 | 3 | Mean ± s.d. |
|---|---|---|---|---|
| Particle size (nm) | 198.0 ± 55.4 | 195.0 ± 50.6 | 203.1 ± 56.0 | 198.7 ± 54.0 |
| Zeta-potential (mV) | −26.40 | −25.90 | −26.80 | −26.40 ± 0.45 |

The data of particle size were given directly as mean ± s.d. by the Nicomp 380ZLS particle sizer.

**Table 2** Pharmacokinetic parameters in rats after intravenous administration of flunarizine solution and flunarizine-loaded LMs

| Parameter | Flunarizine-loaded LMs | Flunarizine solution |
|---|---|---|
| Ke ($Lh^{-1}$) | 0.16 ± 0.04 | 0.11 ± 0.03 |
| t½ (h) | 5.32 ± 2.24 | 6.39 ± 1.70 |
| $AUC_{0-t}$ ($\mu g{\cdot}h\ mL^{-1}$) | 6.13 ± 2.98 | 3.64 ± 0.37 |
| $AUMC_{0-t}$ ($\mu g{\cdot}h^2\ mL^{-1}$)* | 43.71 ± 22.46 | 18.25 ± 6.67 |
| MRT (h) | 7.20 ± 2.86 | 5.65 ± 1.55 |
| CL ($L\ h^{-1}$) | 0.043 ± 0.027 | 0.062 ± 0.008 |
| Vss (L) | 0.272 ± 0.106 | 0.329 ± 0.091 |

The data were mean ± s.d., n = 6. Ke, elimination rate constant; t½, half-life time; $AUC_{0-t}$, area under the concentration–time curve; $AUMC_{0-t}$, area under the cross product of time and plasma concentration–time curve; MRT, mean residence time; CL, clearance; Vss, steady-state apparent volume of distribution. *$P < 0.05$, others $P > 0.05$.

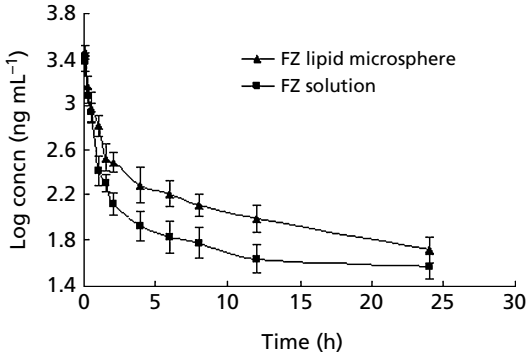


**Figure 2** Mean plasma concentration–time profiles of intravenously administered flunarizine (FZ)-loaded LMs and flunarizine solution to rats (n = 6).

moment method (Table 2). Most of the pharmacokinetic parameters had no statistically significant differences (n = 6, $P > 0.05$), except the $AUMC_{0-t}$ (n = 6, $P < 0.05$). In addition, the $AUC_{0-t}$ for flunarizine-loaded LMs was larger than that of flunarizine solution, and the CL was smaller, which suggested that the availability of the drug was slightly increased.

The curves of the mean plasma concentration–time were used to describe the process in-vivo (Figure 2). The plasma concentrations within 0.5 h (including 0.5 h) and the time point 24 h between flunarizine-loaded LMs and flunarizine solution had no statistically significant differences (n = 6, $P > 0.05$), while other plasma concentrations between the two formulations did have significant differences (n = 6, $P < 0.05$). According to the curve, flunarizine-loaded LMs could maintain a higher concentration over a longer period and thereby prolong the circulation time of flunarizine in rats.

According to one report (Wang & Sun 1997), drug-loaded LMs have a slow-release effect. This is because, in the emulsions, drug is loaded in the oil phase, which may cause a delay in the drug release compared with the aqueous phase. For example, the half-time of $PGE_1$-loaded lipid microspheres was prolonged to maintain the pharmacodynamic action for 24 h, compared with injection of solution (Yu 2002). Such a phenomenon was not remarkably observed in this experiment. The main reason was the drug distribution in the preparation. LMs are composed of three parts – the aqueous phase, the oil–water interfacial film and the oil phase. The entrapment efficiency of flunarizine-loaded LMs was about 95%, so there was little drug distributed in the aqueous phase. Some researchers claim that the main drug-loaded part for a drug that is poorly water-soluble and oil-soluble is the oil–water interfacial film, which is composed of the surface-acting agents, such as lecithin and Tween-80 (Akkar & Rainer 2003). Müller et al (2004) studied the preparation of amphotericin B emulsion using this theory. Thus, in the case of flunarizine, which is not dissolved in the water and oil phase, most of the drug is in the oil–water interfacial film and is more easily released in the blood than from oily droplets. So, LMs do not exhibit a markedly slower release. However the $AUC_{0-t}$(LMs)/$AUC_{0-t}$(solution) was about 168% (Table 2). This shows initially that LMs, as a new drug delivery system, increase the availability of drug to a certain degree.

## Conclusion

In conclusion, the flunarizine-loaded lipid microsphere system composed of 10% oil phase (LCT:MCT = 50:50), 1.2% egg lecithin, 0.2% Tween-80, 2.5% glycerin, 0.3% dl-α-tocopherol, 0.02% EDTA, 0.03% sodium oleate, 0.1% flunarizine and double-distilled water to a volume of 100 mL is the optimal formulation. The content, entrapment efficiency and characteristics all complied with the requirements for lipid microspheres. The pharmacokinetic studies showed that encapsulated flunarizine in lipid microspheres released in-vivo in rats did not significantly alter its pharmacokinetics and there was no significant delayed-release effect. However, the drug availability was slightly increased. On the whole, to increase the concentration of drug in the preparation and reduce the adverse effects by increasing the availability, lipid microspheres, as a new drug delivery system, offer a promising approach for the poorly soluble drug flunarizine.

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