JPP Journal of Pharmacy and Pharmacology

JPP 2006, 58: 1429–1435

Received February 22, 2006
Accepted July 26, 2006
DOI 10.1211/jpp.58.11.0002
ISSN 0022-3573

Formulation and evaluation of nimodipine-loaded lipid microspheres

Jia Yu, Hai Bing He and Xing Tang

Abstract

The purpose of this study was to develop an alternative, improved and better tolerated formulation and investigate the pharmacokinetic profile of the new formulation of nimodipine (NM) compared with nimodipine ethanol solutions. Lipid microspheres (LMs) prepared using lecithin and vegetable oils have attracted a lot of interest owing to their versatile properties, such as non-immunogenicity, being easily biodegradable and exhibiting high entrapment efficiency. NM incorporated in LMs could reduce irritation by avoiding the use of ethanol as a solubilizer. The solubility of NM was also increased by dissolving it in the oil phase. The particle size distribution, zeta potential, entrapment efficacy and assay of the NM-loaded LMs were found to be 188.2 ± 5.4 nm, –31.6 mV, 94.2% and 1.04 mg mL^{-1}, respectively. The preparation was stable for 1 year at 4–10°C. The formulation and some physicochemical properties of NM-loaded LMs were investigated. The pharmacokinetic and biodistribution studies were performed in rats at a dose of 1.2 mg kg^{-1}. From the observed data, there is no obvious retention of NM-loaded LMs in plasma. Moreover, incorporation of NM in LMs did not alter the tissue distribution significantly except for the relatively greater drug accumulation in the liver and spleen. The stimulation studies demonstrate that LMs of NM reduce irritation markedly compared with NM solutions. These results suggest that the LM system is a promising option to replace NM ethanol solutions as an intravenous treatment.

Introduction

Vasospasm of the cerebral arteries due to subarachnoid haemorrhage (SAH) is a major source of delayed ischaemic deficits in patients with ruptured aneurysms, which are still responsible for considerable morbidity and mortality in patients (Mayberg 1998). The mechanisms responsible for delayed vasospasm following SAH are unclear and therefore remain the focus of several animal studies (Dreier et al 2002). Nimodipine (NM) is presently the only available therapy that has been proven to reduce the morbidity and mortality associated with delayed ischaemic deficits in patients with SAH-related vasospasm (Dorsch 2002).

NM (isopropyl-2-methoxyethyl-1,4-dihydro-2,6-dimethyl-4-(3-nitro-phenyl)-3,5-pyridine dicarboxylate) is a dihydropyridine calcium channel blocker with therapeutic effects for cerebrovascular spasm, stroke and migraine (Gelmers 1985; Langley & Sorkin 1989). However, the clinical usefulness of NM is limited by its high first-pass effect in liver, which leads to low oral bioavailability (Muck et al 1996; Blardi et al 2002) and poor brain penetration. Intravenous nimodipine administration is an alternative to oral administration that provides greater bioavailability than oral dosing. Nevertheless, NM ethanol injections may lead to local adverse reactions, such as pain and inflammation at the administration site. There is therefore a need for alternative routes of administration to improve therapeutic effects and reduce irritation.

Lipid microspheres (LMs) are colloidal particles of vegetable oils and lecithin with an average diameter of 200 nm, which are initially used to supply calories to patients unable to obtain adequate nourishment normally. They have no particular adverse effects, even at dose levels of 500 mL. Their usefulness as carriers stems from their ability to incorporate drugs with poor water solubility within the dispersal phase (Müller et al 2004). Thus, direct contact of the drug with body fluids and tissues can be avoided and the drug is released slowly over a prolonged period of time, which may markedly reduce side-effects (Bock & Müller

Department of Pharmaceutics, School of Pharmacy, Shenyang Pharmaceutical University, Shenyang 110016, China

Jia Yu, Hai Bing He, Xing Tang

Correspondence: Xing Tang, 103 Wenhua Road, Shenyang 110016, China. E-mail: tangpharm@yahoo.com.cn

1994). The incorporation of a drug in LMs can also enhance its activity and bioavailability. Prostaglandin E_1, dexamethasone palmitate and flurbiprofen axetil are available as LM pharmaceuticals on the market. As NM is a poorly water-soluble drug, LMs would be a good carrier. The NM-loaded LMs were prepared by dissolving NM in the oil phase in order to increase its solubility and reduce local adverse reactions. Stimulation was greatly reduced by NM-loaded LMs. LMs of NM are therefore a good substitute for intravenous NM ethanol solutions to prevent and treat the neurological defects secondary to cerebral vascular spasm caused by subarachoid haemorrhage. LMs themselves are very stable and can be stored for up to 1 year at room temperature. However, incorporating drugs into LMs can result in an interaction with the emulsifier film, leading to break down of the LMs. Based on this background, the objective of this study was to search for a stable formula using different excipients for parenteral use. The irritation test and pharmacokinetic study were also performed to evaluate the NM-loaded LMs.

Materials and Methods

Materials

NM was purchased from the Tianjin Zhongyang Pharmaceutical Factory, China. NM solution ($0.2\,mg\,mL^{-1}$) was prepared using 23.7% ethanol and 17% PEG 400. Nitrendipine was obtained from the Sandong Xinhua Pharmaceutical Factory, China. Lipoid E80 was purchased from the Germany Lipoid Company. All other chemicals and reagents were of analytical or chromatographic grade.

Solubility of NM in water of different pH and oils

Excess NM was added to centrifuge tubes containing 2 mL of different solvents and the mixtures were equilibrated in a HZQ-C air bath agitator at 37°C for 48 h. After centrifugation at 4000 rpm for 10 min, the supernatant was determined by HPLC.

Preparation of NM-loaded LMs

Lipoid E-80 and NM were dispersed in an oil phase and an aqueous phase consisting of Tween-80, glycerol and sodium oleate. Then, with continuous stirring, the oil phase was slowly added to the aqueous phase at 75°C. This mixture was pre-emulsified using a high-shear mixer (FJ-200, Shanghai Model Factory) at 8000 rpm for 5 min. Finally LMs were obtained by passing the coarse solution through a Niro Soavi NS10012k high-pressure homogenizer (Italy). Next, the pH of the LMs was adjusted to about 8 using 0.1 M hydrochloric acid. Finally, the LMs in the ampoule were sterilized on a water bath at 100°C for 30 min (Yokoyama & Watanabe 1996).

Determination of entrapment efficiency of LMs

Centrifugation of LMs was carried out on a Hitachi ultracentrifuge at 50 000 rpm for 2 h at a temperature of 10°C. The amount of NM in the aqueous phase and LMs was estimated using HPLC. The separation was performed on a 4.6 mm × 200 mm, C18 analytical column (Diamonsil) at room temperature. The mobile phase was 75% methanol/25% purified water (v/v) at a flow rate of $1\,mL\,min^{-1}$ and the detection wavelength was 237 nm (Meng et al 2002).

Dilution stability of LMs

The stability of LMs in two diluting fluids was studied using a reported method (Collins-Gold et al 1990). Briefly, LMs were dispersed in 0.9% NaCl and 5.4% glucose and their particle size distribution, ζ-potential and entrapment efficiency were determined after 0.5, 1, 2, 4, 8 and 12 h. The mean particle size and ζ-potential of the LMs were measured by a ζ-potential/particle sizer NICOMP 380ZLS (Santa Barbara, USA).

Rat paw lick test

In this test, a group of 10 Wistar rats weighing approximately 100 g received a subplantar injection of 0.1 mL NM-loaded LMs and ethanol injection (positive control) into the footpad of their right hind paw. Thereafter, the number of times the animals licked the paw as well as the total time the paw was licked for were monitored over a period of 15 min (Celozzi et al 1980; Lovell 1994).

Rabbit ear vein test

In this test groups of six rabbits weighing 2.0–2.4 kg received an infusion of NM-loaded LMs, NM ethanol solution (positive control) and an equivalent volume of 5% glucose solution (negative control) into their marginal ear vein. The rate of infusion was maintained at 18 drops min^{-1} and the drug was given on four consecutive days at a dose of $2\,mg\,kg^{-1}\,day^{-1}$. Following infusion, visual observations of the vascular reaction were made every day. Forty-eight hours after the last administration, three rabbits from each group were killed and a piece of vascular tissue at the site of injection was removed for histopathological examination. Other rabbits were kept under observation for 1 week.

Pharmacokinetic and tissue distribution studies

A comparative pharmacokinetic study was performed with NM ethanol solutions and NM-loaded LMs (Srinath & Diwan 1998). Male Wistar rats weighing 200 ± 10 g were divided into two groups with six animals in each group. The dosage of NM ethanol solution for humans is $0.2–0.8\,mg\,kg^{-1}\,day^{-1}$ and the dosage for rats was calculated using a skin surface area conversion table. Considering the injecting volume, a dose of $1.2\,mg\,kg^{-1}$ via the femoral vein was selected. At predetermined time points (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), rats were anaesthetized by ether and 0.5 mL blood samples were collected by retro-orbital puncture and centrifuged immediately at 6000 rpm for 10 min to obtain plasma. In the tissue distribution study, six rats from each group were killed by decapitating at predetermined time points (0.5 h, 2 h, 6 h, 12 h and 24 h) and various tissues were removed.

To 200 μL plasma samples, 50 μL nitrendipine methanol solution (3 μg mL^{-1}) was added as the internal standard and extracted with 2 mL extraction solvent (N-hexane/chloroform/isopropanol 57:38:5) by vortexing for 5 min. After centrifugation at 4000 rpm for 10 min, the supernatant was transferred to a conical tube. The separated organic phases were then evaporated to dryness under a gentle stream of nitrogen at 40°C. The residue was reconstituted in 100 μL methanol and then 20 μL was injected onto an HPLC system. Tissues were homogenized by adding a two-fold amount of sodium chloride per 0.5 g tissue and tissue homogenates were processed similarly to plasma samples.

As NM is sensitive to light-induced degradation, all operations except data evaluation were carried out protected from light (Muck & Bode 1994).

The experimental procedures were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

Statistical analysis

The effect of pH on solubility and the effect of oil type on drug solubility were statistically examined using a one-way ANOVA. Statistical analysis of the effect of time and diluent type on particle size and time was determined using the paired-samples *t*-test. The effect of formulation type on the number of animals licking was analysed using Fischer's exact test. The effect of formulation type on various pharmacokinetic parameters, average number of times each rat licked and average total licking time was determined using the Mann–Whitney *U*-test. The pharmacokinetic and tissue distribution results were analysed statistically using Student's independent samples *t*-test. A significance level of $P<0.05$ denoted significance in all cases. All data were analysed using SPSS statistical software.

The blood concentration–time curve was analysed initially with the Pharmacokinetics Program 3p87 edited by the Mathematics Pharmacological Committee, Chinese Academy of Pharmacology, which determined the most appropriate pharmacokinetic model to describe the experimental data. The model was selected on the basis of the residual sum of squares and the minimum Akaike's information criterion value. The area under the curve of concentration vs time (AUC) from zero to the last time point was calculated by the log-linear trapezoidal method. The mean residence time (MRT), serum clearance (Cl), the volume of distribution (V_c) and the elimination rate constant (K_e) of the drug were obtained by a non-compartment analysis based on the statistic moment theory.

Results and Discussion

LM composition

The basic formulation refers to the intralipid on sale, which consisted of oil phase 10 g, emulsifier (e.g. lecithin) 1.2 g, sodium oleate 0.03 g, glycerol 2.25 g and double-distilled water to 100.0 mL. To optimize the formula of the LMs, an L_9 (3^4) orthogonal design experiment was conducted as part of the study with the factors of egg lecithin, oil phase 10% (soybean oil/medium chain fatty acid) and Tween-80. To comply with the requirements for LMs, the appearance, particle size distribution and stability should be taken into consideration by evaluating the formula. The solubilities of NM in water were 1.58, 2.06, 2.68, 3.04, 2.97 and 3.08, with pHs of 2, 4, 6, 7, 10 and 12. NM is nearly insoluble in water of different pHs whereas the solubilities were 5.98, 37.6, 7.13 and 14.5 in soybean oil, medium chain fatty acid, arachis oil and coconut oil, respectively. The solubility in water of a different pH was not statistically significantly different ($P>0.05$), but significant differences were shown in different oils ($P<0.05$). Mixing soybean oil with medium chain fatty acid (MCT) led to a decrease in the viscosity of soybean oil (Jumaa & Müller 2001), the particle size distribution and, simultaneously, to an increase in the drug solubility and entrapment efficiency. However, the stability may be reduced by the addition of too much MCT because of the formation of free oil droplets. A 1:1 mixture of soybean oil/MCT seemed to be the optimal mixture. Under an optical microscope, drug crystals could be seen when insufficient egg lecithin was used as the main emulsifier. However, if there was too much egg lecithin, small particles would be formed by the remaining lecithin and the particle size would exhibit a bimodal distribution. Tween-80 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. However, the toxicity should also be taken into consideration (Sznitowka et al 2001). Different amounts of NM, from 0.02 to 0.2%, were dispersed in the oil phase in order to investigate the load capacity of the LMs. The drug load capacity experiment showed that the LMs were unstable at 0.2% because of the precipitation of the drug crystal. According to the conventional dosage of NM, the LMs should be diluted prior to administration. A high concentration of NM must be diluted in a large amount of medium, leading to a decrease in the entrapment efficiency. From the dilution experiment, the concentration of the drug-loaded LMs should not exceed 0.1% in order to satisfy the demand of entrapment efficiency. The optimal formula based on the experimental data was NM 0.1%, oil phase 10% (soybean oil/MCT 5:5), Tween-80 0.1%, egg lethicin 1.2%, glycerol 2.25%, sodium oleate 0.03% and water to total 100 mL.

Emulsification procedure

The efficiency of the emulsification process was recorded by measuring the mean droplet diameter (Jumaa & Müller 1998; Trotta et al 2002). The results demonstrate a decrease in particle size on increasing the number of cycles initially; however, after the sixth cycle the particle size did not significantly decrease, and the standard deviation increased markedly after the 10th cycle. Repeating the processing or cycling resulted in a decrease in average particle size and a narrowing of the particle size distribution, after which the mean particle size and standard deviation both increased as the process continued. The homogenization pressure also affected the particle size of the LMs. The particle size decreased on increasing the pressure, but too high pressure led to an increase in the standard deviation. This observation is in agreement with a

report showing that the droplet size is a result of breakdown and coalescence and that, for systems containing relatively high percentages of oil, increasing the operational pressure did not always lead to a reduction in the particle size (Coulaloglou & Tavlarides 1997). The homogenization was therefore best performed using 6 to 10 cycles at a pressure of 600–800 bar.

Characterization of LMs

Before sterilization, the particle size was a little larger compared to that after sterilization. It was reported that sterilization was perhaps a re-emulsifying procedure that improved the fluidity and decreased the particle size. However, the absolute value of the ζ-potential decreased after sterilization. Under the high temperature of sterilization, the lipid hydrolysed to a long-chain fatty acid and a new charged long-chain acid radical formed to decrease the ζ-potential. The particle size was 188.2 ± 5.4 nm and the ζ-potential was -31.6 mV. The entrapment efficiency of NM was 94.2%, hence almost all the NM was located in the oil phase.

The average particle size and ζ-potential of the LMs were not statistically significantly affected by diluting with 0.9% NaCl and 5.4% glucose for up to 12 h ($P > 0.05$), but the standard deviation of the particle size distribution increased, suggesting the possibility of aggregation of the LMs. The particle size of the LMs after diluting with NaCl and glucose is displayed in Table 1. The entrapment efficiency decreased rapidly initially, probably because of the destruction of the layer of emulsifier, which led to leakage of the drug. However, the leakage of drug decreased with time and about 80% NM remained in the oil phase after 12 h. These results suggest that the NM-loaded LMs can be safely diluted with 0.9% NaCl or 5.4% glucose during the therapy. The properties of LMs, such as pH, particle size and ζ-potential, did not change significantly during storage. The long-term stability study results show that LMs are stable for 1 year at 4–10°C.

Rat paw lick test

In order to assess the level of pain reduction due to drug delivery in LMs, an animal model called the rat paw lick test was used. The basis of this test is that the more painful the formulation, the greater the number of animals that perform paw licking. In addition, the number of times and the total time an animal licks its paw also increases with pain and/or irritation. Table 2 summarizes the data from the rat paw lick study. When NM ethanol solution was injected, all the animals licked their paws. Each rat licked its paw about 12 times, which corresponds to a total licking time of about 35 s. However, when NM-loaded LMs were administered by injection, only 60% of the animals licked their paws and the number of times the rats licked their paws was reduced to five. This, in turn, equated to a total licking time of about 21 s. This test suggests that an injection of LMs may be only 50% as painful as NM ethanol solutions. There were statistically significant differences in number of animals licking, the average number of times each rat licked and the average total licking time between the two formulations ($P < 0.05$). NM-loaded LMs reduced irritation significantly compared to NM ethanol solutions.

Rabbit ear vein irritation test

The rabbit ear vein irritation test was used to evaluate the irritation of drug formulations following i.v. injection and the results were compared with those of NM ethanol solutions. There was no internal clot, angiorrhexis or inflammatory cell infiltrate, and endothelial tissue and the vessel wall remained intact in all the experimental groups. The histopathologic slide photos of rabbit ear-rim auricular vein are displayed in Figure 1. From macroscopic observation, vascular engorgement and dropsy were seen at the injection site. Furthermore, angiectasia and erythrocyte aggregation were observed at or away from the site of injection of NM ethanol solutions by light microscope. However, these phenomena were not observed in 5% glucose solution and LMs. Based on these observations, the injection of LMs was assumed to be less irritating than the NM solutions.

In-vivo evaluation of NM-loaded LMs

NM is highly lipophilic, which is the major reason for its pharmacokinetic properties. The pharmacokinetic profile of NM is best described by a two-compartment model, reflecting a rapid distribution from blood into tissues, and rapid metabolic clearance from blood (Zhang et al 2004). The linear ranges of NM were 15–1500 and 5–1000 ng mL^{-1} and the inter- and intra-day variations were less than 4 and 6% for plasma and tissue samples, respectively. The relative recoveries of NM from plasma and tissue homogenates were more than 90 and 80%, respectively. The organ concentration of NM following administration of ethanol solutions and LMs is shown in Table 3.

Incorporation of NM into LMs enhanced the total amount of drug accumulation in the liver and spleen. A difference between the drug concentrations obtained with these two formulations was evident after 2 h and then

Table 1 Change in particle size distribution of LMs after dilution with different solutions

	0	0.5 h	1 h	2 h	4 h	8 h	12 h
NaCl (nm)	178.6 ± 60.0	182 ± 63.3	183.5 ± 64.4	188.1 ± 60.4	189.5 ± 64.4	188 ± 65.4	195.9 ± 71.1
Glucose (nm)	178.6 ± 60.0	178.9 ± 65.3	186.7 ± 61.1	187.9 ± 64.4	189.5 ± 59.1	190.4 ± 62.8	190.4 ± 60.5

Values are reported as mean ± s.d., n = 3. $P > 0.05$.

Table 2 Results of rat paw lick test

Formulation tested	Number of animals licking	Average number of times each rat licked	Average total licking time (s)
NM-loaded LMs	60% (6/10)	5	21
NM ethanol solution	100% (10/10)	12	35

Values are reported as mean ± s.d., n = 10. $P < 0.05$.

gradually decreased. There was no statistically significant difference in the concentration of the two formulations in each organ. From the above biodistribution data it is evident that the sites of the highest accumulation of encapsulated NM are the liver and spleen, confirming that its in-vivo fate is similar to that of other colloidal drug delivery systems (Junzo et al 2004). It may be assumed that the size of the LMs is a critical factor in restricting the localization of the drug in these organs. From the pharmacokinetic study it was found that both formulations follow a two-compartment model with a weighting factor of $1/c^2$. Figure 2 shows the plasma concentration–time profiles of NM after intravenous administration of NM-loaded LMs and NM ethanol solution. Pharmacokinetic parameters such as AUC and distribution half-life (two-compartment model) are displayed in Table 4. The overall plasma concentrations of NM on administration of NM-loaded LMs were slightly higher than those after the administration of NM ethanol solution, but the observed differences in the concentration, $T_{1/2}\ \alpha$, $T_{1/2}\ \beta$ (h) and AUC_{0-t} were not statistically significant between the two drug formulations ($P > 0.05$). There was no significant retention of NM-loaded LMs in plasma probably because NM is present not only in the oil phase but also in the oil/water interfacial film (Akkar & Müller 2003). The pharmacokinetics did not change markedly when administration in the form of LMs was compared with ethanol solutions.

Conclusion

In conclusion, the LM system consisting of 0.1% NM, 10% oil phase (soybean oil/MCT 1:1), 1.2% lecithin, 0.1% Tween-80, 2.25% glycerin, 0.03% sodium oleate and water is the optimal formulation of NM-loaded LMs. This mixture is stable for 1 year at a temperature of 4–10°C. The solubility of NM was increased by dissolving it in the oil phase and direct contact of the drug with body fluids and tissues was avoided. The pharmacokinetic studies revealed that encapsulated NM in LMs did not significantly alter the pharmacokinetic parameters

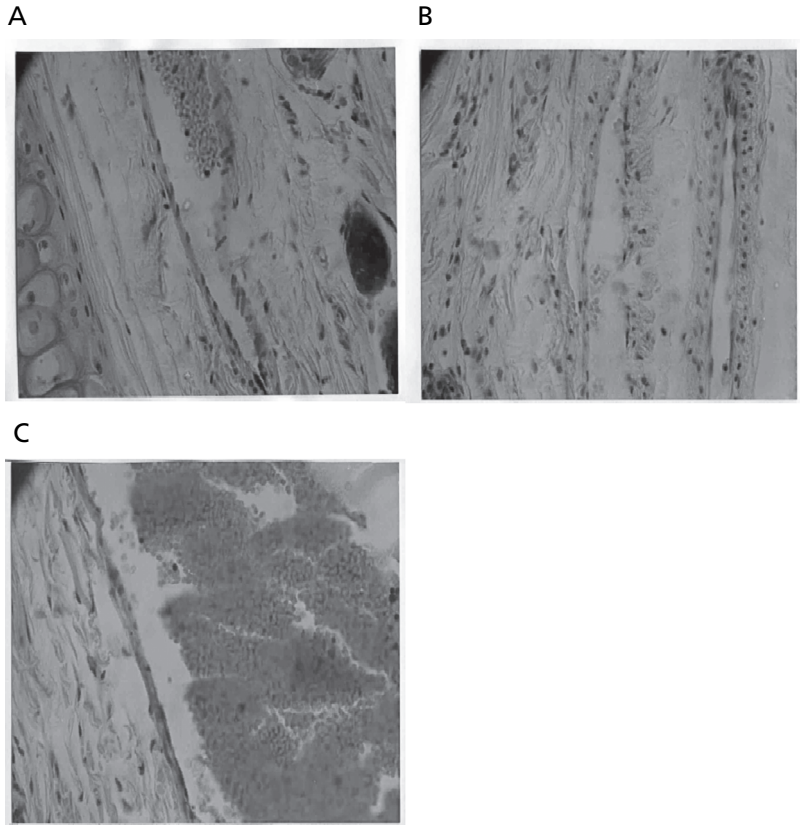

Figure 1 The histopathologic slide photos of rabbit ear-rim auricular vein following the administration of different solutions: (A) 5% glucose solution; (B) NM-loaded LMs; (C) NM ethanol solution.

Table 3 Tissue distribution of NM ethanol solution (ES) and LMs (ng 0.5 g^{-1})

		0.5 h	2 h	6 h	12 h	24 h
Heart	ES	50.33 ± 21.98	56.32 ± 56.20	118.61 ± 67.93	56.85 ± 43.30	34.40 ± 8.78
	LMs	65.76 ± 8.75	75.42 ± 73.32	139.07 ± 134.68	79.97 ± 57.49	52.18 ± 43.83
Liver	ES	27.57 ± 24.97	37.70 ± 33.67	360.77 ± 278.55	331.04 ± 175.03	151.08 ± 119.9
	LMs	29.80 ± 21.27	140.77 ± 140.75	428.16 ± 369.51	300.08 ± 91.42	216.34 ± 171.03
Spleen	ES	14.42 ± 4.76	42.09 ± 33.80	142.15 ± 140.40	38.04 ± 36.03	25.90 ± 7.27
	LMs	32.18 ± 26.9	105.28 ± 103.40	155.46 ± 150.47	104.45 ± 104.19	39.59 ± 20.84
Lung	ES	84.51 ± 69.20	370.50 ± 135.23	231.83 ± 231.70	155.82 ± 115.31	59.53 ± 52.58
	LMs	81.45 ± 53.39	320.17 ± 310.39	195.45 ± 194.23	121.70 ± 112.24	54.04 ± 12.39
Kidney	ES	113.15 ± 28.53	134.89 ± 110.62	292.03 ± 284.81	80.99 ± 56.05	58.83 ± 12.40
	LMs	97.65 ± 91.05	114.17 ± 73.70	294.86 ± 239.36	144.12 ± 61.15	42.04 ± 18.02
Brain	ES	68.35 ± 9.46	247.48 ± 35.37	334.58 ± 186.61	117.70 ± 51.98	112.43 ± 109.95
	LMs	64.07 ± 19.49	260.53 ± 202.62	322.76 ± 243.69	123.99 ± 56.01	129.92 ± 128.65

Values are reported as mean ± s.d., n = 3. $P > 0.05$.

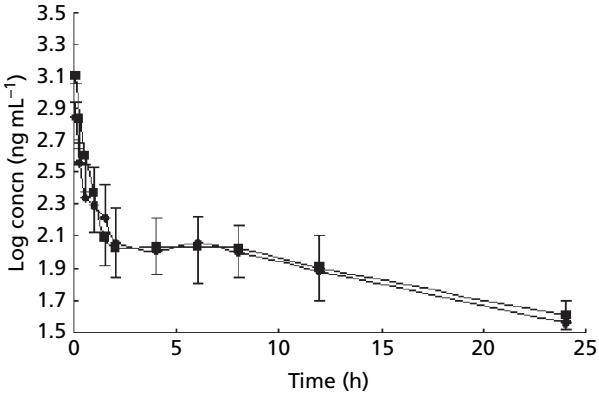

Figure 2 The plasma concentration–time profiles of NM following the administration of 1.2 mg mL^{-1} NM solutions and NM-loaded LMs to fasted Wistar rats (n = 6). ◆, NM solutions; ■, NM-loaded LMs.

and tissue distribution except for the increased accumulation of NM-loaded LMs in reticuloendothelial system-rich areas, such as the liver and spleen. On the whole, incorporation of NM in LMs reduced the adverse effect induced by the high concentration of alcohol in the NM injection and was a good alternative to NM injections. The animal tests used to assess the potential of LMs in reducing pain suggested the possibility of a two- to three-fold pain reduction using LMs. In conclusion, the LM system is a promising approach for the intravenous delivery of NM in order to achieve improved therapeutic effects.

References

Akkar, A., Müller, R. H. (2003) Formulation of intravenous carbamazepine emulsions by SolEmuls technology. *Eur. J. Pharm. Biopharm.* **55**: 305–312

Blardi, P., Urso, R., De Lalla, A., Volpi, L., Perri, T. D., Auteri, A. (2002) Nimodipine: drug pharmacokinetics and plasma adenosine levels in patients affected by cerebral ischemia. *Clin. Pharmacol. Ther*. **72**: 556–561

Bock, T., Müller, B. W. (1994) A novel assay to determine the hemolytic activity of drugs incorporated in colloidal carriers systems. *Pharm. Res*. **11**: 589–591

Celozzi, E., Lotti, V. J., Stapley, E. O., Miller, A. K. (1980) An animal model for assessing pain-on-injection of antibiotics. *J. Pharmacol. Methods* **4**: 285–289

Collins-Gold, L. C., Lyons, R. T., Bartholow, L. C. (1990) Parenteral emulsions for drug delivery. *Adv. Drug Del*. **5**: 189–208

Coulaloglou, C. A., Tavlarides, L. L. (1997) Descriptions of interaction process in agitated liquid–liquid dispersions. *Ind. Chem. Fundament*. **25**: 554–560

Dorsch, N. W. (2002) Therapeutic approaches to vasospasm in subarachnoid hemorrhage. *Curr. Opin. Crit. Care* **8**: 128–133

Dreier, J. P., Windmuller, O., Petzold, G., Lindauer, U., Einhaupl, K. M., Dirnagl, U. (2002) Ischemia triggered by red blood cell products in the subarachnoid space is inhibited by nimodipine administration or moderate volume expansion/hemodilution in rats. *Neurosurgery* **51**: 1457–1465

Gelmers, H. J. (1985) Calcium-channel blockers in the treatment of migraine. *Am. J. Cardiol*. **55**: 139B–143B

Jumaa, M., Müller, B. W. (1998) The effect of oil components and homogenization conditions on the physicochemical properties and stability of parenteral fat emulsions. *Int. J. Pharm*. **163**: 81–89

Table 4 Pharmacokinetic parameters after i.v. administration of NM ethanol solution (ES) and LMs

Parameter	$T_{1/2}\,\alpha$ (h)	$T_{1/2}\,\beta$ (h)	V_c (mg kg^{-1})/(ug mL^{-1})	Cl mg kg^{-1} h^{-1}/(ug mL^{-1})	AUC_{0-t} (ng mL^{-1} × h)	MRT (h)	K (1/h)
LMs	0.26 ± 0.11	21.7 ± 10.5	0.24 ± 0.05	0.13 ± 0.03	2844.9 ± 948.7	7.45 ± 0.96	0.1 ± 0.03
ES	0.34 ± 0.24	21.3 ± 8.4	0.44 ± 0.17	0.19 ± 0.06	2328.9 ± 1004.1	7.36 ± 1.04	0.1 ± 0.05

Values are reported as mean ± s.d., n = 6. $P > 0.05$.

Jumaa, M., Müller, B. W. (2001) Development of a novel parenteral formulation for tetrazepam using a lipid emulsion. *Drug Dev. Ind. Pharm*. **27(10)**: 1115–1121

Junzo, S., Satoru, S., Akiira, S. (2004) A nanometer lipid emulsion, lipid nano-sphere (LNS), as a parenteral drug carrier for passive drug targeting. *Int. J. Pharm*. **273**: 75–83

Langley, M. S., Sorkin, E. M. (1989) Nimodipine: a review of its pharmaco-dynamic and pharmacokinetic properties, and therapeutic potential in cerebrovascular disease. *Drugs* **37**: 669–699

Lovell, M. W., Lohnson, H. W., Hui, H. W., Cannon, J. B., Gupta, P. K., Hsu, C. C. (1994) Less-painful emulsion formulation for intravenous administration of clarithromycin. *Int. J. Pharm*. **109**: 45–57

Mayberg, M. R. (1998) Cerebral vasospasm. *Nurs. Clin. North Am*. **9**: 615–627

Meng, J., Chen, D., Jiang, X., Hu, J. (2002) Study on pharmacokinetics and relative bioavailability of nimodipine tablets. *West China J. Pharm*. **17(1)**: 32–34

Muck, W., Bode, H. (1994) Bioanalytics of nimodipine – an overview of methods. *Pharmazie* **49**: 130–139

Muck, W., Breuel, H. P., Kuhlmann, J. (1996) The influence of age on the pharmacokinetics of nimodipine. *Int. J. Clin. Pharmacol*. **34**: 293–298

Müller, R. H., Schmidt, S., Buttle, I., Akkar, A., Schmitt, J., Bromer, S. (2004) SolEmuls – novel technology for the formulation of i.v. emulsions with poorly soluble drugs. *Int. J. Pharm*. **269**: 293–302

Srinath, P., Diwan, P. V. (1998) Pharmacodynamic and pharmacokinetic evaluation of lipid microspheres of indomethacin. *Pharm. Acta Helv*. **73**: 199–203

Sznitowka, M., Janicki, S., Dabrowska, E., Zurowska-Pryczkowska, K. (2001) Submicronemulsions as drug carriers. Studies on destabilization potential of various drugs. *Eur. J. Pharm. Sci*. **12**: 175–179

Trotta, M., Pattarino, F., Ignoni, T. (2002) Stability of drug-carrier emulsions containing phosphatidylcholine mixtures. *Eur. J. Pharmaceut. Biopharmaceut*. **53**: 203–208

Yokoyama, K., Watanabe, M. (1996) Limethason as a lipid microsphere preparation: an overview. *Adv. Drug Del. Rev*. **20**: 195–201

Zhang, Q., Jiang, X., Jiang, W., Lu, W., Su, L., Shi, Z. (2004) Preparation of nimodipine-loaded microemulsion for intranasal delivery and evaluation on the targeting efficiency to the brain. *Int. J. Pharm*. **275**: 85–96