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Research Article

Liquid Proliposomes of Nimodipine Drug Delivery System: Preparation, Characterization, and Pharmacokinetics

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Abstract. To investigate the possibility of liquid proliposomes being carriers for oral delivery, nimodipine liquid proliposomes-based soft capsules (NPSC) were prepared. Nimodipine proliposomes were characterized by transmission electron microscopy (TEM), conversion rate from proliposomes to liposomes, entrapment efficiency, particle size, and zeta potential. Accelerated stability testing of NPSC was carried out for 3 months at 40±2°C, 75±5% RH. The concentration of nimodipine in plasma of New Zealand rabbits of NPSC, nimodipine soft capsules, and hydrated liposomes was studied. Results showed that nimodipine proliposomes were automatically converted into liposomes when exposed to a water phase in 30 s. The average diameter was 378.6±26.5 nm in distilled water with entrapment efficiency (EE%) of 84.7±5.9%, while the average diameter was 316.9±34.6 nm in 0.1 M hydrochloric acid solution with EE% of 72.8±4.7%. Accelerated stability test showed that there was no change in drug content, particle size, and EE% except for a decrease in dissolution of nimodipine. *In vivo* experiments, areas under the plasma level-time curve of NPSC and nimodipine-hydrated liposomes increased 2.41 and 2.34 times more than that of nimodipine soft capsules, peak concentration increased 2.87 and 2.92 times, time of peak concentration from 0.75 to 2 and 1 h, respectively. Nimodipine-hydrated liposomes presented similar pharmacokinetic parameters compared with NPSC. Results suggested that NPSC offered a potential way to improve oral delivery of nimodipine.

KEY WORDS: liquid proliposomes; nimodipine; pharmacokinetics; soft capsules; stability.

INTRODUCTION

Nimodipine is a dihydropyridine calcium antagonist with therapeutic indications for cerebrovascular spasm, stroke, and migraine (1,2). Nowadays, nimodipine is administered by oral soft capsules and capsules clinically; however, there are several unfavorable properties which limited their clinical efficacy, including the low bioavailability, large dosage and many adverse effects (3,4). Furthermore, the drug is water-insoluble and decomposes easily under the light, resulting in loss of activity (5). So there is a need to find a new way to enhance the solubility and bioavailability of nimodipine.

In recent years, liposomes have received a lot of attention as a drug carrier to improve the therapeutic activity, reduce side effects, and improve stability of drugs by protecting compounds from chemical degradation or transformation (6,7). Among the various routes of liposomes administration, the oral route is advantageous for its versatility, safety, and patient compliance. However, less integrity of liposomes at the site of absorption, physicochemical instability, such as hydrolysis, separation of drug from liposomes, sedimentation, and aggregation limited their utilization for oral delivery of drugs (8).

These problems can be solved by proliposomes. Proliposomes were capable of forming a liposomal suspension upon the addition of a water phase or *in vivo* conditions (9). They offer the opportunity to form liposomes only at the site of delivery, which is more stable during sterilization and storage.

Proliposomes are defined as dry, free-flowing particles at first (9). Liquid proliposomes were developed in recent years. It was reported to be a kind of transparent solution and form drug-loaded liposomes when blank proliposomes were mixed with 0.9% NaCl aqueous solution containing drug (10). Compared with solid proliposomes, the preparation process of liquid proliposomes was simpler and required no specialized devices. Additionally, drug-containing liposomes can automatically generate in short time without applied force, such as sonication or extruders when liquid prolioposomes contacted a water phase. The mean particle size of liposomes hydrated from liquid proliposomes was smaller and could be of unimodal narrow distribution. However, less-relevant studies about liquid proliposomes for oral administration have been reported yet.

The aim of the current study was to develop a kind of liquid proliposomes of nimodipine for improving its oral bioavailability and further increasing the stability of liposomes. Soft capsules were selected as containers for nimodipine proliposomes. On dissolution of the soft capsule shell, proliposomes were released and hydrated to form nimodipine

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liposomes. Proliposomes were characterized by particle size, drug EE%, zeta potential, transmission electron microscopy (TEM), and conversion rate from proliposomes to liposomes. The drug content (in percent), dissolution rate, and disintegration time of nimodipine liquid proliposomes-based soft capsules (NPSC) were evaluated. Water in capsule shells (in percent) and accelerated stability testing of NPSC were also studied for 3 months at 40±2°C, 75±5% RH. Finally, the pharmacokinetics of NPSC was investigated, compared with nimodipine soft capsules and nimodipine-hydrated liposomes.

MATERIALS AND METHODS

Materials

Nimodipine, PEG-400, and dehydrated alcohol were the products of Nanjing Reagent Company. Sodium deoxycholate was obtained from Shanghai Lanji Technology Development Co., Ltd. Poloxamer-188 was from BASF Co., Ltd. Soybean phospholipid was the generous gift of Cargill Texturizing Solutions Deutschland GmbH & Co.KG. All reagents used were of analytical grade or better.

Animals

New Zealand male rabbits with an average weight of 2.1 ±0.42 kg were purchased from Experimental Animal Center of China Pharmaceutical University. The animal studies were in compliance with the university conduct and adhered to the principles of Institutional Animal Care and Use Committee Guidebook (http://en.wikipedia.org).

Preparation of NPSC, Nimodipine Soft Capsules, and Hydrated Liposomes

The preparation method of nimodipine proliposomes used in this study was less venomous and more efficient. A mixture of nimodipine, soybean phospholipids, sodium deoxycholate, poloxamer-188, and PEG-400 was dissolved in dehydrated alcohol by sonication (DL-720, Shanghai China), and then filled into ampules (2 ml) and sealed after oxygen was driven out with aseptic nitrogen gas. It was a light yellow transparent liquid. The optimal ratio was nimodipine/soybean phospholipids/sodium deoxycholate/poloxamer-188/PEG-400/dehydrated alcohol 1:20:0.8:6.7:20:80 (*w*/*w*). The capsule shells were made in the laboratory which consisted of gelatin/glycerol/distilled water 1:0.5:1.3 (*w*/*w*). Proliposomes were filled into the bodies of capsule shells by a syringe. The content of nimodipine in each capsule was about 5 mg. Openings of the capsules were sealed with a hot metal spatula. These capsules were dried for 4 h at ambient temperature and placed in two 20-ml clear glass vials. Each vial was capped, and placed in a desiccator with 25% RH.

The nimodipine soft capsule control was prepared according to the same procedure of NPSC except adding soybean phospholipids, sodium deoxycholate and poloxamer-188. The capsule shell component is also same as the capsule shell of NPSC.

Hydrated liposomes were formed automatically by dropping distilled water to nimodipine proliposomes and shaking the mixture manually for 2 min.

Characterization of Proliposomes

Detemination of Nimodipine

The content of nimodipine in proliposomes was determined by an ultraviolet (UV)–visible detector, and 354 nm was selected as the detected wavelength. Drug and excipients were validated to have no interference with each other. The standard curve was constructed by plotting the concentration of nimodipine to the absorbance: C=75.229A–0.0749 (r= 0.9997). The linearity of the method was studied in the range of 5–45 $\mu g\,ml^{-1}$. Intra- and interday relative standard deviations were less than 1%. All of the absolute recoveries were 98~102%, with all relative standard deviations less than 1%.

Measurement of Size and Zeta Potential

Size and zeta potential of the hydrated liposomes were measured by dynamic light scattering with a Zetasizer 3,000 HS (Malvern, UK). Samples were hydrated with distilled water or 0.1 M hydrochloric acid (HCl) solution for size measurements. The proliposomes were hydrated in 0.1 M HCl solution and then appropriately diluted with distilled water to meet the zeta potential determination requirements of the instrument. The results are expressed as mean ± standard deviation for at least three different batches of each liposome formulation.

Transmission Electron Microscopy

Nimodipine proliposomes were mixed with a water phase to obtain liposomes. The microstructure of liposomes was examined by TEM (H-7000, Hitachi, Japan) with negative stain method (11). Samples were deposited on a carbon-coated copper grid to form a thin liquid film. Negative staining with 2% (*w*/*v*) phosphotungstic acid solution was performed to enhance image quality. After being air-dried at room temperature, the samples were accomplished for the TEM investigation.

Evaluation of Entrapment Efficiency

Unentrapped drug was separated from liposomes by a Sephadex-G50 column (400×15 mm) using distilled water for elution with the flow-rate of 1 $ml\,min^{-1}$, and then the opalescence part of the eluate was collected (12). Nimodipine in the eluate and in the suspensions was determined by UV–visible detector previously described. EE% was calculated with the following formula:

$$EE\% = (I/II) \times 100\% \quad (1)$$

Where I is the amount of drug in eluate and II is the amount of drug in the liposomes suspensions.

Conversion Rate From Proliposomes to Liposomes

Dispersion of liposomes in aqueous media can be determined by its turbidity (τ), the turbidity no longer changed when homogenenous dispersion was formed. There is a proportional relationship between turbidity (τ) and absorbance

(*A*) (13). Hence, this study evaluated the conversion rate from proliposomes to liposomes by measuring the absorbance during conversion process. Samples were prepared by mixing the desired amounts of nimodipine proliposomes with 0.1 M HCl solution of 3.0 ml in a 1-cm path length cuvette. Reference cuvette was filled with 0.1 M HCl solution to automatically subtract the absorbance of buffer. The absorbance was measured using an Agilent UV-8453 spectrophotometer (Agilent, USA) at 600 nm.

Accelerated Stability Test of NPSC

Stability indexes such as physical appearance, drug content in soft capsules, water in shell (in percent), drug dissolution rate, disintegration time of soft capsules, particle size, and encapsulation efficiency of liposomes were evaluated after storage of 3 months at 40±2°C, 75±5% RH.

Pharmacokinetics Studies in Rabbits

Pharmacokinetic Design

Eighteen New Zealand male rabbits with an average weight of 2.1±0.42 kg were used in this study. The rabbits were divided into three groups and fasted overnight before the experiments with free access to water. Each animal received a dose in one of the following dosage forms: (1) the nimodipine soft capsules group was supplied with nimodipine soft capsules at a dose of 8 mg kg^{-1}; (2) the NPSC group was given NPSC at a dose of 8 mg kg^{-1}; and (3) the nimodipine-hydrated liposomes group was offered nimodipine-hydrated liposomes at a dose of 8 mg kg^{-1}.

After oral administration of NPSC, nimodipine soft capsules, and nimodipine-hydrated liposomes, 2 ml of blood samples were collected from the marginal ear vein of each group at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, and 12 h. Plasma was separated by centrifugation at 10,000×*g* for 5 min and stored at −20°C until analysis.

Plasma Sample Preparation

Nitrendipine was used as the internal standard substance. Twenty microliters of nitrendipine solution (5 μg ml^{-1}) was added into 1 ml of plasma, which was then vortex-mixed for 30 s. Six milliliters of solution of ether/*N*-hexane=1:1 was added and vortex-mixed for 2 min. Then the mixture was centrifuged at 3,500×*g* for 20 min, the upper layer solution was transferred to a clean centrifuge tube and dried under nitrogen at 50°C. The residue was reconstituted with methanol (100 μl) and centrifuged at 12,000×*g* for 10 min. Aliquots (20 μl) of the supernate were injected into the HPLC system for analysis.

The method was validated by adding various quantities of nimodipine and equivalence nitrendipine to the blank rabbit plasma. The resulting concentrations of nimodipine were 5, 10, 20, 40, 80, 160, and 320 ng ml^{-1} and the concentration of nitrendipine was always 100 ng ml^{-1}. Nimodipine was extracted from the plasma using the liquid phase extraction method described above. The HPLC method was evaluated for selectivity, linearity, and precision.

Chromatographic System

HPLC system was applied to detect the nimodipine concentration in plasma. The stationary phase, μBondapak C18 column (150×4.6 mm, 5 μm), with a guard column (45×4.6 mm, 5 μm) was kept at 30°C. The mobile phase was a mixture of methanol/distilled water=60:40, and the flow rate was 1.0 ml min^{-1}. The detection wavelength was 237 nm.

Statistics and Data Analysis

Standard compartmental pharmacokinetic parameters (±SD) were calculated using the pharmacokinetic programs DAS 2.0 with a compartment model. Values were reported as mean±SD.

RESULTS AND DISCUSSION

Characterization of Proliposomes

Measurement of Size and Zeta Potential

Proliposomes were hydrated with distilled water and 0.1 M HCl solution to obtain liposomes. 0.1 M HCl solution was selected as hydration medium to simulate normal gastric juice. The liposomes were in nanometer size range both in distilled water and 0.1 M HCl solution. The average size of hydrated liposomes was 378.6±26.5 nm (n=3) with polydispersity index of 0.42 in distilled water and 316.9±34.6 nm (n=3) with polydispersity index of 0.49 in 0.1 M HCl solution. The size in 0.1 M HCl solution was a little smaller than that in distilled water. The reason might be that some liposomes were disrupted in the acidic environment (14).

Zeta potential measurements give information about the surface properties of the liposomes. Commonly, zeta potential can be an index to the stability of the nanospheres (15). There was stronger repellent interaction among nanospheres with higher absolute value of zeta potential which results in higher stability. The average zeta potential was −23.6±6.2 mV. The zeta potential data allowed predicting a good stability of the preparations since it was previously reported that a negative zeta potential higher than 20 mV was sufficient to prevent vesicle coalescence (16,17).

Transmission Electron Microscopy

TEM photos of the proliposomes after hydration alone or within soft capsules in different hydration mediums was shown in Fig. 1. The liposome vesicles as shown under TEM were all discrete spherical particles with distinct boundaries, which confirmed the formation of liposomes from proliposomes. However, it was noticed that the particle size obtained from TEM is significantly smaller as compared with particle size measured by dynamic light scattering technique. The reason is that the two methods are based on different sample preparation processes and different principles (18,19).

Nimodipine liposomes were successfully formed from its proliposomes in 0.1 M HCl solution as show in Fig. 1b; they were well-identified spheres; however, the size distribution was inhomogenous. It indicated that some liposomes might be disrupted in this acidic solution. Figure 1c, d showed that the disintegration

of soft capsule shells had not affected the formation of liposomes, and the same intact spherical morphology of liposomes was observed. In addition, sodium deoxycholate incorporated into the formulation could facilitate liposome formation, for that it was reported that lipids containing bile salts can readily transform into vesicular or mixed micelles in the gastrointestinal environment (20–24). It could be assumed that proliposomes would convert into liposomes upon contact with physiological fluids present in the body after oral administration.

Evaluation of Entrapment Efficiency

The average EE% of nimodipine liposomes hydrated in distilled water was 84.7±5.9% (n=3) and 72.8±4.7% (n=3) in 0.1 MHCl solution. The high EE% is probably due to the lipophilic character of nimodipine. Although there was a decrease of EE% in 0.1 MHCl solution, more than 70% of the originally entrapped nimodipine was still retained in liposomes incorporated. The decrease might be caused by disruption of some liposomes in the low pH environment which then resulted in drug leakage from liposomes. It was in agreement with the conjecture mentioned above.

Conversion Rate from Proliposomes to Liposomes

Figure 2 shows the turbidity change during the progress of liposomes formation from proliposomes in 0.1 MHCl solution. Proliposomes were transparent liquid before hydration, however, an evident turbidity change was observed after distilled water was added. There was the greatest absorbance at 30 s and no more increase, suggesting a progressive and rapid conversion of proliposomes to liposomes. It could be assumed that proliposomes would progressively and rapidly convert into liposomes upon contact with physiological fluids present in the body after oral administration.

Accelerated Stability Test of Proliposomes-Based Soft Capsules

Results of accelerated stability test were shown in Table I. In the conditions of 40±2°C, 75±5% RH for 3 months, nimodipine proliposomes wrapped in soft capsules still appeared transparent, and liposomes suspension was formed automatically when the proliposomes contacted with a water phase. The EE% and particle size of the reconstituted liposomes had no significant difference after storage of 3 months. So the nimodipine proliposomes were stable at 40±2°C, 75±5% RH for 3 months. It was considered that the soft capsule shells improved stability of nimodipine proliposomes which are highly susceptible to oxidation and hydrolysis.

However, there was a decrease in dissolution of nimodipine. Figure 3 shows the dissolution profile of nimodipine from proliposomes wrapped in soft capsules. The dissolution of nimodipine was as high as 92.7% in 60 min at 0 day, but

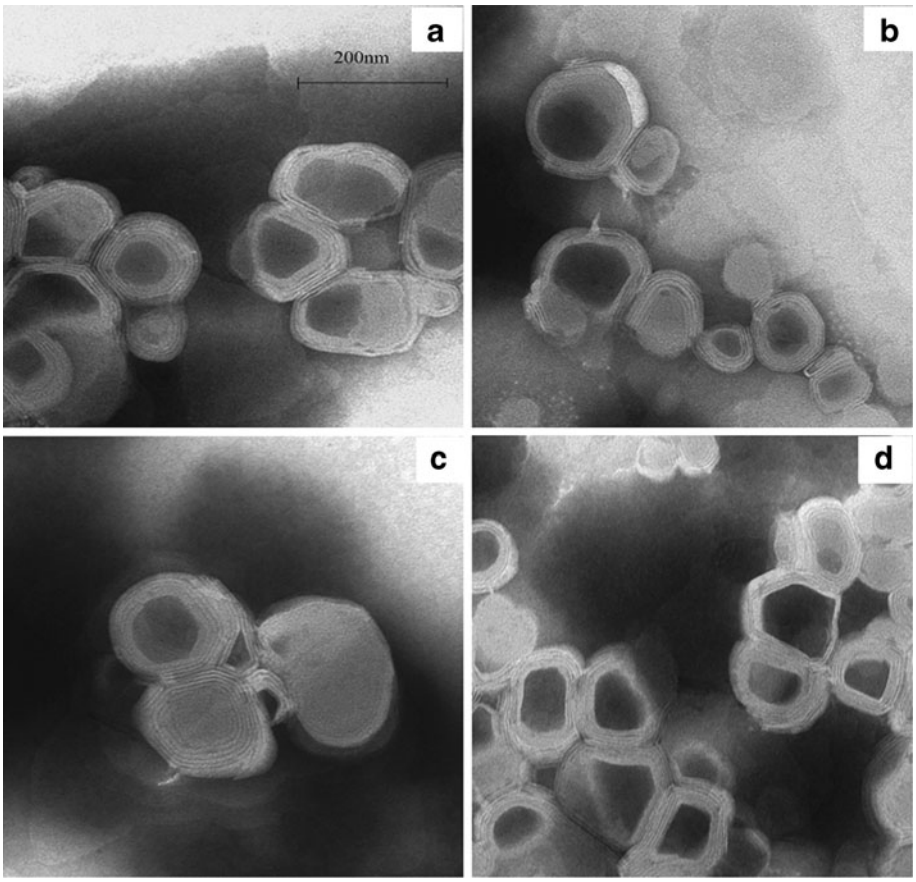

Fig. 1. Transmission electron micrographs of nimodipine-hydrated liposomes (**a** proliposomes hydrated with distilled water, **b** proliposomes hydrated with 0.1 MHCl solution, **c** soft capsules disintegrated in distilled water, and **d** soft capsules disintegrated in 0.1 MHCl solution)

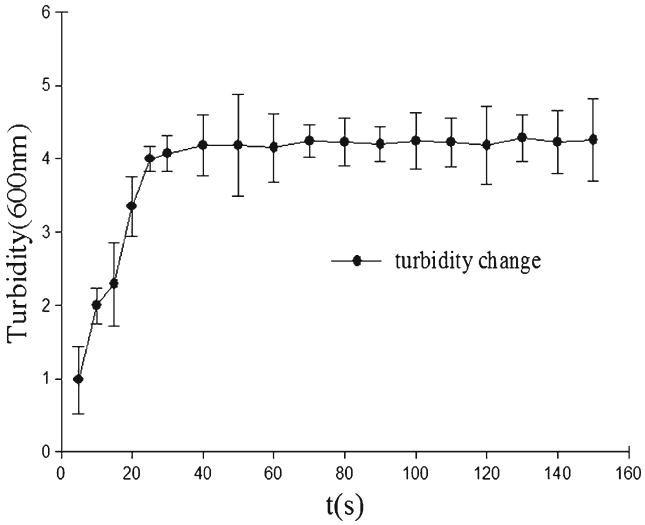

Fig. 2. Turbidity measured for nimodipine proliposomes hydration process with 0.1 MHCl solution. Each point is the mean of at least three experiments (*bars* represent SD values)

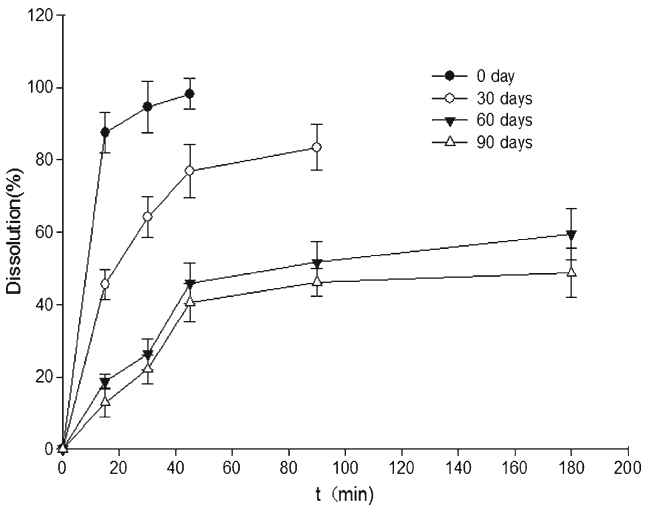

Fig. 3. Dissolution curves of NPSC in accelerated stability test (n=6)

there was a significant decrease after 30 days, and the dissolution of NPSC was <60% after 60 days.

It was considered that the dissolution failure was possibly caused by cross-linking effects in gelatin. The slow dissolution due to cross-linking effects in gelatin is a common problem that appeared in storage of soft capsules (25). Soft gelatin capsules undergo conformational change and cross-linking when stored under high-humidity conditions, and then the cross-linking process causes formation of a swollen, rubbery, water-insoluble membrane which restricts drug release during dissolution testing. It was proved by the significantly longer disintegration time of NPSC. Addition of antioxidants or replacement of plasticizer in the capsule shell formulation may improve the disintegration, which need a further investigation.

Pharmacokinetics Studies in Rabbits

The standard curve ranging from 5 to 320 $ng ml^{-1}$ was linear (r=0.9995). All of the absolute recoveries were above 90%, with all RSD less than 10%, which were within the acceptable limits to meet the guidelines for bioanalytical methods.

Mean plasma concentration-time profiles of NPSC, nimodipine soft capsules and nimodipine-hydrated liposomes in rabbits are shown in Fig. 4. As seen in the graph, plasma nimodipine concentrations were significantly higher in rabbits administrated NPSC and hydrated liposomes than in those administrated nimodipine soft capsules, at all time points. The peak concentration (C_{max}) and time of peak concentration (T_{max}) were obtained directly from the individual plasma nimodipine concentration *vs.* time profiles. The C_{max} value of the NPSC group (141.36±7.34 $mg L^{-1}$) and hydrated liposomes group (143.61±6.75 $mg L^{-1}$) were higher than that obtained with nimodipine soft capsules (49.22±16.36 $mg L^{-1}$). Moreover, NPSC gave a T_{max} of 2 h and hydrated liposomes 1 h, compared with 0.75 h for nimodipine soft capsules, the delayed T_{max} with NPSC and nimodipine-hydrated liposomes were possibly due to the slow release of drug from hydrated liposomes. The similar plasma concentration-time profiles and comparable T_{max} and C_{max} of the NPSC and hydrated liposomes further reflected that proliposomes could turn into intact liposomes after administration and then behave the same way as normal liposomes.

The pharmacokinetic parameters are summarized in Table II. When formulations containing liposomal nimodipine were administered, some pharmacokinetic parameters were entirely different from nimodipine soft capsules. Compared with nimodipine soft capsules, an area under the plasma level-time curve ($AUC_{0-\infty}$) of 395.88 $mg L^{-1}h$ obtained from the NPSC treatment represented a significant 2.41-fold increase in AUC, confirming slower nimodipine distribution and elimination from the plasma of nimodipine-encapsulated liposomes. Higher MRT (1.54-fold) and lower clearance rate (36.7%) were acquired after the administration of NPSC in comparison with nimodipine soft capsules. Vd for NPSC was

Table I. Results of NPSC in Accelerated Stability Test (n=6)

Index	0 day	30 days	60 days	90 days
Content (%)	98.6±1.3	97.8±3.6	96.4±3.4	96.1±3.8
Water in shell (%)	16.5±0.4	13.8±0.3[a]	12.6±0.2[a]	11.4±0.3[a]
Entrapment efficiency (%)	83.25±1.6	80.16±1.8	79.75±3.4	81.43±2.0
Particle size (nm)	385.3±8.4	378.7±21.5	392.6±4.8	384.9±13.6
Dissolution (%)	92.7±1.1	79.1±5.2[a]	45.5±5.1[a]	36.3±5.4[a]
Disintegration time (min)	7.4±0.5	53.3±5.5[a]	>60[a]	>60[a]

Data are expressed as mean±SD
[a] p<0.01 *vs.* 0 day

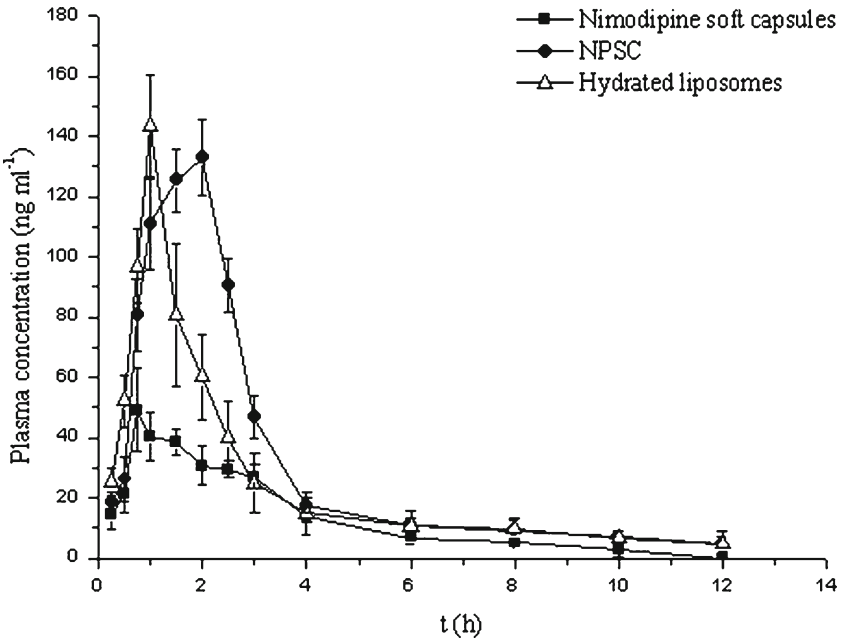

Fig. 4. Mean plasma concentration-time profile of nimodipine following oral administration of nimodipine soft capsules, NPSC, and hydrated liposomes in rabbits (n=6)

slightly larger than that of nimodipine soft capsules but with no significant difference. Moreover, with nimodipine-hydrated liposomes, the pharmacokinetic parameters were similar to that with NPSC, demonstrating that liposomes formed from the NPSC experience similar processes *in vivo* as hydrated liposomes.

These results indicated that the encapsulation of nimodipine in the liposomes leads to a substantial improvement in nimodipine absorption (26–28). This can be explained from several aspects: first, nimodipine in liposome form could be taken up through the GI tract, where particle size plays a dominant role (29). A liposome size of approximately 300 nm allows for efficient uptake in the intestine, particularly in the lymphoid sections of this tissue (30), and therefore escapes from the first-pass metabolism in the liver (31). Second, the surfactants involved in the formulations could affect the membrane permeability and solubility of drugs (32). Third, by embedded into the phospholipid bilayer, exposure of nimodipine to bacterial destruction, as well as enzymatic degradation during absorption process could be reduced. Moreover, liposomes exhibited adhesion towards the epithelial mucosal surface of the small intestine, which prolonged contact time of drug with the intestinal wall (33). Accordingly, it seems that encapsulation of nimodipine as NPSC could highly improve oral bioavailability and extend circulation time in the body.

CONCLUSIONS

In this study, a kind of liquid nimodipine proliposomes for oral administration was prepared by a more efficient and simpler method. Drug-loaded liposomes may be formed with a rapid hydration rate as disintegration of soft capsules in stomach after oral administration. As containers for the proliposomes, soft capsules could protect the proliposomes from destruction of acidic gastric fluid and improve storage stability by isolating proliposomes from water, air, and other outside unfavorable factors. In accelerated stability testing, although there was a decrease in dissolution of nimodipine which is due to soft capsule shells, nimodipine proliposomes were qualified. This liquid proliposomes-based soft capsules formulation did improve the oral bioavailability of nimodipine in New Zealand rabbits and offer a new approach to liposomes for oral administration. A further study of the soft capsule shells will be carried out in the future.

Table II. The Main Pharmacokinetic Parameters of NPSC, Nimodipine Soft Capsules, and Hydrated Liposomes in Rabbits (n=6)

Parameter	NPSC	Nimodipine soft capsules	Nimodipine-hydrated liposomes
T_{max} (h)	2.00±0.00	0.75±0.00	1.00±0.00
C_{max} (mgL^{-1})	141.36±7.34	49.22±16.36[a]	143.61±6.75[b]
$AUC_{0-\infty}$ (mgL^{-1}h)	395.88±25.84	164.17±27.22[a]	383.85±24.13[b]
$MRT_{0-\infty}$ (h)	5.84±0.98	3.79±0.03[a]	5.44±1.27[b]
CL (Lh^{-1}kg^{-1})	0.018±0.0014	0.049±0.0083[a]	0.031±0.0088[b]
Vd (Lkg^{-1})	0.19±0.038	0.15±0.042[b]	0.21±0.066[b]

Data are expressed as mean±SD
NPSC nimodipine liquid proliposomes-based soft capsules, T_{max} time of peak concentration, C_{max} peak concentration, $AUC_{0-\infty}$ areas under the plasma level-time curve, $MRT_{0-\infty}$ mean risidence time, *CL* clearance rate, *Vd* volume of distribution
[a] Significant difference *vs.* NPSC (p<0.05)
[b] No significant difference *vs.* NPSC (p>0.05)

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REFERENCES

1. Gelmers HJ. Calcium-channel blockers in the treatment of migraine. Am J Cardiol. 1985;55:139B–43B.
2. Langley MS, Sorkin EM. Nimodipine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in cerebrovascular disease. Drugs. 1989;37:669–99.
3. He Z, Zhong D, Chen X, Liu X, Tang X, Zhao L. Development of a dissolution medium for nimodipine soft capsules based on bioavailability evaluation. Eur J Pharm Sci. 2004;21(4):487–91.
4. Grunenberg A, Keil B, Henck JO. Polymorphism in binary mixture, as exemplified by nimodipine. Int J Pharm. 1995;118(1):11–21.
5. Berson JA, Brown E. Studies in the synthesis of cortisone. J Am Chem Soc. 1955;77(2):447–50.
6. Mi Z, Burke TG. Marked interspecies variations concerning the interactions of camptothecin with serum albumins, a frequency-domain fluorescence spectroscopic study. Biochemistry. 1994;33 (42):12540–5.
7. Gabizon A, Dagan A, Goren D, Barenholz Y, Fuks Z. Liposomes as *in vivo* carriers of adriamycin: reduced cardiac uptake and preserved antitumor activity in mice. Cancer Res. 1982;42:4734–9.
8. Rajesh K, Gupta RB, Betageri GV. Formulation, characterization, and *in vitro* release of glyburide from proliposomal beads. Drug Deliv. 2001;8:25–7.
9. Payne NI, Timmis P, Ambrose CV, Warel MD. Proliposomes: a novel solution to an old problem. J Pharm Sci. 1986;75:325–9.
10. Junping W, Maitani Y, Takayama K, Nagai T. *In vivo* evaluation of doxorubicin carried with long circulating and remote loading proliposome. Int J Pharm. 2000;203:61–9.
11. Dayan N, Touitou E. Carriers for skin delivery of trihexyphe-nidyl HCl: ethosomes *vs.* liposomes. Biomaterials. 2000;21:1879–85.
12. Gao XL, Ji XM. Determining the trap efficiency of liposome using Sephadex column chromatography. J China Pharm. 2003;38(7):515–7.
13. Matsuzaki K, Murase O, Sugishita K, *et al.* Optical characterization of liposomes by right angle light scattering and turbidity measurement. Biochim Biophys Acta. 2000;1467(1):219.
14. Brocks DR, Betageri GV. Enhanced oral absorption of halofantrine enantiomers after liposomal encapsulation. J Pharm Pharmacol. 2002;54:1049–53.
15. Chansiri G, Lyons RT, Patel MV, Hem SL. Effect of surface charge on the stability of oil/water emulsions during steam sterilization. J Pharm Sci. 1999;88(4):454–8.
16. Lyklema J, Fleer GJ. Zeta electrical contributions to the effect of macro-molecules on colloid stability. Colloid Surf. 1987;25:357–68.
17. Wiacek A, Chibowski E. Zeta potential, effective diameter and multimodal size distribution in oil/water emulsion. Colloid Surf. 1999;159:253–61.
18. Mehnert W, Mader K. Solid lipid nanoparticles. Production characterization and applications. Adv Drug Deliv Rev. 2001;47:165–96.
19. Liu J, Gong T, Wang CG, Zhong ZR, Zhang ZR. Solid lipid nanoparticles loaded with insulin by sodium cholate-phosphatidylcholine-based mixed micelles: preparation and characterization. Int J Pharm. 2007;340:153–62.
20. Kossena GA, Boyd BJ, Porter CJ, Channan WN. Separation and characterization of the colloidal phases produced on digestion of common formulation lipids and assessment of their impact on the apparent solubility of selected poorly water-soluble drugs. J Pharm Sci. 2003;92:634–48.
21. Fahr A, van Hoogevest P, May S, Bergstrand N, Mathew L, Leigh S. Transfer of lipophilic drugs between liposomal membranes and biological interfaces: consequences for drug delivery. Eur J Pharm Sci. 2005;26:251–65.
22. Chen Y, Lu Y, Chen J, Lai J, Sun J, Hu F, *et al.* Enhanced bioavailability of the poorly water-soluble drug fenofibrate by using liposomes containing a bile salt. Int J Pharm. 2009;376: 153–60.
23. Hildebrand A, Beyer K, Neubert R, Garidel P, Blume A. Temperature dependence of the interaction of cholate and deoxycholate with fluid model membranes and their solubilization into mixed micelles. Colloid Surf B Biointerfaces. 2003;32:335–51.
24. Porter CJ, Trevaskis NL, Charman WN. Lipid and lipid-based formulations: optimizing the oral delivery of lipophilic drugs. Nat Rev Drug Discov. 2007;6:231–48.
25. Digenis GA, Gold TB, Shah VP. Cross linking of gelatin within capsules and its relevance to their *in vitro–in vivo* performance. J Pharm Sci. 1994;83(7):915–21.
26. Iwanaga K, Ono S, Narioka K, Morimoto K, Kakemi M, Yamashita S, *et al.* Oral delivery of insulin by using surface coating liposomes. Improvement of stability of insulin in GI tract. Int J Pharm. 1997;157:73–80.
27. Xu H, He L, Nie S, Guan J, Zhang X, Yang X, *et al.* Optimized preparation of vinpocetine proliposomes by a novel method and *in vivo* evaluation of its pharmacokinetics in New Zealand rabbits. J Contr Release. 2009;140:61–8.
28. Gupta S, Pal Singh R, Lokwani P, Yadav S, Gupta SK. Vesicular system as targeted drug delivery system: an overview. Int J Pharm Tech. 2011;3:987–1021.
29. Hussain N, Jaitley V, Florence AT. Recent advances in the understanding of uptake of microparticulates across the gastrointestinal lymphatics. Adv Drug Deliv Rev. 2001;50:107–42.
30. Stuart DD, Allen TM. A new liposomal formulation for antisense oligodeoxynucleotides with small size, high incorporation efficiency and good stability. Biochim Biophys Acta. 2000;146: 3219–29.
31. Yuan H, Chen J, Du YZ, Hu FQ, Zeng S, Zhao HL. Studies on oral absorption of stearic acid SLN by a novel fluorometric method. Colloid Surf B. 2007;58:157–64.
32. Gursoy RN, Benita S. Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs. Biomed Pharmacother. 2004;58:173–82.
33. Lim SJ, Lee MK, Kim CK. Altered chemical and biological activities of all-trans retinoic acid incorporated in solid lipid nanoparticle powders. J Contr Release. 2004;100:53–61.