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Preparation, Characterization, and Pharmacokinetics of Sterically Stabilized Nimodipine-Containing Liposomes

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ABSTRACT Nimodipine is a dihydropyridine calcium antagonist used in clinical trials in the treatment of ischemic damage in subarachnoid hemorrhage and commercially available as nimotop® intravenous infusion solution and tablets. However, due to its poor solubility in water, intravenous administration depends on the use of the dehydrated alcohol to achieve a clinically relevant concentrated infusion solution while the low bioavailability of the nimotop® tablets were far away from content. We have prepared a well-characterized novel lyophilized liposome-based nimodipine formulation that is sterile and easy-to-use. Of the several formulations examined, nimodipine-liposomes composed of ePC/CHOL 20:3 and co-surfactant poloxamer 188/sodium deoxycholate/ePC/3:0.3:5 were chosen for further studies. This composition was found to give more stable liposomes than other formulations. It gave 89.9% entrapment efficiency and particle size of 200 nm after lyophilization. The pharmacokinetic parameters following orally and intravenously administration to New Zealand rabbits were determined and compared with those of commercial nimodipine formulations. Encapsulation of nimodipine in liposomes produced marked differences over those of commercial preparations with an increased C_{max}, prolonged elimination half-life, and an increased value for AUC. The obtained values for mean residence time (MRT) indicated that nimodipine remains longer for liposomal formulation. Thus an optimum i.v. liposome formulation for nimodipine can be developed for an alternative to the commercial nimodipine preparations.

KEYWORDS Nimodipine, Liposomes, Lyophilization, Pharmacokinetics

INTRODUCTION

Vasospasm of the cerebral arteries due to subarachnoid hemorrhage is a major source of delayed ischemic deficits in patients with ruptured aneurysms (Mayberg, 1998). Currently, nimodipine is the only therapy available that has been proven to reduce the morbidity and mortality associated with

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delayed ischemic deficits in patients (Dorsch, 2002). As a result, it is the benchmark which new therapies will be tested against. Future investigations that compare experimental therapies to nimodipine are essential precursors to an effective, comprehensive treatment plan for subarachnoid hemorrhage.

Nimodipine was practically insoluble in water (Grunenberg et al., 1995), and not appropriate so far for oral or intravenous administration. In clinical practices, standard method of dosing called for a 60 mg oral dose of nimodipine to be given every 4 h for 21 days (Toyota, 1999). The frequency of this dosing regimen reflected the low bioavailability of orally-administered nimodipine due to slow drug dissolution, drug decomposition in stomach acid (He et al., 2004), and the high first-pass metabolism of nimodipine in liver (Ramsch et al., 1985). Pharmacokinetic studies showed that the bioavailability of orally-administered nimodipine was between 4 and 13% in healthy subjects (Ramsch et al., 1985). Intravenous nimodipine administration was an alternative of oral administration which could provide greater bioavailability. However, because of the poor solubility in water, the current nimotop® i.v. infusion consisted of nimodipine solubilized in 25% (v/v) dehydrated alcohol. Patients were generally required to be admitted to the hospital overnight because of long infusion time and caused additional inconvenience to the patients. Nimotop® infusion solution must be administered by means of an infusion pump in the bypass together with the recommended infusion solution via 3-way stop cock to the central catheter, and the ratio of nimodipine solution to concomitant infusion solution should be maintained at 1 to 4 by volume to ensure appropriate dilution of nimodipine i.v. This was in order to avoid the possibility of precipitating nimodipine with resulting in crystal formation seen in vitro tests at higher dilutions. Furthermore, nimotop® was associated with several stability and compatibility issues, such as possibility of drug precipitation upon dilution (Feng & Yang, 2003), light sensitivity (Ragno, G., 1995), and non-specific adsorption to infusion micro-pump (Qi et al., 2003). The aforementioned stability and compatibility issues present a number of practical problems with respect to the special requirement of a filter device and use of non-plasticized containers and infusion sets during drug storage and infusion.

Given all the drawbacks of the commercial products, it is thus apparent that there is a need for a better nimodipine formulation that is easy to use and may be more efficacious than nimotop®. The primary goal of this work was to develop a nimodipine formulation using a better-tolerated drug delivery system to deliver nimodipine. Various approaches or drug delivery systems of formulating nimodipine, including emulsions (Zhang et al., 2004), slow-release pellets (Perez-Trepichio & Jones, 1996), subcutaneous administration (Laslo et al., 2004), intranasal administration (Zhang & Jiang, 2005), and liposome (Schlossmann et al., 1985) have been reported. Among the drug delivery systems, liposomes represent a mature, versatile technology with considerable potential for entrapment of both lipophilic and hydrophilic drugs (Vemuri & Rhodes, 1995). It has been shown that liposome delivery systems could enhance drug solubility, reduce toxicity, and improve stability of drugs by protecting compounds from chemical degradation or transformation (Mi & Burke, 1994). Several liposomal products have been proven to be more effective, less toxic, and exhibited improved pharmacokinetic and pharmacodynamic profiles than free drugs (Thomson & Montvale, 2003); however, the number of commercially available liposomal products was still limited (Zhang & Pawelchak, 2000). To be useful as a pharmaceutical product, the liposomal formulation should have a high drug to lipid ratio in order to reduce unnecessary lipid load to the patients, a relatively higher drug entrapment to lower free drug in the product, and a scalable manufacturing process.

The objectives of the present study were (1) to develop a pharmaceutically acceptable formulation, (2) to fully characterize the formulation, and (3) to evaluate pharmacokinetics of single dose of nimodipine following oral and intranasal administration.

MATERIALS AND METHODS

Chemicals

Nimodipine was provided by Shandong Xinhua Pharmaceutical Company Limited, China. Egg yolk phosphatidylcholine (ePC, E 80) was purchased from Lipoid GmbH, Germany. Polysorbate-80 was provided by Shanghai Shenyu Pharmaceutical & Chemical Co., Ltd., China. Cholesterol was purchased from China Medicine Shanghai Chemical Reagent Corporation, China and Triton X-100 was from Sigma, Zhenjiang

Green Bio-engineering Co., Ltd. All other reagents and solvents used were of analytical grade. Distilled water was used throughout this study.

Preparation of Nimodipine Liposome

Liposome vesicles containing nimodipine were prepared by lipid-dripping method (Deamer & Bangham, 1976) with modifications. Briefly, required amounts of phospholipid and cholesterol with different quantities of surfactants (ePC: CHOL mass ratio = 20:3 in Table 1) were dissolved in absolute ethanol to be a lipid solution and dripped into a stirred and thermostated cell (60°C) of cryoprotective agent mannitol aqueous solution at a rate of 2 mL per 10 min. After equally dispersed for about 30 min, high-pressure homogenizer (APV2000, APV Co., Silkeborg, Denmark) was carried out to reduce the size of the liposomes for 3–5 cycles until the dispersion system became homogeneous, transparent, and slightly opalescent. The resulting liposome-based drug formulation was then sterile filtered through 0.1 μm filter and filled into 25 mL vials in aliquots of 7 mL. The filtered formulation in vials was lyophilized using Tofflon lyo-5 (sip+cip) lyophilizer (Genesis, The Fulong Company, Shanghai, China).

The lyophilization cycle consisted of cooling the solution down to –45°C for 6 h, primary drying for 24 h at –25°C under 0.4 Pa pressure, and ramp from –25°C to –5°C for 8 h under 7 Pa chamber pressure. Second drying was performed at 25°C for 6 h under 7 Pa chamber pressure. The chambers were removed and the vials were closed with rubber caps and stored at 4°C until further treatments.

The lyophilized liposomes (freeze-dried cakes) were reconstructed with 5% mannitol solution to its original volume before use.

Characterization of Liposomes

Vesicle Size Measurement

Immediately after preparation, nimodipine-loading liposomes were examined for possible aggregation by visual inspection. Thereafter, mean diameter and particle size distribution of the liposomes were determined using dynamic light scattering (DLS) technique with a Zeta Potential/Particle Sizer 3000 HS (Particle Sizing Systems, Malven Co., Worcestershire, UK) equipped with auto dilution function. The laser in this

TABLE 1 **Characteristics of Nimodipine-carrying Liposomes[a] (nimodipine:ePC: CHOL Mass Ratio = 1:20:3)**

Surfactant	Surfactant: ePC (w/w)	Average particle size[d] (nm)	Zeta potential (mv)	Stability[b]	Entrapment efficiency[c] (%)
Poloxamer 188	1:5	125.9	–48.8	24 hs	94.9
	2:5	99.7	–37.6	24 hs	87.9
	3:5	54.3	–31.1	24 hs	84.9

Surfactant: ePC(w/w)	Surfactant: ePC (w/w)		Average particle size[d] (nm)	Zeta potential (mv)	Stability[b]	Entrapment efficiency[c] (%)
Poloxamer 188: ePC = 3:5	Span-40	0.2:5	102.7	–25.5	8 hs	88.9
		0.6:5	117.9	–23.3	8 hs	83.2
	Span-80	0.2:5	106.3	–18.9	8 hs	83.7
		0.6:5	204.1	–17.1	8 hs	80.2
	Tween 80	1:5	80.6	–29.3	24 hs	78.6
		2:5	50.9	–23.3	24 hs	74.7
	Sodium deoxycholate	0.24:5	73.1	–36.3	24 hs	83.5
		0.3:5	53.3	–38.3	24 hs	89.9

[a]Values were means of three experiments and standard deviations (not reported) were below 5% of the mean values.

[b]Short-term stability studies of the liposomal formulations after reconstituted and eight-fold diluted were judged as drug leakage and maintenance of uniform size both at 2–8°C and room temperature.

[c]Ratio between drug:lipid weight ratio by equilibrium dialysis.

[d]The particle size was determined before freeze-drying.

equipment was operated at 532 nm using a 90° angle between incident and scattered beams. Polystyrene bead standards were used to verify the performance of the instrument prior to sample measurement. Data were analyzed in terms of intensity, volume, and number distributions and reported as number weighted distribution as mean of at least two replicates.

Negative-stain Transmission Electron Microscope (TEM) Observation

It has been previously demonstrated that TEM techniques applied to negatively stained liposomes constituted appropriate methods to study the formation and morphology of liposome structure (Memoli et al., 1995). A Hitachi (Japan) H-7000 transmission electron microscope operating at 75 kV was used. Samples were deposited on carbon film coated Cu grids. Negative staining with 0.5% phosphotungstic acid solution was performed to enhance image quality.

Drug Entrapment Efficiency Measurement by RP-HPLC Assay

Encapsulation efficiency was determined by equilibrium dialysis as described before (Schneider et al., 1995) using a Dianorm system with Diachema dialysis membranes (cut-off 8000–10000, China Medicine Shanghai Chemical Reagent Corporation, Shanghai, China). The diluted liposome dispersion (1:100) was dialyzed against a 20% ethanol solution for 4 h. After destroyed by 5% aqueous solution of Triton X-100, the concentration of nimodipine in the liposomes were determined by RP-HPLC consisting of a reversed-phase column (Hypersil ODS, 250 mm × 4.6 mm, 5 μm, Dalian Elite Analytical Instrument Co., Ltd., Da Lian, China). The mobile phase was methanol-water-tetrahydrofuran (40:30:30 v/v). The detection wavelength was 237 nm. The flow rate was 1 mL/min and the column temperature was 40°C. The encapsulation efficiency was calculated as a fraction of drug in the liposome pellet expressed as a percentage of total drug content.

Pharmacokinetics Studies

Fourteen New Zealand white male rabbits with an average weight of 2.5 kg were used in this study. The rabbits were fasted overnight but were allowed free access to water. Each animal received a dose in one of the following dosage forms: (1) nimotop® oral tablet at dose of 8 mg/kg (n = 5); (2) nimotop® i.v. infusion solution 0.4 mg/kg per hour (Nimotop S, Bayer AG) was administered for 60 minutes (n = 5); and (3) nimodipine liposomal formulation for i.v. infusion prepared was administered as the infusion solution (n = 5). The oral doses were administered using polyethylene tube while the marginal ear vein was used for the i.v. dosing with the aid of implanted cannula for collecting blood samples. The samples (about 2 ml) were collected in heparinized tubes prior to and at intervals of 0.25 h post oral administration and prior to and up to 5.0 h after the beginning of i.v. infusion administration. The samples were stored at −4°C until HPLC analysis (Zhang et al., 2004).

Pharmacokinetic parameters for nimodipine following administration of commercial and liposomal preparations were determined from the concentration-time data. A computer program 3P87 (Administration of Health, Beijing, China) was used for the fitting of pharmacokinetic models (Zhang et al., 2000). The maximum blood concentration (C_{max}) and the time to reach this maximum (T_{max}) were obtained directly from the individual concentration-time profiles. The area under the concentration-time curve (AUC) and the area under the moment curve (AUMC) were estimated by the linear trapezoidal rule and extrapolated to infinity using standard methods. The mean residence time (MRT) was calculated as the ratio of $AUMC_{0-\infty}$ to $AUC_{0-\infty}$. The data were analyzed for statistical significance by the t-test ($P < 0.05$). All results were expressed as the mean ± standard deviation (S.D.).

RESULTS

Formulation Development

Nimodipine liposome formulation was prepared by different surfactant ratios in phospholipid suspension. The liposomal compositions used to evaluate in vitro short-term stability and in vivo pharmacokinetics were optimized to obtain maximal stability and nimodipine incorporation. Ethanol-dripping, followed by high-pressure homogenization, was found to be a feasible preparation method for homogeneous small vesicles.

First of all, we tried to prepare the liposome with poloxamer 188 at different concentrations. The smaller size of the liposomes were present at concentration of 3:5 (poloxamer: ePC, w:w), while the entrapment efficiency was unsatisfactory. Then co-surfactants have

to be used. It was not possible to prepare liposomes of this composition with span-40 and span-80 because the preparation was unstable and a drug precipitate was observed, which was verified by the high values of zeta potential (Table 1). Tween-80 could be useful to the stability of liposome, but the entrapment efficiency was slightly decreased. The smaller vesicles were present at the higher concentration of sodium deoxycholate. Finally, the formulation composed of co-surfactant, sodium deoxycholate/poloxamer 188/ePC 0.3:3:5 (w/w) showed smaller size particle and good stability as well as high drug entrapment efficiency 89.9% (Table 1) and were selected for further studies. Figure 1A shows the size distribution of the nimodipine liposomes before dehydration and after rehydration in the presence of mannitol at the concentrations of 5%.

After rehydrated, the liposomes were dispersed homogeneously in the 5% mannitol aqueous phase again. Resulting from fusioning on the periphery or aggregation of several independent liposomes, dry cake after rehydration gave rise to approximately spherical or elliptical large aggregates ranging from 200 to 250 nm (Fig. 1B), consistent with the hydrodynamic diameters measured by DLS (Fig. 1(A)). Although the mean particle size of the liposome was increased from 53.3 nm prior to lyophilization to 214.2 nm after redispersion in the case of 5% mannitol, about 4.02 times larger than the original dispersions, mannitol did not cause significant differences in particle size depending on its concentration that were similar to those reported previously (Kim et al., 2005).

Since nimodipine is usually given to the patients as intravenous infusion, further dilution of the product is necessary to adjust the dose and drug infusion rate at a given time. The result of the dilution study demonstrated that 8-fold diluted nimodipine-liposome products stored at 2–8°C and room temperature was physically stable for up to 24 h (Table 1). At both temperatures, the mean liposome vesicle diameter remained unchanged at the end of study. No precipitation or drug crystals were observed during and at the end of study. Nimodipine and lipid concentrations remained unchanged at both temperature conditions over the course of the stability study. The entrapment efficiency remained the same regardless of the storage time and condition. There was no appreciable change in pH for the diluted samples over the 24 h study period at room temperature and 2–8°C. It can be concluded that the reconstituted nimodipine-liposome products can be

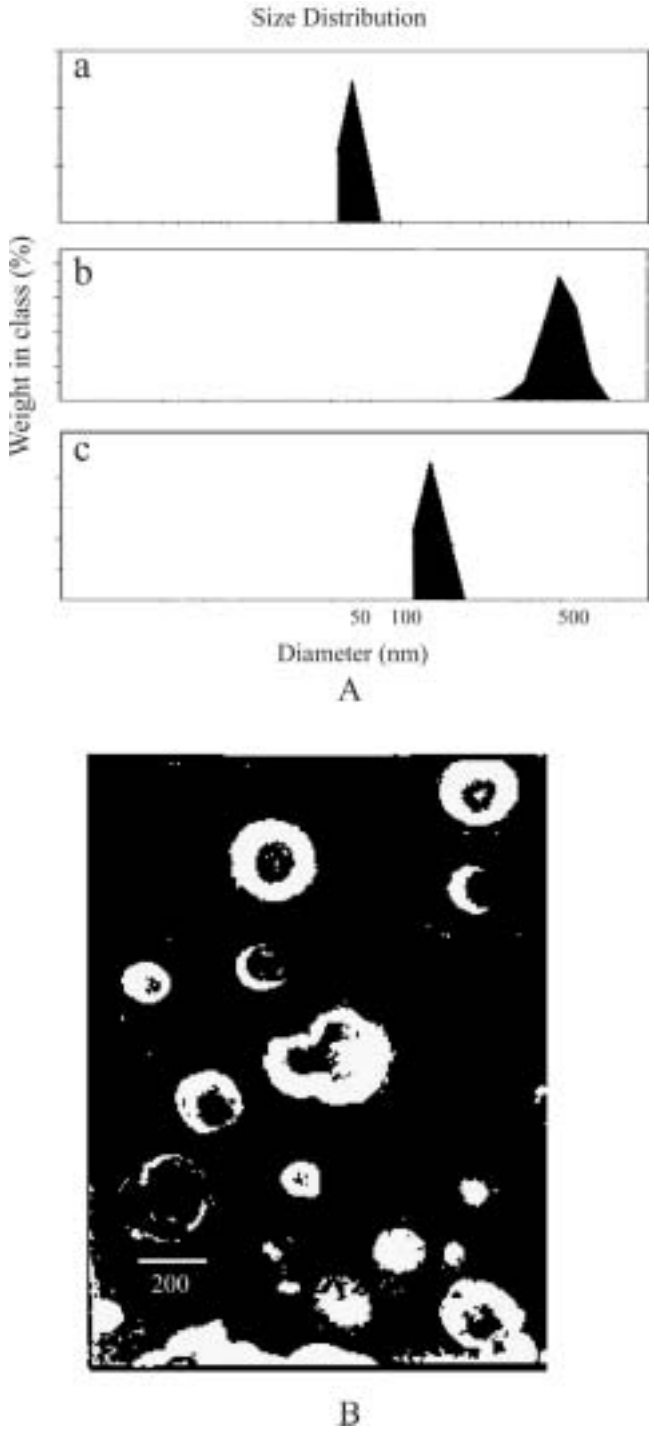

FIGURE 1 **A: Apparent Size Distributions of Nimodipine-carrying Liposomes Before Freeze-drying and After Rehydration in the Presence of 5% Mannitol. a: Before Freeze-drying; b: Rehydration with 0 mM Mannitol; c: Rehydration with 5% Mannitol. B: Transmission Electron Micrographs of Negative Staining Samples of Nimodipine-loaded Vesicles Rehydrated in an Aqueous Dispersion of 5% Mannitol.**

further diluted up to 8-fold and be used within 24 h at clinical setting.

Pharmacokinetics

The mean plasma concentration vs. time profile of nimodipine following oral and i.v. administration are shown in Fig. 2 and the pharmacokinetic parameters calculated in Table 2. Both i.v. routes gave significant differences from oral administration in every pharmacokinetic parameter studied ($P < 0.05$). The absolute bioavailability, which was defined as $(AUC_{oral}/DOSE_{oral})/(AUC_{i.v.}/DOSE_{i.v.})$, was 13.17 ± 6.14% for oral tablets. The low bioavailability of nimodipine from the tablets could be due to a lower dissolution rate of drug particles as Toyota (1999) reported.

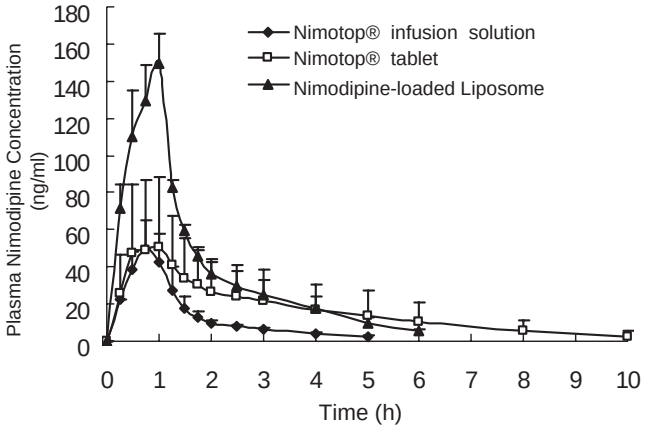

FIGURE 2 **Mean Plasma Concentration vs. Time Profile of Nimodipine Following Oral Administration of Nimodipine Tablet at Dose of 8 mg/kg (*n* = 5) and I.V. Infusion of Nimodipine Infusion Solution (*n* = 5) and Liposomes at Dose of 0.4 mg/kg for 60 Min, Respectively (*n* = 5). Time (0.25, 0.50, 1.0, 1.50, 1.75, 2.0, 3.0, 4.0, 6.0, 8.0, and 10.0 h), Refrigerated, and Immediately Centrifuged (5 min, 10,000 × g). Each Point Represents the Mean ± S.D.**

Encapsulation of nimodipine in liposomes produced a significant change in drug pharmacokinetic parameters. Table 2 shows that nimodipine liposome gave $t_{1/2}$ β of 1.67 ± 0.70 h as terminal elimination half-life, compared with 0.97 ± 0.16 h for free drug, indicating a longer elimination half-life for nimodipine in liposome formulation. It appears that nimodipne in liposomal formulation could be given intravenously with a long duration of action due to high drug and liposomal stability. The Cl and AUC values confirmed this trend. Liposomal nimodipine resulted in 3.53 times increase in AUC, significantly increased from 66.44 ± 17.13 ng·h/l for the free drug i.v. infusion solution to 234.8 ± 4.94 ng·h/l for the liposomes ($p < 0.05$), and 72.02% decrease in Cl compared with nimodipine infusion solution ($P < 0.05$). In addition, MRT of nimodipine-liposomes was longer than that of nimodipine infusion solution although statistically not significant ($P > 0.05$).

TABLE 2 **Pharmacokinetic Parameters for Nimodipine After Administration of Three Dosage Forms: Oral Nimodipine Tablet (OT), Intravenous Nimodipine Infusion Solution (INI), and Intravenous Liposomes (IVL)[a]**

Parameter	OT	INI	IVL
Dose (mg/kg)	8	0.4	0.4
$AUC_{0-\infty}$(ng·h/l)	173.5 ± 136.1	66.44 ± 17.13	234.8 ± 4.94*
AUMC (ng·h^2)	593.7 ± 483.0	90.69 ± 21.33	445.4 ± 66.80
F[b] (%)	13.17 ± 6.14	—	—
Cl (l/h)	0.12 ± 0.11[c]	(15.26 ± 5.44) × 10^{-3}	(4.27 ± 0.08) × 10^{-3}*
V_c (ml)	390.80 ± 171.10[d]	7.67 ± 1.53	2.83 ± 0.42
t_{max} (h)	0.75	1	1
C_{max} (ng/ml)	50.36 ± 37.90	49.95 ± 15.07	149.4 ± 16.46*
$t_{½}$ α (h)	2.30 ± 0.61	0.97 ± 0.16	1.67 ± 0.70
$t_{½}$ β (h)	0.057 ± 0.14	0.21 ± 0.08	0.27 ± 0.037
MRT (h)	3.40 ± 0.60	1.37 ± 0.083	1.90 ± 0.26

AUC, area under curve; AUMC, area under mean curve; F, absolute bioavailability; Cl, clearance; $t_{1/2}$ α, α half-life; $t_{1/2}$ β, β half-life; V_c, distribution volume of central compartment; MRT, mean residence time; *significantly different from INI ($P < 0.05$).

[a]Each value represents the mean ± S.D. ($n = 5$).

[b]$F = (AUC_{OT}/DOSE_{OT})/(AUC_{INI}/DOSE_{INI})$.

[c]Cl/F = oral clearance.

[d]V_c/F = apparent volumes of distribution after oral administration.

DISCUSSION

Several formulations were investigated in terms of lipid to drug ratio, drug concentration, drug entrapment, and short-term physical stability. Upon further optimization, a lead formulation of liposome-based nimodipine was developed. The ePC:CHOL and sodium deoxycholate: poloxamer 188:ePC weight percent ratios were 20:3 and 0.3:3:5, respectively. Since lipid components in the liposomes were heat labile, steam sterilization of liposomes was not a viable approach. To achieve sterile filterable liposome formulation, the size of nimodipine-liposome, before freeze-drying, was reduced to about 50 nm by high-pressure homogenation to allow for aseptic filtration through 0.1 μm membrane filter. Consequently, ethanol-dripping, followed by high-pressure homogenization, was found to be a feasible method to be large scale produced of liposomes in pharmaceutical industry.

Nowadays, non-ionic surfactants are being widely employed because they are readily incorporated as co-surfactants into liposomes. The presence of poloxamer molecules within liposome bilayers may sterically inhibit contact between two vesicles and subsequently prevent aggregation and fusion. As poloxamer concentration was increased, vesicles may have been increasingly inhibited from associating with other vesicles during bilayer repair (Glavas-Dodov et al., 2005). Castile et al. (2001) showed that bilayer permeability of ePC was increased by the presence of poloxamers, possibly due to the formation of "pores" or regions of enhanced membrane fluidity caused by inclusion of poloxamer within the bilayer. In addition, the strong effect of cholate on the lecithin chain order could most directly be interpreted as due to an increase in the average cross-sectional area available to the alkyl chains in the lamellae. There was a dramatic decrease of the order in the lamellar phase on addition of cholate. Ulmius et al. (1982) have found that the presence of cholate, added in the bilayer reconstitution process, could give rise to artifacts dominating over the effect of the drug itself on the chain order. A further consequence of a cholate molecule solubilized flat on the bilayer surface was that a local curvature would be induced. These would account for the correlation between the increasing presences of small vesicles at a higher concentration of poloxamer and cholate.

Phospholipids in liposomes were known to be sensitive to hydrolysis and oxidation in aqueous medium. Liposomes can be hydrolyzed to form lysophospholipids and free fatty acids. The lysophospholipids can be further hydrolyzed to glycerophospho-compounds and fatty acids (Zhang & Pawelchak, 2000). The hydrolytic degradation may change the rigidity of liposomal bilayers, retention of entrapped drug, and alter liposome size and distribution. In order to enhance the chemical and physical stability of the liposome formulation, freeze-drying was used to remove free water from the formulation to minimize lipid hydrolysis in this study. However, it was a considerable challenge to preserve the structural integrity of liposome during the dehydration/rehydration process. In the absence of any protective agents, vesicle fusion and leakage of internal aqueous contents of liposomes can occur (Harrigan et al., 1990). Sugars have been shown to act as protective agents during dehydration/rehydration of liposomes to prevent vesicle fusion and retention of encapsulated compounds within liposomes (Madden et al., 1985). In this study, the cryoprotective substance mannitol seems to protect the liposomes during lyophilization against dehydration damage. This was reflected by the existence of some intact liposome structures observed in the TEM image (Fig. 1B). Nevertheless, some of the nimodipine-carrying liposomes also undergo changes during lyophilization due to limited protection. Upon rehydration, new liposomes were formed and result in vesicle size growth (Fig. 1).

The negative staining electron microscopy images of the reconstituted nimodipine were presented in Fig. 1B. The liposome vesicles as shown under electron microscope were discrete particles with sharp boundaries that range in size from 200 to 250 nm which matches the results obtained from particle size measurements using DLS technique. After lyophilization and reconstitution with 5% mannitol solvent, the mean liposome vesicle size increased by about 4 times as compared to the mean diameter of the pre-lyophilization samples. The vesicle size distribution of the samples was mono-model (Gaussian) distribution.

The pharmacokinetic parameters of oral administration of nimodipine, i.v. infusion administration of nimodipine infusion solution, and liposome have been calculated. Oral-administration producing unsatisfactory results was not surprising, considering the important first-pass effect of hepatic metabolism on nimodipine following oral administration (Ramsch et al., 1985; Vinge et al., 1986). The data were consistent with these previously published observations. In contrast to orally-administered nimodipine, i.v. infusion doses of

nimodipine led to significantly higher C_{max}, $t_{1/2}$ β, MRT, and AUC values. The most likely explanation for the difference in oral and i.v. administration was that i.v. administration of nimodipine bypasses oral pre-systemic elimination (hepatic and intestinal), thereby avoiding the large first-pass effect (Vinge et al., 1986). In other words, a large portion of the nimodipine that was given orally was metabolized in the gut before it reached the systemic circulation.

The most interesting observation regarding these pharmacokinetic data was that incorporation of nimodipine in liposomes produced better drug solubilization in the blood circulation after i.v. infusion than that showed by the commercial infusion. Indeed, plasma concentration and some pharmacokinetic parameters, such as AUC, AUMC, MRT (which better describe the amount of drug available for the therapeutic effect over time), showed the higher values in nimodipine-liposome. Furthermore, some pharmacokinetic features of nimodipine liposomes, such as clearance and $t_{1/2}$ □, would be related to the so-called long-ciruculating or stealth liposomes described in the literature (Daemen et al., 1997). The low reduced $t_{1/2}$ β in the nimodipine case appeared to be related to the reduced clearance rate and, hence, to the reduced uptake of liposomal drug by the elements of the mononuclear phagocytic system (MPS).

CONCLUSION

We have developed a sterile, stable, lyophilized liposomal formulation which was a viable alternative to the nimotop®, due to its small and uniform liposome size and easy-to-use character. The formulation process used for nimodipine-loading liposome was simple and scalable. The novel, sterile, and lyophilized nimodipine-carrying liposome appears to offer advantages of better safety (avoidance of hypersensitivity) and improved patient compliance (shorter infusion time and less time spent in the treatment). Additional work is needed to verify the in vivo pharmaceutical efficacy of nimodipine-liposome in different conditions.

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