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Preparation and Characterization of a Submicron Lipid Emulsion of Docetaxel: Submicron Lipid Emulsion of Docetaxel

Kun Gao, Jin Sun, Kai Liu, Xiaohong Liu, and Zhonggui He
Department of Biopharmaceutics, School of Pharmacy, Shenyang Pharmaceutical University, Shenyang, China

Docetaxel, a widely used anticancer agent, has sparingly low aqueous solubility, thus Tween 80 and ethanol need to be added into its formulation, probably resulting in the toxic effects. In this study, we aimed to utilize submicron lipid emulsions as a carrier of docetaxel to avoid these potential toxic vehicles. Preformulation study was performed for rational emulsions formulation design, including drug solubility, distribution between oil and water, and degradation kinetics. Supersaturated submicron lipid emulsion of docetaxel was prepared by temperature elevation method. Soya oil and Miglyol 812 can incorporate docetaxel up to 1.0% (drug to lipid ratio) and were used as the oil phase of emulsions. The optimal formulation of docetaxel is composed of 10% oil phase, 1.2% soybean lecithin, 0.3% Pluoronic F68, and 0.4 or 0.8 mg/mL docetaxel, with particle size in the nanometer range, entrapment efficiency more than 90%, and is physicochemically stable at 4 and 25°C for 6 months. Animal studies showed that docetaxel emulsion has significantly higher area under the curve (AUC) and C_{max} in rats compared to its micellar solution. The results suggested that the submicron lipid emulsion is a promising intravenous carrier for docetaxel in place of its present commercially available docetaxel micellar solution with potential toxic effects.

Keywords submicron lipid emulsion; docetaxel; stability; degradation kinetics; animal study

INTRODUCTION

Docetaxel (*N*-debenzoyl-*N*-tert-butoxycarbonyl-10-deacetyl) (Taxotere®), a semisynthetic analog of paclitaxel (Figure 1), is prepared from a nontoxic precursor compound that is extracted from needles of European yew tree (*Taxus baccata* L.) (Bissery, Guenar, Gueritte-Voegelein, & Lavelle, 1991) and is clinically effective against cancer, such as ovarian carcinoma, breast, lung, and head/neck cancer (Capri, Terenzi, Fulfaro, & Gianni, 1996; Rowinsky, 1997). It takes effect, similar to that of paclitaxel, by binding to microtubules and inhibiting microtubule depolymerization to free tubulin through the stabilization of the polymer. This disrupts the equilibrium within the microtubule system leading to mitotic arrest in the G_2M phase of the cell cycle and ultimately to cell death (Gueritte-Voegelein et al., 1991; Ringel & Horwitz, 1991; Schiff, Fant, & Horwitz, 1979). Docetaxel has an approximately 2-fold higher affinity than paclitaxel, but its very low aqueous solubility is one of the major barriers in the development of an intravenous dosage form. At present, docetaxel is formulated as a micellar solution using Tween 80 (polysorbate 80) solution with 50% absolute ethanol.

Before intravenous infusion, the solution must be diluted in the physiological saline or 5% glucose solution to prepare 0.3–0.9 mg/mL docetaxel micellar solution (Vaishampayan, Parchment, Jasti, & Hussain, 1999). Because Tween 80 has been observed to cause hematolysis and hypersensitivity reactions, routine premedication with glucocorticoids must be used to prevent their incidence, such as Corson (Earhart, 1999; Fulton & Spencer, 1996). Therefore, the development of a safer intravenous formulation devoid of Tween 80 is an important issue for good clinical treatment. Lately, alternative dosage forms have been suggested, including liposomes (Immordino et al., 2003), cyclodextrins (Grosse, Bressolle, & Pinguet, 1998), and nanoparticles (Musumeci et al., 2006). But there are several limitations of these current formulations, such as lower encapsulation efficiency, limited solubilized capacity, poor physicochemical stability, and a complex preparation procedure. Submicron lipid emulsion as a drug carrier has many favorable properties, such as biocompatibility, physical stability, ease of preparation, and it has been used as parenteral nutritional supplements for more than 30 years (Wretlind, 1981). Lipid emulsions can sequester a drug from direct contact with body fluids and tissues and present a number of advantages, for example, enhance the solubilization of poorly water-soluble drugs, increase the stability of hydrolytically susceptible compounds, or reduce irritation and toxicity of intravenously administered drugs, and they have the potential for

Address correspondence to Zhonggui He, Mailbox 59#, Department of Biopharmaceutics, School of Pharmacy, Shenyang Pharmaceutical University, No. 103 Wenhua Road, Shenyang 110016, China. E-mail: hezhonggui@gmail.com

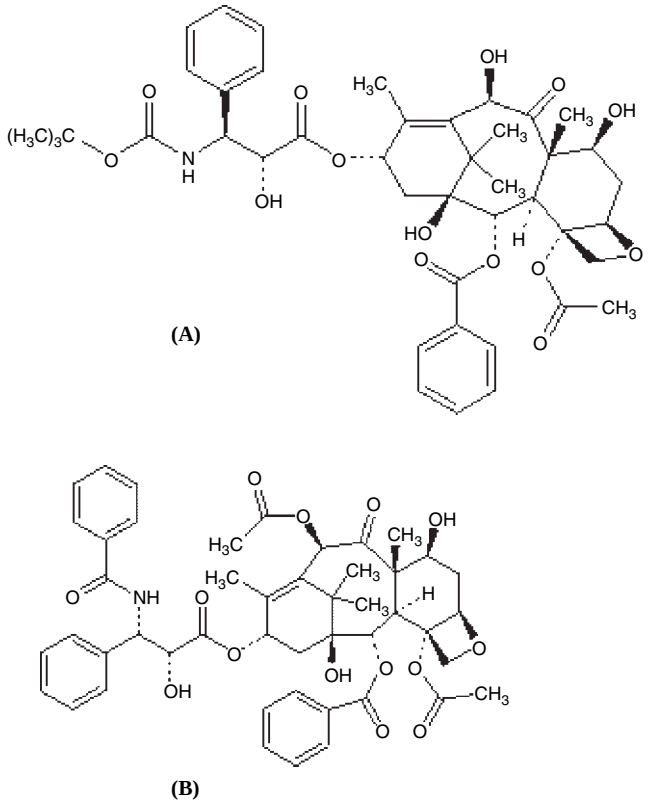

FIGURE 1. Chemical structures of docetaxel (A) and paclitaxel (B).

sustained drug release (Prankerd & Stella, 1990; Wang & Cory, 1999). Paclitaxel has been incorporated in a lipid emulsion for parenteral administration (Han, Davis, Papandreou, Melia, & Washington, 2004; Lundberg, 1997; Tarr, Sanbandam, & Yalkowsky, 1987), and a lipophilic prodrug of paclitaxel has also been synthesized for high loading capacity in lipid carrier preparations (Lundberg, Risovic, Ramaswamy, & Wasan, 2003).

The main purpose of this study was to incorporate docetaxel in a submicron lipid emulsion. Preformulation studies, including aqueous solubility, distribution between oil (the lipid phase in emulsion) and water, and stability, were carried out for the guidance of designing formulation. Docetaxel is instable at alkaline solution while lipid emulsion instable at low pH condition. So, the influences of initial docetaxel concentration and pH on its degradation were studied in aqueous solutions to select a proper pH range in which lipid emulsion stability should be guaranteed. We selected a mixture of soybean oil and medium chain triglyceride (MCT), namely Miglyol 812, as the oil phase, and soybean lecithin and poloxamer 188 (a polyoxyethylene–polyoxypropylene polymer) as surfactants, which are acceptable for intravenous administration. Although doctaxel was found to be poorly soluble in the oil phase (<0.5 mg/mL at 25°C), which can achieve a maximal drug concentration of approximately 0.05 mg/mL in a 10% lipid emulsion or 0.1 mg/mL in a 20% lipid emulsion by conventional de novo emulsion preparation method, we hypothesized that it may be effectively incorporated in the lipid emulsion, and the drug loading capacity of the lipid emulsion may be enhanced by temperature elevation method (Levy & Benita, 1989). Animal studies were performed to roughly investigate the pharmacokinetics of this emulsion. The results suggested that docetaxel could be effectively incorporated in the lipid emulsion, and the docetaxel lipid submicron emulsion was physically and chemically stable for at least 6 months and could increase the effective plasma concentration of docetaxel in rats and is a promising intravenous carrier in place of the clinically docetaxel micellar solution potentially eliciting toxic effects.

MATERIALS AND METHODS

Materials

Docetaxel anhydrous (99.7% purity) was obtained from Shanghai Jinhe Bio-Technology Co., Ltd. (Shanghai, China). Soya oil was from Jiangxi Goldencrabapple Medicinal Oil Co., Ltd. (Yushan, China). Glycerol was obtained from Shanghai Better Chemical Co., Ltd. and vitamin E was from Qingdao Zhongzhi Pharmaceutical Science and Technology Co., Ltd. (Qingdao, China). Oleic acid was purchased from Yixing Chemical reagent Co. Ltd. (Yixing, China). Miglyol 812 (DAC, oleum neutrale; CTFA, caprylic/capric triglyceride [caprylic acid, C8; capric acid, C10] MCTs) was provided by Caelo (Hilden, Germany). Pluoronic F68 (poloxamer 188) was donated by BASF (D-Ludwigshafen, Germany). Soybean lecithin was purchased from Sigma (St. Louis, MO, USA). The other chemicals were of analytical reagent grade.

Solubility of Docetaxel in Aqueous Solution

An excess amount of docetaxel was added to double-distilled water or phosphate buffer at various pH values and shaken for 48 h at 25°C. The mixture was centrifuged at 8000 × *g* for 20 min (Hamada, Ishiara, Masuoka, Mikuni, & Nakajima, 2006), and the supernatant was passed through a 0.45-μm membrane filter. The drug concentration in filtrate was determined by high-performance liquid chromatography (HPLC) directly.

Distribution of Docetaxel between Oil (Lipid) and Water

Excess oil (soya oil, Miglyol 812 or mixture of them at various amount ratio) was added to double-distilled water and mixed for 24 h (at 25°C) to saturate each other. Then two phases were separated and used as lipid phase and aqueous phase. About 20 mL of oil (dissolved proper amount docetaxel) and aqueous solution were mixed. These mixtures were thoroughly shaken for 24 h (at 25°C) and then centrifuged at 4,000 rpm for 15 min to fully separate aqueous phase from the lipid phase. Portion of oil phase layer was then transferred and 1:10 diluted with dichloromethane, and then analyzed by HPLC method to determine drug concentration. Aqueous phase was directly analyzed by HPLC.

Degradation Kinetics of Docetaxel

Docetaxel was dissolved in absolute ethanol to prepare a stock solution at a concentration of 2 mg/mL. The final concentration for stability samples was 40 μg/mL, which was obtained by mixing a 2:98 ratio of stock solution with appropriate experimental solution. The pH values of 0.05 mol/L phosphate buffer used as experimental solution for measurement of the rate-pH profile of degradation of docetaxel were as follows: pH 1.2, 3.0, 5.0, 7.0, and 8.0. All pH values were measured using a pH meter equipped with a combination electrode, which was calibrated with primary buffer solution of pH 4.01, 6.86, and 9.18. The ionic strength of these buffer solutions was adjusted to 0.2. Stability samples were sealed in screw-topped test tubes and then laid in a thermostat bath at 70°C (Stable to ± 0.5°C) (Guo et al., 2007). Tubes were withdrawn at appropriate intervals. Samples were diluted 1:1 with ice-cold ethanol rapidly and cooled in ice to quench the reaction. Solutions were stored at –20°C for analysis within 5 h. About 20 μL of samples was injected into HPLC column for analysis. The effect of initial drug concentration on degradation was performed by using phosphate buffer (pH 5.0) at a drug concentration from 20 to 60 μg/mL at 70°C.

Docetaxel Submicron Lipid Emulsion Preparation

The aqueous phase and oil phase were separately prepared. The aqueous phase consisted of double-distilled water, soybean lecithin (1.2–2% of total emulsion), isotonic moderator glycerol (2.25% of total emulsion), and coemulsifier Pluoronic F68 (0.3–0.5% of total emulsion). The oil phase consisted of oil (mixture of soya oil and Miglyol 812 at a mass ratio of 1:4) (10–20% of total emulsion), stabilizer oleinic acid (0.3% of total emulsion), oxidation-resistant vitamin E (0.05% of total emulsion), and docetaxel (0.4 to 1.0 mg/mL of total emulsion). The two phases were further heated to 65°C, and the oil phase must be stirred at 65°C to make docetaxel completely dissolve in oil phase. After soybean lecithin was dispersed uniformly in aqueous phase, oil phase was dropped into aqueous phase and mixed using a high-shear homogenizer at 10,000 rpm (FJ-200, Shanghai Specimen Formwork Co.) for 10 min (Wang, Sung, Hu, Yeh, & Fang, 2006) at 65°C to prepare coarse emulsion. Then the coarse emulsion was further homogenized through a high-pressure homogenizer (YSNM-1500 nanomizer system, Japan) five times at an operational pressure of 75 Mpa at 40°C to obtain the final emulsion. After adjusting the pH value with HCl or NaOH solution, the preparation was filtered through a sterile Coster® 0.22-μm filter, filled with N_2 and sealed in glass bottles that were then wrapped in aluminum foil under asepsis condition. To evaluate the effect of pH value on the stability of preparation, the pH value of final emulsion was adjusted in the range from 3.0 to 7.5, and the final preparation was heated for 30 min at 100°C to accelerate the drug degradation and assess the physical stability of emulsion.

Particle Size and Zeta Potential Analysis

Particle size was measured by photon correlation spectroscopy (PCS) using a Nicomp™ 380 particle sizing system (Santa Barbara, CA, USA). The operating software employs two mathematical approaches for data analysis, and two kinds of distribution were obtained, namely Gaussian and Nicomp distribution. The measuring range was approximately 3 nm–3 μm, and before measurement, emulsion samples were diluted 1:5,000 in highly purified water, which had been passed through 0.22-μm filter. In order to assess whether Gaussian distribution was reasonable, a fit error parameter was determined which was less than 3. Through analysis the Gaussian distribution was considered reliable and then the mean diameter and standard deviation were reported to each sample. Otherwise, the data were analyzed using Nicomp distribution and the mean size, and the percentage of each size was reported.

Zeta potential of the vesicle was determined using a Nicomp™ 380 electrophoretic light scattering (ELS) apparatus. The ELS technique was based on the scattering of light from particles that moved in liquid under the influence of an applied electric field. For the results obtained here, samples were diluted by 1:3,000 with 0.01% NaCl solution that had also been passed through 0.22-μm filter.

Drug Entrapment in Lipid Emulsion

The entrapment efficiency (EE %) was calculated by the percentage of docetaxel incorporated into oil phase relative to the amount of docetaxel in total emulsion. Two methods were used for this measurement, namely ultrafiltration and ultracentrifugation method (Wang et al., 2006). Total emulsion was divided into two portions. One portion was used for determining the unentrapped drug that was separated from the emulsion by ultrafiltration or ultracentrifugation method. The other portion was used for determining the amount of docetaxel in total emulsion. The amount of unentrapped and originally total docetaxel was determined by HPLC.

Ultrafiltration

After the separation of the lipid vesicle by ultrafiltration/centrifugation technique through VIVASPIN 4 filters (molecular weight cut-off 10 kDa), unentrapped docetaxel was determined in the ultrafiltrate. To correct the nonspecific adsorption of the drug molecular to the ultrafiltration membrane, the correction factor *f* was introduced, which was determined by ultrafiltering the docetaxel standard solution. The correction factor *f* was calculated by Equation 1, where *C* is the concentration of docetaxel.

$$f = \frac{C_{\text{filtrate}}}{C_{\text{total}}} \tag{1}$$

The unentrapped drug concentrations were corrected by the *f* value.

For EE % determination, 4 mL of drug-loaded emulsion was added to the VIVASPIN 4 and centrifuged for 1 h at 3,000 rpm. The concentration of docetaxel in filtrate and total emulsion was determined by HPLC. The EE% was calculated by Equation 2, where C is the concentration of docetaxel and V is the volume of aqueous phase or total emulsion.

$$EE\% = \left(1 - \frac{C_{\text{filtrate}}/f}{C_{\text{total}}} \times \frac{V_{\text{aqueous}}}{V_{\text{total}}}\right) \times 100 \quad (2)$$

Ultracentrifugation

Three milliters of emulsion was pipeted into polyallomer tube and centrifuged at 162,000 × g and 4°C for 2 h in a Micro CS120GXL Ultracentrifuge (Hitachi, Tokyo, Japan) to separate the incorporated drug from the nonincorporated drug. The subnatant aqueous layer was analyzed by HPLC for nonincorporated drug concentration to determine the EE %. The EE % was calculated by Equation 2, where f was equal to 1.

Stability Studies

Thermal Destruction

Emulsions at various pH values, about 4 mL sealed in 10-mL glass bottles wrapped in aluminum foil, were heated at 100°C for 30 min followed by natural cooling to room temperature. The emulsions were visually examined and sampled for particle size, zeta potential, entrapment efficiency, and docetaxel concentration analysis.

Shaking Test

Emulsions were shaken on a shaker (HZQ-C; Harbin, China) at the setting of 30 at room temperature. The emulsions were visually examined and sampled for particle size analysis at predetermined time intervals.

Stability of Docetaxel Emulsions in Plasma

It was anticipated that future in vivo evaluations would be carried out in a variety of animal models. Stability of emulsions was studied ex vivo in mouse, rat, dog, and human plasma as a preliminary study. About 0.5 mL aliquots of emulsion were mixed with 2 mL plasma at 37°C for 6 h. The samples were visually examined, and particle size was then measured using PCS and compared with the original emulsions.

Real-Time Stability

Docetaxel emulsions were stored at 4 and 25°C for real-time stability assessment. Emulsion particle size and size distribution were determined periodically for physical stability evaluation, and chemical stability was evaluated by docetaxel concentration determination at appropriate intervals.

Drug Analysis (HPLC)

Docetaxel concentration was analyzed using a RP-HPLC system. The equipment, a Shimadzu HPLC system (Kyoto, Japan), consisted of an LC-10AT pump and an SPD-10A UV-VIS detector. A Diamonsil ODS C18 column (200 × 4.6 mm, 5 μm) was used. The mobile phase was methanol–water (75:25), the flow rate is 1.0 mL/min, and detection was at 230 nm (Loos, Verweij, Nooter, Stoter, & Sparreboom, 1997). For determination of docetaxel concentration in total emulsion, emulsions were directly dissolved in absolute ethanol or isopropanol by 20 times dilution.

Animal Studies

Animal studies were performed on male Wistar rats weighing 180–200 g, which were fasted overnight for 12–14 h with free access to water. The animal experimentation was approved by the Committee of Ethics of Animal Experimentation of Shenyang Pharmaceutical University. Rats (n = 3) received an intravenous injection of 12 mg/kg docetaxel lipid emulsion or solution in the tail vein. Docetaxel solution was prepared by dissolving docetaxel in Tween 80 at 40 mg/mL and then diluted 1:4 with ethanol 13% (w/v). Before administration, the solution was diluted with physiological buffer to the desired concentration (1.0 mg/mL). Blood samples were taken from the retro-orbital plexus at various times. Plasma was sampled and detected by LC-MS analysis. Plasma pharmacokinetic parameters were obtained from the plasma concentration–time data of each experiment with statistical moment algorithm.

RESULTS AND DISCUSSION

Drug Solubility

The equilibrium aqueous solubility of docetaxel at various pH values is shown in Table 1. The aqueous solubility at 25°C was determined to be 4.93 μg/mL. When phosphate-buffered solution was used as the solvent, docetaxel solubility increased at higher pH values but was still very low. The results suggested that the change of pH could not significantly increase

TABLE 1
Aqueous Solubility of Docetaxel at 25°C

pH Value	Solubility[a] (μg/mL)
5.00	2.09 ± 0.05
5.73	3.76 ± 0.07
6.50	4.17 ± 0.07
7.10	4.29 ± 0.04
7.32	4.33 ± 0.08
8.00	5.02 ± 0.10
Purified water	4.93 ± 0.11

[a]$M \pm SD$ (n = 3).

the solubility of docetaxel, because of its hydrophobic chemical structure (Figure 1). In addition, docetaxel dose not contain the ionized functional groups that allow for pH alternation and salt formation to increase its solubility. In clinic, docetaxel is administrated at a concentration from 0.3 to 0.9 mg/mL. To achieve this concentration, docetaxel is formulated in current commercially available micellar solution (Taxotere®) containing Tween 80 and dehydrated ethanol. To avoid using these potentially toxic vehicles, a new intravenous carrier for docetaxel is urgently needed, such as submicron lipid emulsion.

Distribution of Docetaxel between Oil and Water

Soya oil and Miglyol 812 (MCT) were used to study the distribution characteristics of docetaxel between oil and water for the guidance to the lipid emulsion formulation design, because soya oil and MCT were the most widely used oil phase in current commercial lipid emulsion preparations. The results suggested that distribution coefficient of docetaxel between oil and water increased with the increasing percentage of MCT in the oil phase (Figure 2). Based on Figure 2, we selected mixtures of soya oil and MCT (1:4, wt/wt) or MCT alone as the oil phase to prepare a docetaxel emulsion by temperature elevation method.

Degradation Kinetics of Docetaxel

Degradation of docetaxel in phosphate buffer at various pH values was investigated and a reaction rate constant, *k*, was calculated (Guo et al., 2007; Tokumura et al., 1985). The degradation of docetaxel followed pseudo-first-order kinetics because a linear relationship between logarithmic remaining percentage of docetaxel and degradation time existed as shown in Figure 3. The apparent first-order rate constant was calculated from the slope of each straight line, and the results are shown in Table 2.

The degradation rate constant of docetaxel increased when decreasing pH from 5.0 to 1.2. Docetaxel could not be detected after a 10-min reaction in pH 1.2 buffer at 70°C, indicating that docetaxel is very unstable under strong acid conditions. By contrast, the degradation rate constant of docetaxel increased with increasing pH above pH 5.0. Therefore, around pH 5.0 is the optimal pH for increased docetaxel stability. As the degradation of docetaxel was affected significantly by pH, pH value of the formulations has a significant influence on the stability of docetaxel preparations. Accordingly, pH screening sheds a light on formulation design for drugs with pH-sensitive stability.

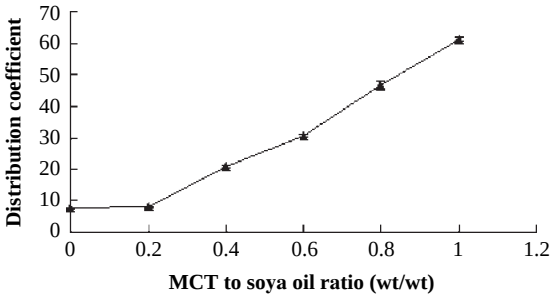

FIGURE 2. Distribution coefficients of docetaxel between oil and water at different medium chain triglyceride (MCT) to soya oil ratios. Each value represented as $M \pm SD$.

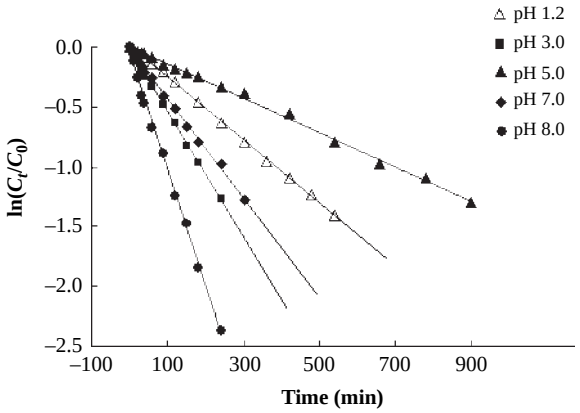

FIGURE 3. Pseudo-first-order plots for degradation of docetaxel at 25 (open symbols) and 70°C (filled symbols) in phosphate-buffered solutions at various pH values.

TABLE 2
Apparent Pseudo-First-Order Rate Constant for Degradation of Docetaxel in Phosphate Buffer

pH	k (min^{-1})
1.2	2.59×10^{-3}[a]
3.0	5.35×10^{-3}[b]
5.0	1.42×10^{-3}[b]
7.0	4.26×10^{-3}[b]
8.0	1.01×10^{-2}[b]

[a]25°C.
[b]70°C.

The influence of the initial concentration of docetaxel on the degradation rate is shown in Figure 4. The initial concentration of docetaxel, ranging from 20 to 60 μg/mL, has no effect on degradation kinetics at pH 5.0.

Preparation of Docetaxel Supersaturated Emulsions

In this study, we used mixtures of soya oil and MCT (1:4, w/w) or MCT alone as the oil phase to prepare the docetaxel supersaturated emulsion by temperature elevation method (Levy & Benita, 1989). Although docetaxel was observed to be more soluble in MCT than in soya oil, the solubility was still very low (<0.5 mg/mL at 25°C) (data not shown). Hence, by the conventional de novo emulsion preparation method, one can achieve the maximal drug concentration of approximately 0.05 mg/mL in a 10% MCT emulsion or 0.1 mg/mL in a 20% MCT emulsion.

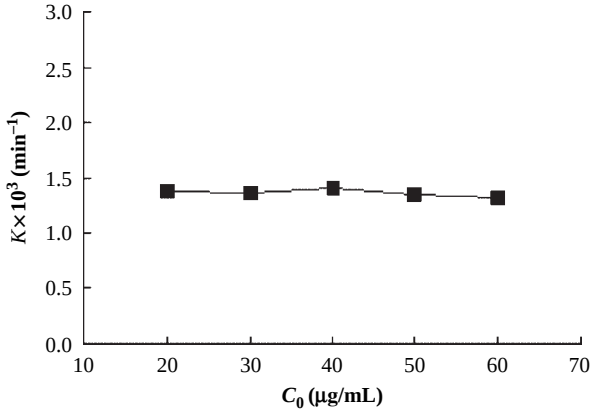

FIGURE 4. Degradation rate constants of docetaxel at different initial concentrations in phosphate buffer at pH 5.0 and 70°C.

This is much lower than the desired product concentration in clinic, 0.3–0.9 mg/mL. In general, the drug loading of emulsions is determined by the solubility of the drug in the internal oil phase (Benita & Levy, 1993). When a higher drug loading in emulsions is desired with a certain lipid amount, a conventional lipid emulsion for docetaxel may not be possible because of solubility limitation. Currently, temperature elevation method is usually used to enhance the solubility of drug in the internal oil phase to prepare a high drug-loading supersaturated emulsion (Levy & Benita, 1989). It was thought that upon emulsification of the supersaturated oil phase, the drug might partition to the oil/water interface of the emulsion particles or it might precipitate to reduce the supersaturation of the internal oil phase (Wang & Cory, 1999). We have observed that the color of emulsions became more yellow after heating when MCT was used alone. Hence, mixtures of soya oil and MCT (1:4, wt/wt) were used as the oil phase to prepare emulsions.

Phospholipids (PHLs) are usually the first candidates as emulsifier because of their biocompatibility and long-time application in commercial intravenous fat emulsion. Pluoronic F68 (Poloxamer 188) is also widely used as coemulsifier in intravenous formulations. Table 3 shows the effect of the amount of oil and surfactants (soybean lecithin and Pluoronic F68) on properties of emulsions containing 0.4 mg/mL docetaxel. No precipitation or pellets were observed in all formulations by ultracentrifugation, suggesting that docetaxel did not precipitate in the emulsions. When reducing oil content, mean droplet size diminished slightly. In all formulations (Nos 1–7), amounts of oil and surfactants had no significant effect on droplet size distribution, zeta potential, and drug entrapment efficiency. Commercial intravenous fat emulsions usually have mean droplet sizes between 100 and 500 nm and is generally required to be smaller than 1 μm. Therefore, mean droplet sizes of emulsions (Nos 1–7) were suitable for intravenous applications. To reduce the lipid amount and contents of surfactants, No. 7 formulation, 10% lipid emulsion was adopted to carry out the further studies.

Loading Capacity of Docetaxel in Emulsions

Docetaxel was incorporated in 10% lipid emulsions at a concentration range from 0.4 to 1.0 mg/mL. As shown in Table 4, No. 7 formulation could entrap docetaxel at a concentration up to 1.0 mg/mL without drug precipitation. Particle size, zeta potential, and drug entrapment efficiency were not affected by increasing load of docetaxel. Hence, this emulsion may be a promising option for docetaxel intravenous administration.

Effect of Drug Load on Particle Size Distribution and Zeta Potential

The potential effect of entrapping the supersaturated oil phase in emulsions is that drug precipitation could occur

TABLE 3
The Effectiveness of Selected Oil Phase Ratio and Emulsifiers in Docetaxel Emulsions

Formulation	Oil Phase % [a]	PHL %	F68 %	Particle Size[b] (nm)	Zeta Potential (mv)	EE% [b,c]	Drug Precipitation[d]
No. 1	20	2.0	0.5	184.8 ± 64.5	–21.4	95.4 ± 2.4	–
No. 2	15	2.0	0.5	156.7 ± 55.9	–22.8	94.8 ± 3.5	–
No. 3	10	2.0	0.5	160.8 ± 7`1.5	–22.4	93.6 ± 3.8	–
No. 4	10	1.6	0.5	156.1 ± 57.9	–21.7	94.0 ± 3.2	–
No. 5	10	1.2	0.5	161.4 ± 69.5	–19.9	95.4 ± 2.3	–
No. 6	10	1.2	0.4	158.1 ± 57.8	–21.3	94.4 ± 2.6	–
No. 7	10	1.2	0.3	162.9 ± 78.3	–20.1	95.6 ± 2.4	–

0.05% vitamin E, 0.3% oleinic acid, 2.25% glycerol, 0.4 mg/mL docetaxel.
[a]Mixtures of soya oil and MCT (1:4, wt/wt).
[b]$M \pm SD$.
[c]Determined by ultracentrifugation method.
[d]After centrifuged at 162,000 × g and 4°C for 2 h.

TABLE 4
The Influences of Drug Load on the Properties of Docetaxel Emulsions

Concentration of Drug (mg/mL)	Particle Size[a] (nm)	Zeta Potential (mv)	EE % [a,b]	Drug Precipitation[c]
0.4	155.4 ± 61.2	−19.5	95.3 ± 1.7	–
0.8	159.5 ± 52.7	−20.8	96.6 ± 1.1	–
1.0	161.3 ± 66.2	−21.5	96.1 ± 0.5	–

No. 7 formulation mentioned in Table 3.
[a]$M \pm SD$.
[b]Determined by ultracentrifugation method.
[c]After centrifuged at 162,000 × g and 4°C for 2 h.

or drug load might affect the particle size distribution and destabilize the emulsions. Therefore, the particle size distribution was investigated as a function of drug load (Figure 5). It shows that relative to emulsion placebos, addition of docetaxel did not affect the mean particle size and size distribution of emulsions either before or after heating at 100°C for 30 min. It is possible that drug load may change the zeta potential of the emulsion particles because of the partition of the drug to oil/water interface of the emulsion particles (Wang & Cory, 1999). The zeta potential of 10% lipid emulsion as a function of drug load is also shown in Figure 5. The zeta potential was not affected by addition of docetaxel, because docetaxel dose not contain functional groups that can ionize in emulsions.

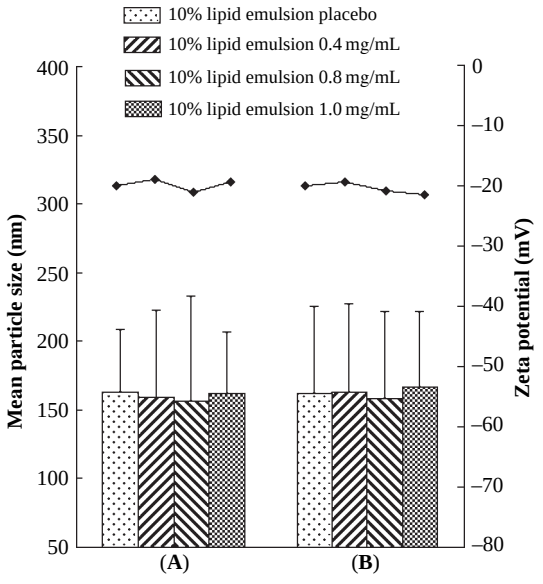

FIGURE 5. Particle size and zeta potential of 10% lipid emulsion as a function of drug load before (A) and after (B) heating for 30 min at 100°C. Bars represent mean particle diameter + *SD*; lines represent zeta potential.

Stability Studies

Thermal Destruction (Effect of pH on the Stability)

The stability of docetaxel emulsions to autoclaving was evaluated by autoclaving at 121°C for 20 min. But we found that a large amount of drug was lost after autoclaving, so docetaxel emulsions at various pH values were heated at 100°C for 30 min to inspect the effect of pH on the stability of emulsions.

Table 5 lists particle size distribution before and after heating, along with drug concentration and entrapment efficiency, which was determined by ultracentrifugation and ultrafiltration methods simultaneously. Regardless of drug load, particle size distribution of the emulsions at various pH values were essentially unaffected by heating, demonstrating excellent physical stability of docetaxel emulsions against heat. Concentration analysis of docetaxel by HPLC showed that the extent of drug loss was closely related to the pH values of emulsions. This suggests that the excess drug in supersaturated oil phase may largely distribute to the oil/water interface and can be affected by pH values in the aqueous phase. Above pH 5.0, the higher the pH, the higher the percent of drug loss, and the case is an inverse proportion to that at pH below 4.0. The similar percents of drug loss from pH 4.0 to 5.0 suggest that docetaxel emulsions should be controlled at pH from 4.0 to 5.0. In addition, pH values of emulsions did not dramatically affect the EE % of docetaxel as listed in Table 5. Higher EE % determined by ultrafiltration method compared to that by ultracentrifugation may attribute to the incomplete separation between oil phase and aqueous phase in ultracentrifugation. Despite this, EE % determined by ultracentrifugation is still very high, up to 90%, which can be used in entrapment efficiency determination. Although not the focus of this report, the chemical stability of docetaxel has been improved by selecting proper sterilization equipment, such as the one with the square wave heating and cooling capability.

Shaking Test

The physical stability of submicron lipid emulsion of docetaxel was evaluated by a shaking test. As shown in Figure 6, shaking of the emulsions for up to 12 h produced no significant change in mean droplet diameter, suggesting that the emulsions are physically stable to shaking.

TABLE 5
Effects of pH Values on the Physicochemical Stability of Docetaxel Emulsions

pH	Particle Size Distribution[a] (nm)		Concentration[a] (μg/mL)			Entrapment Efficiency[a] (%)			
						Before Heating		After Heating	
	Before Heating	After Heating	Before Heating	After Heating	Residue (%)	Ultracentrifugation	Ultrafiltration	Ultracentrifugation	Ultrafiltration
3.0	157.4 ± 61.7	155.4 ± 61.2	403.6 ± 4.0	304.3 ± 3.5	75.4	95.5 ± 1.3	97.7 ± 0.1		
3.5	159.3 ± 52.1	161.4 ± 62.8	403.6 ± 3.4	374.2 ± 2.9	92.7	93.1 ± 2.2	97.6 ± 0.2	94.6 ± 1.1	96.0 ± 1.6
4.0	155.3 ± 62.1	159.5 ± 44.8	408.3 ± 10.9	390.3 ± 3.9	95.6	94.8 ± 3.1	97.8 ± 0.1	93.7 ± 3.4	97.7 ± 0.1
4.5	158.4 ± 59.2	162.5 ± 49.8	399.5 ± 6.0	388.8 ± 3.9	97.3	94.0 ± 2.4	97.5 ± 0.3	93.4 ± 1.4	98.0 ± 0.6
5.0	163.5 ± 44.7	163.6 ± 64.3	404.9 ± 10.0	389.6 ± 4.7	96.2	95.7 ± 3.6	97.7 ± 0.2	97.1 ± 1.0	97.7 ± 0.1
5.5	162.4 ± 71.3	159.5 ± 51.7	398.4 ± 8.3	371.9 ± 5.9	93.4	93.5 ± 2.1	97.5 ± 0.3	94.6 ± 1.9	96.8 ± 0.7
6.0	163.2 ± 56.7	158.4 ± 47.6	401.9 ± 2.2	377.4 ± 2.7	93.9	94.9 ± 1.7	97.7 ± 0.2	94.4 ± 3.0	97.7 ± 0.1
6.5	154.8 ± 61.3	155.7 ± 76.9	398.2 ± 8.3	276.5 ± 5.4	69.4	94.5 ± 1.0	97.6 ± 0.1		
7.0	159.5 ± 56.4	165.8 ± 48.9	404.5 ± 4.9	185.2 ± 2.5	45.8	94.1 ± 3.0	97.5 ± 0.1		
7.5	161.8 ± 67.1	159.4 ± 61.0	405.7 ± 6.9	125.3 ± 1.6	30.9	93.7 ± 3.7	97.6 ± 0.3		

10 % lipid emulsions containing docetaxel 0.4 mg/mL.
[a]*M* ± *SD*.

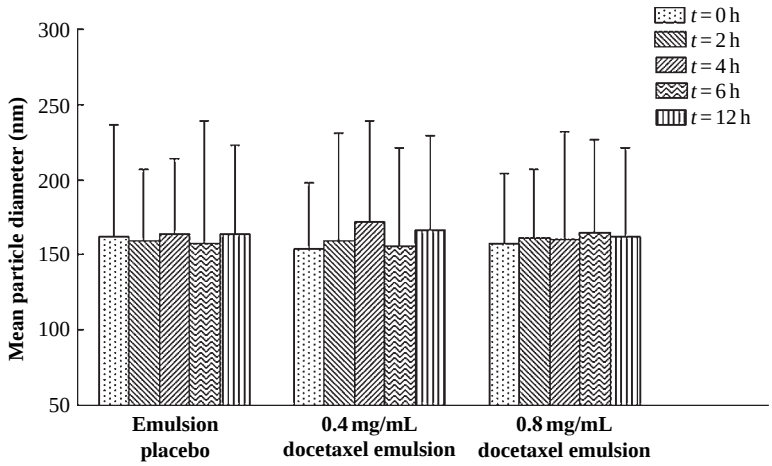

FIGURE 6. Effect of shaking on the stability of submicron lipid emulsions of docetaxel. Bars represent mean particle diameter + *SD*.

Stability of Emulsions in Plasma

Emulsion stability in plasma is clearly an important factor for intravenous applications. The possible flocculation and coalescence of emulsions in plasma may block the lung capillaries and cause some other adverse effects. We investigated the stability of the emulsion in mouse, rat, dog, and human plasma, and no flocculation was observed in different plasma. After incubation in different plasma at 37°C for 6 h, the mean droplet size of docetaxel emulsions increased slightly at drug concentration of either 0.4 or 0.8 mg/mL but still below 200 nm (Figure 7).

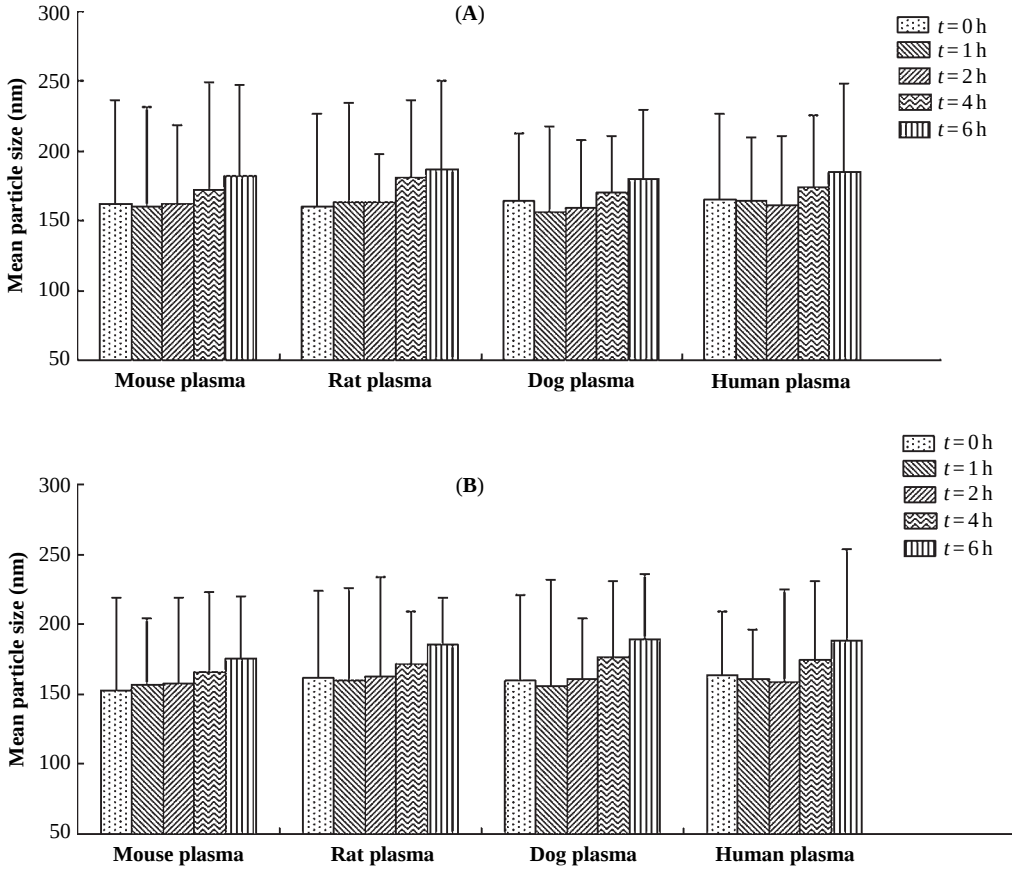

FIGURE 7. Stability of submicron lipid emulsions of docetaxel at concentrations of 0.4 (A) and 0.8 mg/mL (B). Bars represent mean particle diameter + *SD*.

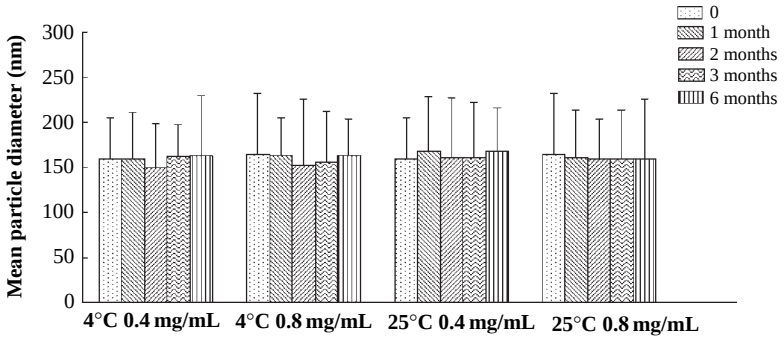

FIGURE 8. Long-term physical stability of submicron lipid emulsions of docetaxel. Bars represent mean particle diameter + *SD*.

Real-Time Stability

The physical stability of the docetaxel emulsions after long-term storage is shown in Figure 8, in which the particle size distributions of two emulsions (10% lipid emulsions of docetaxel at 0.4 and 0.8 mg/mL) stored at 4 and 25°C for up to 6 months are reported. Compared to the particle size distributions at time zero, no change was noted for two emulsions. The data demonstrated that docetaxel supersaturated lipid emulsions have long-term physical stability at 4 and 25°C.

The chemical stability of docetaxel emulsions at 4 and 25°C was also evaluated by HPLC analysis of docetaxel in emulsions (Figure 9). The concentration of docetaxel in emulsions has no significant reduction for either 0.4 or 0.8 mg/mL docetaxel emulsions. These data demonstrated that although the supersaturated docetaxel emulsion is thermodynamically unstable at 121°C, long-term chemical stability at 4 and 25°C could be achieved with proper formulation, such as the pH values, surfactants.

Animal Studies

Docetaxel formulated in lipid emulsion has a significantly greater area under the curve (AUC), 436.2 against 142.5 μg min/mL, higher C_{max}, 36.6 against 8.1 μg/mL, lower systemic clearance, 4.32 against 14.05 mL/min, and lower steady-state volume of distribution (V_{ss}), 309.6 against 1273.4 mL, with a corresponding higher plasma concentration compared to docetaxel solution (Figure 10). These findings suggested that incorporation of docetaxel into lipid emulsion may increase its effective plasma concentration, potentially as a result of the lower release of drug from emulsion droplets.

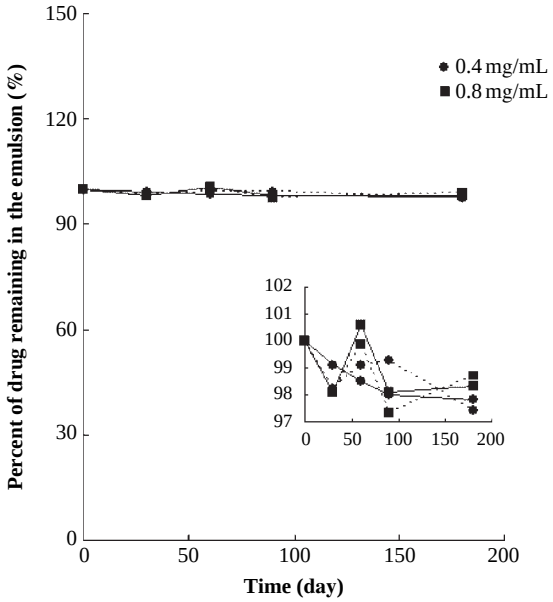

FIGURE 9. Long-term chemical stability of submicron lipid emulsions of docetaxel. Dotted line, 4°C; solid line, 25°C.

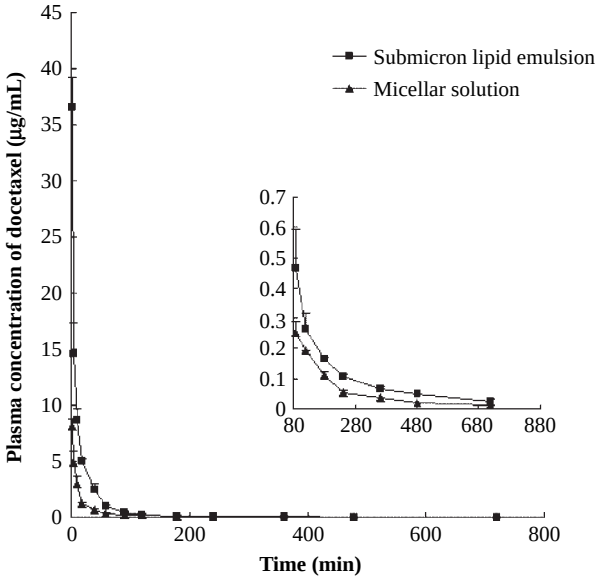

FIGURE 10. The plasma docetaxel concentration–time profiles after intravenous administration of 12 mg/kg dose of docetaxel submicron lipid emulsion and micellar solution. Data are expressed as *M* + *SD*.

CONCLUSIONS

Preformulation investigation suggested that docetaxel is a sparingly soluble drug and pH alternation cannot increase the solubility of docetaxel significantly. Docetaxel is relatively stable in weak acid condition around pH 5.0, and the degradation rate of docetaxel increased above and below pH 5.0. Although docetaxel was observed to be more soluble in MCT than soya oil, the solubility was still very low (< 0.5 mg/mL). By the temperature elevation method and controlled pH value, a high drug loading and stable supersaturated submicron emulsion of docetaxel have been prepared. The resultant emulsions are in the nanometer size and are physically and chemically stable at 4 and 25°C for 6 months. Animal studies suggested that docetaxel submicron lipid emulsion could significantly increase the effective docetaxel plasma concentration. These results indicated that the submicron lipid emulsion obtained in this study is a promising option for docetaxel to replace clinical miceller solutions as an intravenous treatment.

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