Drug Development and Industrial Pharmacy, 2011; 37(5): 597–605

ISSN 0363-9045 print/ISSN 1520-5762 online
DOI: 10.3109/03639045.2010.533276

informa
healthcare

ORIGINAL ARTICLE

Phospholipid–Tween 80 mixed micelles as an intravenous delivery carrier for paclitaxel

Huijun Liang[1,*], Qiuxia Yang[1,*], Li Deng[1], Jianzhong Lu[2], and Jianming Chen[1]

[1]*School of Pharmacy, Second Military Medical University, Shanghai, China, and* [2]*School of Pharmacy, Fudan University, Shanghai, China*

Abstract

A novel mixed micelle made of Tween 80 and soybean phospholipids (S80) was prepared and used as the delivery system for paclitaxel (PTX), with the purpose of improving the stability, therapeutic index, and security of PTX in comparison with Taxol® injection. The micelle size, morphological features, dilution stability, and critical micelle concentration (CMC) were measured. The *in vitro* antitumor activity, pharmacokinetics, and hemolysis effect of the optimal PTX-loaded mixed micelles (PTX-M) were evaluated and compared with Taxol®. The results showed that PTX-M was more stable than Taxol® upon dilution. PTX-M had a higher antitumor efficacy against HeLa and A549 cells than that of Taxol®. The plasma AUC of PTX-M was 1.3-fold higher than that of Taxol® and the hemolysis test revealed that PTX-M was safe for intravenous injection. In conclusion, PTX-M had a higher dilution stability and antitumor efficacy than Taxol®, but significantly reduced the toxicity while improving the bioavailability of PTX. Therefore, Tween 80–S80 mixed micelles could be a promising drug carrier for intravenous administration of PTX.

Keywords: Mixed micelles, paclitaxel, stability, antitumor activity, pharmacokinetics, hemolysis

Introduction

Paclitaxel (PTX), a diterpenoid derived from *Taxus brevifolia*, has been demonstrated significant activity in clinical trials against a wide variety of tumors, including refractory ovarian cancer, metastatic breast cancer, non-small cell lung cancer, AIDS-related Kaposi's sarcoma, head and neck malignancies, and other cancers (Zhang et al., 2008). Due to its poor water solubility (<1 μg/mL), PTX is currently formulated in a 50:50 (v/v) mixture of Cremophor EL/absolute ethanol as Taxol®, which must be further diluted with saline or 5% glucose solution before intravenous (i.v.) administration (Fjällskog et al., 1993). However, Taxol® has numbers of associated side effects attributed to the presence of Cremophor EL including hypersensitivity, nephrotoxicity, and neurotoxicity (Szebeni et al., 1998; Liggins et al., 2002; Singla et al., 2002; Wu et al., 2010). In addition, the precipitation of drug occurs after Taxol® is diluted with an aqueous solution (Alkan-Onyuksel et al., 1994; Sautou-Miranda et al., 1999; Singla et al., 2002; Zhang et al., 2005). To address these concerns, several other delivery systems have been developed, including nanospheres, liposomes, cyclodextrin complexes, emulsions, lipid-based nanospheres, water-soluble prodrugs, and micelles (Gao et al., 2002; Singla et al., 2002).

Among those delivery systems, mixed micelles as the promising drug carriers have been paid more attention in recent years. Mixed micelles have a core-shell structure that enables the poorly soluble drugs to be incorporated, thus improving their bioavailability, and protects from inactivation in biological media (Liggins et al., 2002). Due to their small particle size (approximately 5–50 nm), mixed micelles can spontaneously accumulate in pathological areas with damaged ("leaky") vasculature, such as infarcts and tumors (Gabizon, 1995; Yuan et al., 1995), via the enhanced permeability and retention (EPR) effect (Maeda et al., 2000, 2001). In addition, mixed micelles can enhance micelle stability and drug-loading efficiency, compared with those micelles with the individual components (Gao et al., 2005).

*These two authors contributed equally to this work.

Address for Correspondence: Jianming Chen, School of Pharmacy, Second Military Medical University, Shanghai 200433, China. E-mail:yjcjm@163.com

(Received 06 January 2010; revised 10 October 2010; accepted 15 October 2010)

Suitable phospholipid–surfactant mixtures have recently attracted a significant interest due to their potential industrial applications (Simões et al., 2005). Such carriers in convenient aggregate form are stabilized by phospholipids and made adaptable by surfactants. However, mixtures of phospholipids and surfactants can self-assemble in either liposomes or mixed micelles, depending on the composition. Liposomes are vesicular structures consisting of bilayers that form spontaneously when phospholipids are dispersed in water. Depending on the processing conditions and the chemical composition, liposomes are formed with one or several concentric bilayers. Liposomes are different from phospholipid mixed micelles structurally in that they have a bilayer membrane, whereas the phospholipid mixed micelles are composed of monolayers.

Phospholipids are nontoxic and biocompatible, which meet the requirements of intravenous preparations. It has been reported that PTX showed a much higher solubility in mixed micellar solutions of lecithin (Sznitowska et al., 2008). Polyoxyethylene sorbitan monooleate (Tween 80) is often used as a solubilizing and stabilizing agent in medicinal and pharmaceutical preparations (Wang et al., 2008), owing to its attractive cost and relatively low toxicity (Haque et al., 1999). The *in vivo* study indicates that Tween 80, to a certain extent, is a more acceptable excipient for the insoluble agents compared with Cremophor EL (van Tellingen et al., 1999). Tween 80 is used to solubilize several other anticancer drugs, including etoposide as well as the cyclopropylpyrroloindole compounds such as adozelesin, bizelesin, and carzelesin (van Zuylen et al., 2001). The semisynthetic taxane analog has been formulated for clinical use, and 80 mg of docetaxel is dissolved in 2 mL of Tween 80. The interaction of Tween 80 and phospholipid has been reported previously (Fadda et al., 1998; Cevc, 2004; Simões et al., 2005). Similarly, phospholipid–surfactant systems have been widely investigated for several years. However, at present, mixtures such as delivery carriers for PTX are rarely described.

The aim of this study was to develop Tween 80–soybean phospholipid (S80) mixed micellar systems as the potential carrier for PTX. The PTX-loaded mixed micelles (PTX-M) were prepared, and particle size, morphology, dilution stability, and critical micelle concentration (CMC) were measured. Compared with Taxol®, the antitumor efficacy, pharmacokinetics, and safety of PTX-M were evaluated.

Materials and methods

Materials

PTX was purchased from Meilian pharmaceutical Co. Ltd. (Chongqing, China). Taxol® (Anzatax Injection Concentrate, 30 mg/5 mL) was produced by FH Faulding & Co. Ltd. (Melbourne, Australia). S80 was obtained from Shanghai Taiwei Co. Ltd. (Shanghai, China). Tween 80 was purchased from Shanghai Shenyu Pharmaceutical & Chemical Co. Ltd. (Shanghai, China). Pyrene was bought from Sigma (St. Louis, MO). Water used was double-distilled. Acetonitrile and tetrahydrofuran were of HPLC grade, and other reagents were of analytical grade.

Preparation of PTX-M

Various amounts of S80 were completely dissolved in about 0.3 mL of dehydrated ethanol, then mixed with 0.5 mL Tween 80 and diluted to 1.0 mL with dehydrated ethanol. Thereafter, 6 mg of PTX was added, and the resulting mixture was gently vortexed for 30 min following sonication for 10 min. The PTX-M was obtained.

Characterization of PTX -M

Micelle size determination

Micelles were diluted 10-fold with water. The particle size of PTX-M was measured by dynamic light scattering (DLS) using Zetasizer (Malvern, UK) at 25°C. The detection range was from 2 to 5000 nm. Each sample was analyzed in triplicate.

Morphological features

The morphology of samples was observed with transmission electron microscope (TEM) (JEM-2010; JEOL, Japan). A drop of sample after dilution was placed onto a carbon-coated copper grid to form a thin liquid film. The films on the grid were negatively stained with 0.1% (w/v) phosphotungstic acids. After excess solution was removed, the sample was air-dried at room temperature.

Dilution stability

Samples were diluted 10-fold with 5% glucose solution and placed at room temperature. After 3 days, the diluted samples were filtered through 0.22-μm nylon (polyamide) membrane filters (Millipore Co., Bedford, MA) and the amount of PTX remaining in solution was assayed using HPLC. The mobile phase consisted of acetonitrile and water (60:40, v/v). The flow rate was 1.0 mL/min and the detection wavelength was 227 nm.

CMC determination

Pyrene fluorescence method was used for CMC determination. A stock solution of pyrene (5×10^{-5} M) was prepared in acetone and stored at 4°C until use. The fluorescence emission spectra of pyrene in different micelle solutions were measured using fluorometer (F-2000; HITACHI Co., Japan). The excitation wavelength was 335 nm and the fluorescence intensities were measured at 373 nm (band 1) and 384 nm (band 3).

In vitro antitumor efficacy

Cell culture

HeLa cells (human cervix carcinoma cell lines) and A549 cells (human lung adenocarcinoma cell lines) were obtained from the American Type Culture Collection (Rockville, MD), and were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Grand Island, NY) containing 10% (v/v) heat-inactivated fetal bovine serum,

100 IU/mL penicillin G, 0.25 μg/mL amphotericin B, and 100 μg/mL streptomycin at 37°C with 5% CO_2/95% air humidified atmosphere.

In vitro cytotoxicity

In vitro cytotoxicity was evaluated by MTT assay (Nishiyama et al., 2001; Lee et al., 2003; Wang et al., 2005; Dabholkar et al., 2006; Li et al., 2008; Danhier et al., 2009). The method is based on the fact that living cells reduce MTT to formazan. The cytotoxicity was measured following the absorbance of the degraded MTT (formazan) at 492 nm using a MCC/340 ELIZA Reader (Lab Systems, Finland).

HeLa and A549 cell lines were exposed to the media containing increasing concentrations of Taxol®, PTX-M, Cremophor® EL, and drug-free Tween 80–S80 micelles for 24 h, followed by MTT assay to evaluate their mortality. The IC_{50} values, the concentrations of various preparations at which the cell growth inhibition was 50% compared with untreated control cells, were estimated according to the dose–response curves.

Pharmacokinetics of PTX-M

Chromatographic conditions

The plasma concentrations of PTX were determined with an HPLC method. The HPLC system comprised of a G1311A quaternary pump (1100 series), G1322A online degasser (1100 series), and autosampler (1100 series) (Agilent, Palo Alto, CA). A Diamonsil C_{18} column (5 μm, 250 mm × 4.6 mm; Dikma, Shanghai, China) was used as analytical column. The mobile phase was a mixture of acetonitrile:water:tetrahydrofuran (55:45:8, v/v/v). The flow rate was 1.0 mL/min and effluent was monitored at 227 nm.

Pharmacokinetics of PTX-M in rats

All experimental procedures were reviewed and approved by the Institutional Animal Ethical Committee and were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals. Twelve Sprague-Dawley (SD) rats (250 ± 20 g), which were supplied by the Laboratory Animal Center of Second Military Medical University, were randomly divided into two groups. Taxol® and PTX-M were administered intravenously through the tail vein at a dose of 5 mg/kg. Blood samples (0.5 mL) were collected into heparinized tubes from the plexus venous in the eye ground at 0 (pre-dose), 5, 15, 30 min, 1, 2, 4, 6, 8, 12, and 24 h after intravenous administration. The plasma was obtained following centrifugation at 10,000 rpm for 5 min and stored at –20°C until analysis.

A liquid–liquid phase extraction procedure with tertiary butyl methyl ether was used for the extraction of PTX from plasma. Three milliliters of tertiary butyl methyl ether were added to 0.20 mL plasma samples, and the resulting mixture was vortexed for 2 min. After centrifugation at 15,000 rpm for 3 min, the organic layer was collected, and then 2.00 mL of which were transferred to a clear tapered centrifuging tube and evaporated under nitrogen at 40°C. The residue was re-dissolved in 100 μL mobile phase solution and 20 μL aliquots of the supernatant were injected into the HPLC system.

The pharmacokinetic parameters and the compartment model were computed by software program 3p87 (Chinese Society of Mathematical Pharmacology, China). The Student's *t*-test was used for comparison of the pharmacokinetic parameters in different formulations. For this purpose, the level of significance was set $\alpha = 0.05$. All results were expressed as the mean ± SD.

Hemolysis test

Rabbit blood was used to test the hemolysis effect of PTX-M. In brief, 10 mL of rabbit blood was obtained from arteria cruralis and the fibrinogen was removed by stirring with glass rod. Rabbit blood cells (RBC) were rinsed several times in aqueous NaCl solution (0.9%, w/v) by centrifugation for 3 min at 2000 rpm until supernatants were colorless, and diluted with aqueous NaCl solution (1:50, v/v) to obtain 2% erythrocyte standard dispersion.

Different amounts of PTX-M and Taxol® with volume of 0.25, 0.5, 0.75, and 1 mL were added into four tubes with 2.5 mL of 2% erythrocyte dispersion in each. Then, adequate amounts of 0.9% isotonic NaCl solution were added in every tube to obtain a final volume of 5 mL. To obtain 0% and 100% hemolysis controls, 2.5 mL of 2% erythrocyte dispersion was added to 2.5 mL of 0.9% isotonic NaCl solution and 2.5 mL of distilled water, respectively. All samples were incubated for 1 h at 37°C, and then followed for 5 min at 0°C to stop hemolysis. After centrifugation, the absorbance of supernatants was determined at 415 nm with UV–visible spectrophotometer (Agilent 8453 spectrophotometer). The hemolysis rate (HR) was determined according to the following equation:

$$\mathrm{HR}(\%) = \frac{(\mathrm{Abs} - \mathrm{Abs}_0)}{(\mathrm{Abs}_{100} - \mathrm{Abs}_0)} \times 100\%$$

#1Abs, Abs_{100}, and Abs_0 were the absorbances of the samples, a solution of 100% hemolysis, and a solution of 0% hemolysis, respectively.

Results and discussion

Preparation of PTX-M

To investigate the interaction between S80 and Tween 80, PTX-M with different concentrations of S80 (0, 40, 80, 120, 160, and 200 mg/mL) were prepared and a standard orthogonal array matrix $L_9(3^4)$ was constructed with three factors and three levels to select optimum formulation. The mean diameters and the PTX content in 10-fold diluted micellar solutions were labeled as *S* and Cp, respectively. Then *W* (*W* = Cp/*S*) was taken as an index, the content of S80, the amount of Tween 80, and the volume of ethanol were chosen as the influential factors (labeled as A, B, and C). According to the orthogonal

design, the optimal formulation PTX-M was obtained as follows: 6 mg of PTX, 120 mg of S80, 0.5 mL of Tween 80, and 0.5 mL of dehydrated ethanol. This optimized PTX-M was chosen to compare the antitumor efficacy, pharmacokinetics, and safety with Taxol®.

The use of micelles for solubilization of poorly soluble anticancer drugs had attracted much attention recently, and majority of the micellar formulations were polymeric micelles. Synthesis or structural modification of the polymer was necessary before preparation of micelles, which made the preparation process of micelle more complicated (Gaucher et al., 2005; Huh et al., 2005; Rijcken et al., 2007; Yi et al., 2007; Kollipara et al., 2010). In our study, S80 and Tween 80 were the most commonly used excipients in pharmaceutical industry; micelles from S80–Tween 80 were rather of low cost, and easy and convenient to produce. In addition, S80 could enhance the stability of the PTX-M (Cevc, 2004; Simões et al., 2004) and Tween 80 made the PTX-M adaptable (Cevc et al., 2003).

Characterization of PTX-M

Micelle size

The effects of the amount of S80 on micelle size were investigated. The mean diameters and size distribution of various micellar formulations were shown in Figure 1. The average size of Tween 80 single micelle as well as PTX-M with the concentration of S80 lower than 120 mg/mL was about 10–20 nm, and only one peak was observed (Figure 1A–1D). As the concentration of S80 was higher than 160 mg/mL, a bimodal size distribution patterns was observed, as depicted in Figure 1E and 1F. This result was attributed to the micelle-to-vesicle transition (MVT). MVT was usually examined and used as the technique of detergent removal from mixed micelles (Ollivon et al., 2000; Yin et al., 2006; Stuart et al., 2007). In this study, the detergent removal was replaced with the increase of phospholipid. Before the addition of S80, the size distribution had a single peak with an average size of 10 nm, which indicated that Tween 80 could form a single micelle. With the increase of the amount of S80, S80 was gradually solubilized through Tween 80 inserting into its bilayer. The bilayer was saturated (Stuart et al., 2007) when the concentration of S80 was 120 mg/mL, which resulted in the occurrence of a complete solubilization and Tween 80–S80 mixed micelles with only one peak (Danhier et al., 2009). Upon a further increase of the S80 concentration, Tween 80 was insufficient to solubilize the S80 and the excessive S80 could self-assemble and form the vesicles with a diameter of 100–200 nm. Therefore, the vesicles coexisted with Tween 80–S80 mixed micelles, which resulted in a bimodal size distribution. Many investigations had suggested that the MVT occurred in phospholipid–surfactant mixed systems (Leng et al., 2003; Miyahara et al., 2004).

Morphological characterization

TEM image showed that the morphology of Tween 80 micelles was spherical in shape with a smooth surface (Figure 2A). After forming mixed micelles, the shape of

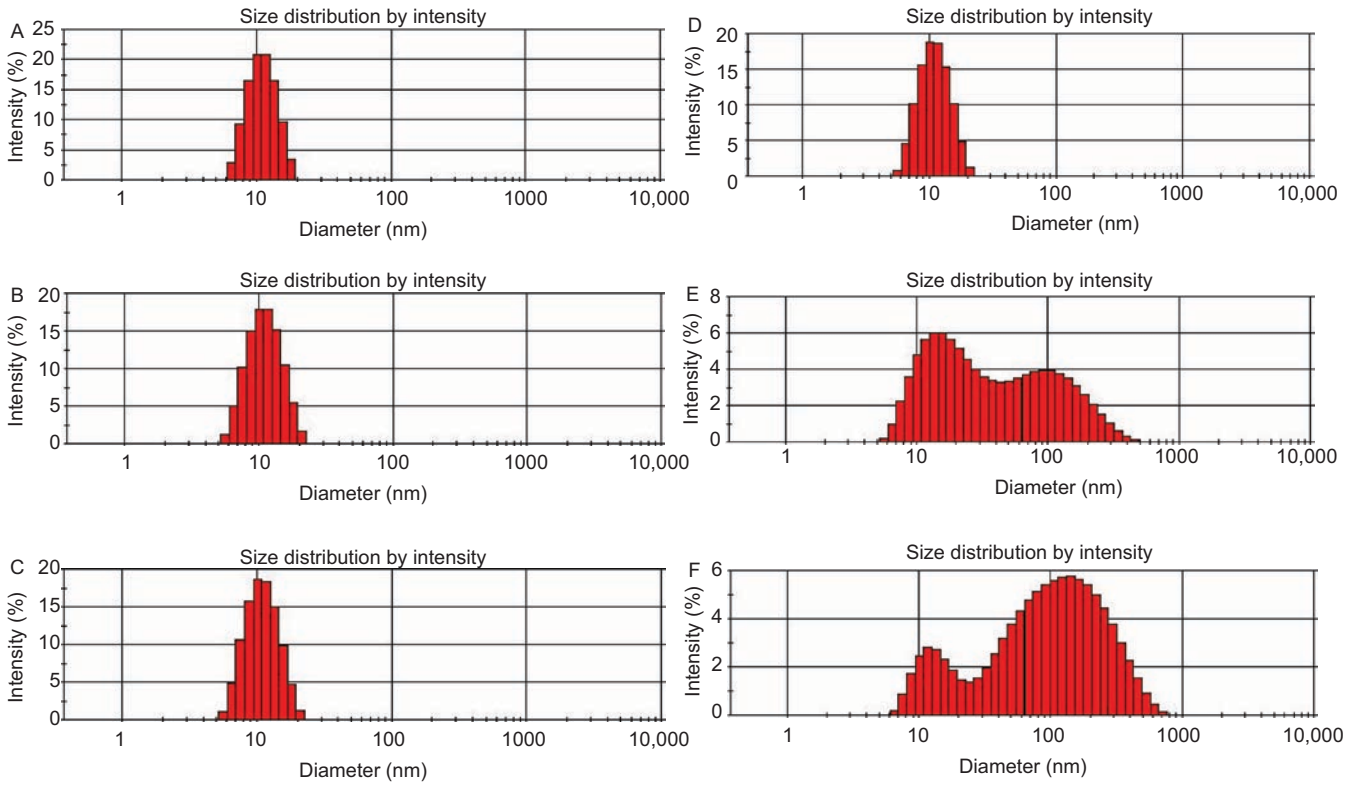

Figure 1. Micelle size and size distribution of Tween 80 single micelle (A), PTX-M containing 40 mg/mL S80 (B), PTX-M containing 80 mg/mL S80 (C), PTX-M containing 120 mg/mL S80 (D), PTX-M containing 160 mg/mL S80 (E), and PTX-M containing 200 mg/mL S80 (F).

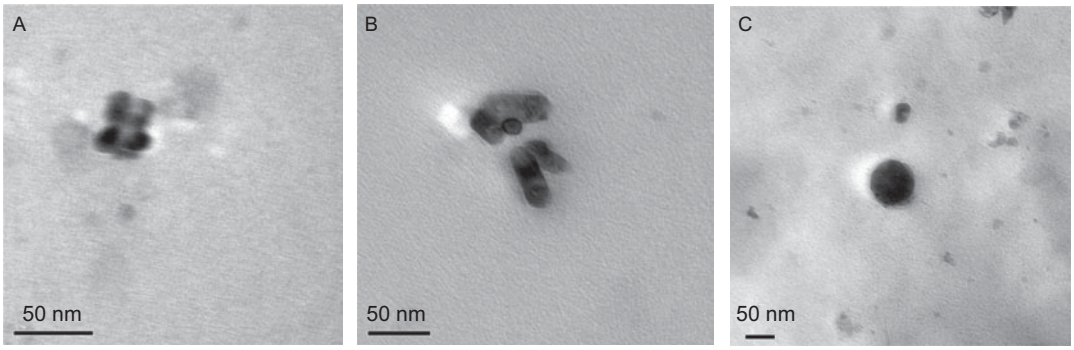

Figure 2. Transmission electron microscopic (TEM) images of Tween 80 single micelle (A), PTX-M containing 120 mg/mL S80 (B), and PTX-M containing 160 mg/mL S80 (C).

micelles changed into cylinders (Figure 2B). And finally, the excessive S80 spontaneously aggregated into spherical vesicles with larger size (Figure 2C). These results were in agreement with those described previously (Yin et al., 2006). This microstructural transformation could also be explained by MVT. When S80 was at the concentration of 120 mg/mL, Tween 80 molecules were just incorporated within all the S80 bilayer, only mixed micelles existed in the system and demonstrated cylindrical in shape. And finally, the morphology reverted to sphere because the excessive S80 could self-assemble and form larger vesicles.

Dilution stability

The PTX content in various 10-fold diluted micellar solutions was determined to investigate the effect of dilution on the stability of micelles (Figure 3). Figure 3 showed that upon a 10-fold dilution of Taxol® by 5% glucose solution, the encapsulated PTX was completely released into the aqueous environment, although 98% of the encapsulated drug was retained in the cores of optimized PTX-M. These data suggested that upon intravenous injections, PTX-M containing S80 were more stable toward dilution than pure Tween 80 micelles and Taxol® injection. As the concentration of S80 was increased, the dilution stability of PTX-M was markedly improved.

Dilution stability was very important for a drug-delivery system because one of the major differences between the *in vitro* and *in vivo* conditions was the dilution effect under *in vivo* administration. A drug-encapsulated micelle was injected into the circulation, which resulted in a many-fold dilution and the micelle dissociation into monomers. Therefore, the use of stable micelles would offer advantages (Gao et al., 2005). The dilution stability of PTX-M was attributed to the presence of S80 (Simões et al., 2005). With the increase of S80, Tween 80 inserted into the bilayer of S80 and mixed micelles with highly hydrophobic cores gradually formed (Ollivon et al., 2000; Yin et al., 2006; Stuart et al., 2007; Danhier et al., 2009). This provided a larger hydrophobic cargo space for PTX. PTX spontaneously participated into micelle cores from the aqueous environment. It was known that hydrophobic micelles were likely to exhibit a higher solubilization capacity than the hydrophilic ones (Sezgin et al., 2006;

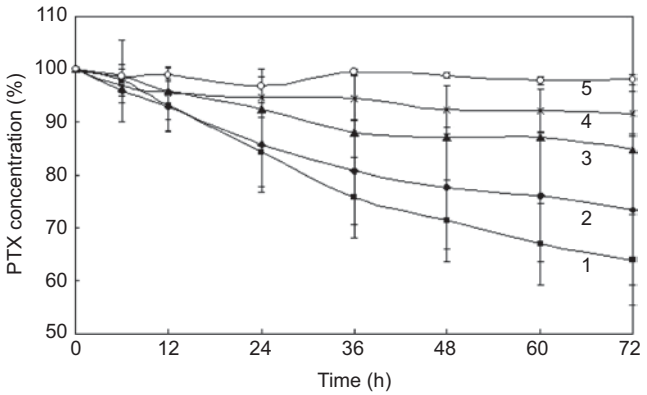

Figure 3. Time courses of PTX concentration in various 10-fold diluted micellar solutions. (1) Tween 80 single micelle, (2) Taxol®, (3) PTX-M containing 40 mg/mL S80, (4) PTX-M containing 80 mg/mL S80, and (5) PTX-M containing 120 mg/mL S80.

Yang et al., 2007), and increased hydrophobicity of mixed micelle cores resulted in the enhanced stability upon dilution (Gao et al., 2005). The addition of S80 increased hydrophobicity of mixed micelle cores, which resulted in the enhanced stability upon dilution. Gao et al. (2005) and Li et al. (2008) had also demonstrated that mixed micelles increased micelle stability and drug-loading efficiency.

CMC values

As for CMC determination, pyrene method was chosen due to its functional, versatile, and easy application (Sezgin et al., 2006). This method utilizes pyrene's low solubility in water. Thus, pyrene preferred to participate in hydrophobic microenvironment (Lukyanov et al., 2004; Khatua et al., 2006; Sezgin et al., 2006; Yang et al., 2007). The CMC values of micelles were presented in Table 1. It was obviously seen that CMC values were decreased with the increase of the concentration of S80 in micelles. The formation of micelles above the CMC value was associated with the appearance of a hydrophobic phase (micelle core) that solubilized pyrene, thus increasing the solution fluorescence. Data shown in Table 1 confirmed the enhancement of the hydrophobic environment as S80 with higher hydrophobicity was introduced into the mixed micelles. The lower CMC

Table 1. The critical micelle concentration (CMC) values of Tween 80 single micelle and PTX-M containing different concentration of S80.

	PTX-M			
Concentration of S80 (mg/mL)	0	40	80	120
CMC × 10^{-3} (mmol/L)	2.334	1.743	1.716	1.198

values of Tween 80–S80 mixed micelles compared with that of Tween 80 single micelle suggested its strong tendency to form micelles. The introduction of S80 might have reduced electrostatic repulsion among ionic head groups, thus lowering the CMC and increasing the intermolecular polymerization to form more stable micelle (Khatua et al., 2006).

In general, the increase in hydrophobicity of micelle core decreased the polarity of microenvironment. The intensity ratio I_1/I_3 of the first (373 nm) and the third (384 nm) vibronic peaks of the pyrene fluorescence spectrum was very sensitive to solvent polarity and therefore had been widely used as a measure of the polarity of the microenvironment of the probe (Aguiar et al., 2003; Bakshi et al., 2005). Normally, a high and low I_1/I_3 ratio indicated polar and nonpolar environment, respectively. The polarity ratio decreased with the increase of S80 as shown in Figure 4. The I_1/I_3 ratio for PTX-M was lower than that of Taxol®. This might be due to more ordering at the interface of the mixed micelles than that in Taxol®. The ordering of the aggregate interface reduced the degree of water penetration into the hydrophobic micelle core and led to an increase in hydrophobic interactions between the polymer chains in the micelle core, and thus provided some advantages in particle stability toward dilution compared with Taxol® (Khatua et al., 2006; Li et al., 2008).

In vitro antitumor activity

In vitro cytotoxicity of PTX-M and Taxol® against HeLa and A549 cells was compared and illustrated in Figure 5. The cytotoxicity of Cremophor® EL and PTX-free Tween 80–S80 micelles was not negligible, as shown in Figure 5A. Cremophor® EL exhibited higher cytotoxicity than PTX-free Tween 80–S80 micelles. The possible mechanisms contributing to the cytotoxicity of Cremophor® EL was ascribed to the formation of free radicals by peroxidation of polyunsaturated fatty acids (oleic acid, one of the constituents of Cremophore EL®) and/or a direct perturbing effect in the cell membrane causing fluidity and leakage (Gelderblom et al., 2001). Though oleic acid was also present in Tween 80, the lower cytotoxicity of PTX-free Tween 80–S80 micelles might be due to Tween 80 inserting into the bilayer of S80 and reducing the chance of Tween 80 directly exposing to cell membrane.

Figure 5B showed the IC_{50} values of Taxol® and PTX-M for the HeLa and A549 cells. PTX-M exhibited superior activity to that of Taxol® in the case of the inhibition of the growth of HeLa and A549 cells. One possible explanation of the data could be greater cellular uptake of PTX-M via phagocytosis or fusion processes of phospholipid

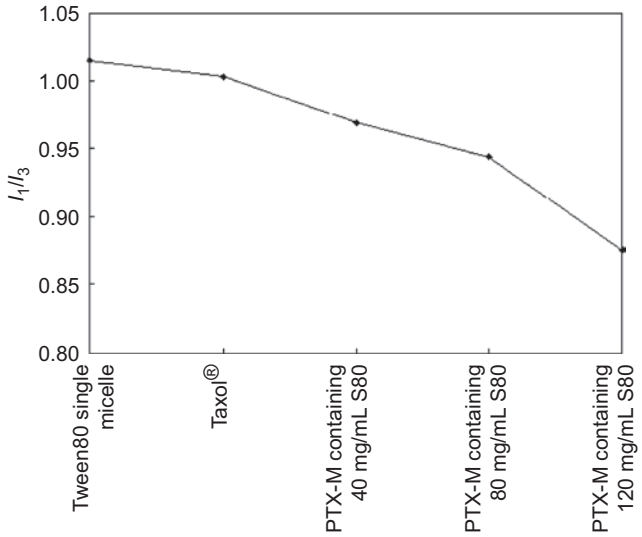

Figure 4. The I_1/I_3 ratios of Taxol®, Tween 80 single micelle, and PTX-M containing different concentrations of S80.

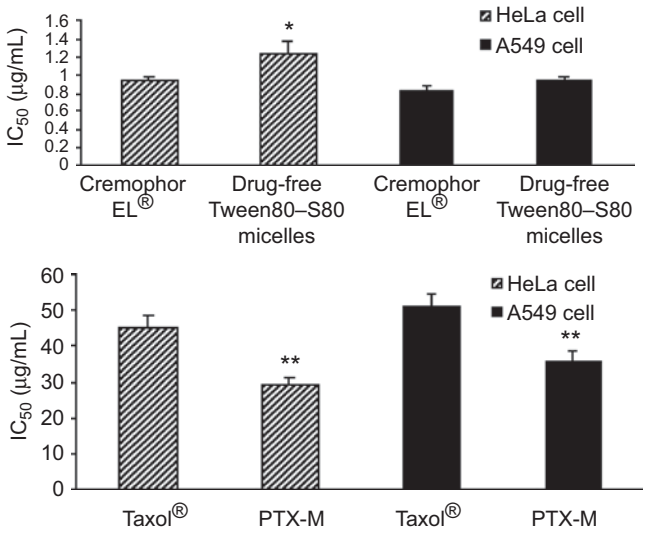

Figure 5. IC_{50} values of Cremophor EL®, drug-free Tween 80–S80 micelles (A) and Taxol®, optimal PTX-M (B) against HeLa cells and A549 cells (*$P < 0.05$, **$P < 0.01$).

micelles (Koo et al., 2005). Tween 80 could solubilize fatty molecules and bilayer membranes (Simões et al., 2005), which resulted in the enhanced permeability of the cell membrane to PTX. Therefore, it might be suggested that Tween 80–S80 micelles maintained the pharmacological activity of PTX and efficiently delivered PTX to the cells. However, more works needed to be done to investigate the various mechanisms of cellular uptake of drug-containing Tween 80–S80 mixed micelles.

Pharmacokinetics of PTX-M

PTX in plasma was completely separated under analytical conditions, and standard curves ranging from 0.05 to 100 μg/mL were linear ($r = 0.9998$). The results attained from recovery of high, middle, and low concentrations were 88.07%, 101.32%, and 96.85%, respectively. The

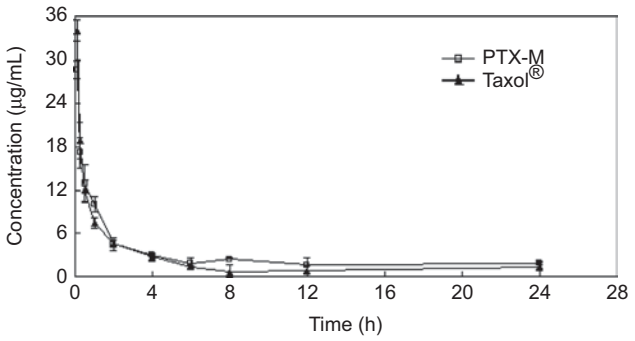

Figure 6. Mean plasma concentration–time curves of PTX in rats after i.v. administration of the optimal PTX-M and Taxol®.

relative standard deviation (RSD) for inter-days were 1.71%, 2.17%, and 1.03%, respectively, the RSD. for intra-days were 2.27%, 0.94%, and 1.87%, respectively, which showed that recovery and RSD for inter-days or intra-days were satisfactory.

The plasma concentration–time profiles of PTX after i.v. administration of PTX-M and Taxol® were shown in Figure 6. The related pharmacokinetic parameters of PTX in the two formulations were listed in Table 2. The pharmacokinetic behaviors for PTX in PTX-M and Taxol® were found to be similar. The plasma concentration of PTX delivered by the mixed micelles was slightly higher than Taxol® during the experimental period except the first 15 min. The AUC of PTX-M was higher (1.3-fold) than that of Taxol® ($P < 0.05$). A rapid decline in concentrations of PTX in two formulations represented a distribution phase. The $t_{1/2\alpha}$ (distribution half-life) showed no significant difference ($P > 0.05$) between the two groups. The $t_{1/2\beta}$ (elimination half-life) of PTX-M and Taxol® were 6.62 and 6.11 h, respectively. The other parameters were no significant differences between the two formulations ($P > 0.05$). These data indicated that PTX-M exhibited comparable bioavailability with that observed with Taxol®. The slightly higher AUC in plasma of PTX-M than Taxol® might be attributed to its interaction with the lipoprotein pool (Lundberg et al., 2003).

Hemolysis test

To determine the safe of the PTX-M formulation for intravenous administration, the hemolytic potential of the formulations were evaluated and compared with Taxol®. The PTX concentration in this experiment was in the range of 0.3–1.2 mg/mL because the Taxol® was diluted with 0.9% sodium chloride or 5% glucose to concentrations of 30–120 mg/100 mL for intravenous administration in clinical practice. The hemolytic effect of PTX-M and Taxol® were shown in Table 3. The HR of PTX-M was significantly lower than that of Taxol® in each tested concentration ($P < 0.01$), and with the increase of PTX concentration, the HR difference between the two groups was more significant. As for PTX-M, the hemolytic effect was hardly observed at the concentration of 0.3 mg/mL; even at the highest concentration of 1.2 mg/mL, the HR was no more than 20%. However, the HR of Taxol® almost reached 80% at the concentration of 1.2 mg/mL.

Table 2. Pharmacokinetic parameters of PTX in the two formulations after i.v. administration.

Parameters	Taxol®	PTX-M
V (L/kg)	0.14 ± 0.01	0.18 ± 0.04
$t_{1/2a}$ (h)	0.40 ± 0.27	0.46 ± 0.13
$t_{1/2\beta}$ (h)	6.11 ± 0.54	6.62 ± 3.35
K_{21} (h^{-1})	0.43 ± 0.18	0.34 ± 0.12
K_{12} (h^{-1})	0.46 ± 0.12	0.32 ± 0.07
K_{10} (h^{-1})	1.32 ± 0.79	1.62 ± 0.72
AUC (mg/L h)	56.57 ± 11.64	72.76 ± 15.03*
Cl (L/h/kg)	0.09 ± 0.02	0.07 ± 0.01

Data represented mean value ± SD, $n = 6$.
*$P < 0.05$, compared with Taxol®.

Table 3. *In vitro* hemolysis rate (HR) of the optimal PTX-M and Taxol® ($n = 3$, mean ± SD).

PTX concentration (mg/mL)	PTX-M (%)	Taxol® (%)
0.3	1.23 ± 0.02	4.91 ± 0.01
0.6	5.77 ± 0.1	8.23 ± 0.8
0.9	12.9 ± 0.8	68.43 ± 3.7
1.2	19.41 ± 1.2	78.01 ± 2.9

The lower HR of Tween 80–S80 micelles might be due to a protective effect of the phospholipids. Lichtenberger et al. (1983) suggested that phospholipids protected membrane from being adsorbed as a monolayer on to the surface and created a hydrophobic barrier, which resulted in reduced hemolysis. Martin et al. (1981) had demonstrated that the formation of mixed micelles between bile salt and phospholipids could protect the erythrocyte membranes against bile salt-induced damage. Tween 80 would incorporate in the lipophilic core or in the interface, which would limit the direct contact of the Tween 80 with the erythrocyte membranes and would accordingly result in a reduction of the hemolytic activity. Therefore, the results suggested that PTX-M was much safer than Taxol® for intravenous administration.

Conclusions

A mixed micelle delivery system for PTX based on phospholipid–Tween 80 was developed and characterized as an effective alternative to Taxol®. The mixed micelles made of Tween 80–S80 increased the stability toward dilution. Additionally, pharmacokinetic and pharmacological assessments demonstrated that PTX-M with optimal formulation had the higher antitumor efficacy than Taxol®, but significantly reduced the toxic effects and improved the bioavailability of PTX. These results indicated that Tween 80–S80 mixed micelles could decrease the disadvantages of conventional delivery systems containing Cremophor EL and dehydrated alcohol and appear to be a better possible approach that would bypass the limitations of current delivery system and provide a desirable therapeutic efficacy.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

Aguiar J, Carpena P, Molina-Bolívar JA, Ruiz CC. (2003). On the determination of the critical micelle concentration by the pyrene 1:3 ratio method. J Colloid Interface Sci 258:116–122.

Alkan-Onyuksel H, Ramakrishnan S, Chai HB, Pezzuto JM. (1994). A mixed micellar formulation suitable for the parenteral administration of Taxol. Pharm Res 11:206–212.

Bakshi MS, Singh J, Kaur G. (2005). Antagonistic mixing behavior of cationic gemini surfactants and triblock polymers in mixed micelles. J Colloid Interface Sci 285:403–412.

Cevc G. (2004). Lipid vesicles and other colloids as drug carriers on the skin. Adv Drug Deliv Rev 56:675–711.

Cevc G, Gebauer D. (2003). Hydration-driven transport of deformable lipid vesicles through fine pores and the skin barrier. Biophys J 84:1010–1024.

Dabholkar RD, Sawant RM, Mongayt DA, Devarajan PV, Torchilin VP. (2006). Polyethylene glycol–phosphatidylethanolamine conjugate (PEG–PE)-based mixed micelles: some properties, loading with paclitaxel, and modulation of P-glycoprotein-mediated efflux. Int J Pharm 315:148–157.

Danhier F, Lecouturier N, Vroman B, Jérôme C, Marchand-Brynaert J, Feron O, Préat V. (2009). Paclitaxel-loaded PEGylated PLGA-based nanoparticles: *in vitro* and *in vivo* evaluation. J Control Release 133:11–17.

Fadda M, Baroli BM, Maccioni AM, Sinico C, Valent D, Alhaiqu F. (1998). Phospholipid–detergent systems: effects of polysorbates on the release of liposomal caffeine. II Farmaco 53:650–654.

Fjällskog ML, Frii L, Bergh J. (1993). Is Cremophor EL, solvent for paclitaxel, cytotoxic? Lancet 342:873.

Gabizon AA. (1995). Liposome circulation time and tumor targeting: implications for cancer chemotherapy. Adv Drug Deliv Rev 16:285–294.

Gao ZG, Fain HD, Rapoport N. (2005). Controlled and targeted tumor chemotherapy by micellar-encapsulated drug and ultrasound. J Control Release 102:203–222.

Gao ZG, Lukyanov AN, Singhal A, Torchilin VP. (2002). Diacyl lipid polymer micelles as nanocarriers for poorly soluble anticancer drugs. Nano Letters 2:979–982.

Gaucher G, Dufresne MH, Sant VP, Kang N, Maysinger D, Leroux JC. (2005). Block copolymer micelles: preparation, characterization and application in drug delivery. J Control Release 109:169–188.

Gelderblom H, Verweij J, Nooter K, Sparreboom A. (2001). Cremophor EL: the drawbacks and advantages of vehicle selection for drug formulation. Eur J Cancer 37:1590–1598.

Haque ME, Das AR, Moulik SP. (1999). Mixed micelles of sodium deoxycholate and polyoxyethylene sorbitan monooleate (Tween 80). J Colloid Interface Sci 217:1–7.

Huh KM, Lee SC, Cho YW, Lee J, Jeong JH, Park K. (2005). Hydrotropic polymer micelle system for delivery of paclitaxel. J Control Release 101:59–68.

Khatua D, Gupta A, Dey J. (2006). Characterization of micelle formation of dodecyldimethyl-*N*-2-phenoxyethylammonium bromide in aqueous solution. J Colloid Interface Sci 298:451–456.

Kollipara S, Bende G, Movva S, Saha R. (2010). Application of rotatable central composite design in the preparation and optimization of poly(lactic-*co*-glycolic acid) nanoparticles for controlled delivery of paclitaxel. Drug Dev Ind Pharm 36:1377–1387.

Koo OM, Rubinstein I, Onyuksel H. (2005). Camptothecin in sterically stabilized phospholipid micelles: a novel nanomedicine. Nanomedicine 1:77–84.

Lee CS, Kim C, Chan Kwon I, Chung H, Young Jeong S. (2003). Polymeric micelles of poly(2-ethyl-2-oxazoline)-block-poly(epsilon-caprolactone) copolymer as a carrier for paclitaxel. J Control Release 89:437–446.

Leng J, Egelhaaf SU, Cates ME. (2003). Kinetics of the micelle-to-vesicle transition: aqueous lecithin–bile salt mixtures. Biophys J 85:1624–1646.

Li L, Tan YB. (2008). Preparation and properties of mixed micelles made of Pluronic polymer and PEG–PE. J Colloid Interface Sci 317:326–331.

Lichtenberger LM, Graziani LA, Dial EJ, Butler BD, Hills BA. (1983). Role of surface-active phospholipids in gastric cytoprotection. Science 219:1327–1329.

Liggins RT, Burt HM. (2002). Polyether–polyester diblock copolymers for the preparation of paclitaxel loaded polymeric micelle formulations. Adv Drug Deliv Rev 54:191–202.

Lukyanov AN, Torchilin VP. (2004). Micelles from lipid derivatives of water-soluble polymers as delivery systems for poorly soluble drugs. Adv Drug Deliv Rev 56:1273–1289.

Lundberg BB, Risovic V, Ramaswamy M, Wasan KM. (2003). A lipophilic paclitaxel derivative incorporated in a lipid emulsion for parenteral administration. J Control Release 86:93–100.

Maeda H, Sawa T, Konno T. (2001). Mechanism of tumor-targeted delivery of macromolecular drugs, including the EPR effect in solid tumor and clinical overview of the prototype polymeric drug SMANCS. J Control Release 74:47–61.

Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. (2000). Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. J Control Release 65:271–284.

Martin GP, Marriott C. (1981). Membrane damage by bile salts: the protective function of phospholipids. J Pharm Pharmacol 33:754–759.

Miyahara M, Kawasaki H, Garamus VM, Nemoto N, Kakehashi R, Tanaka S, Annaka M, Maeda H. (2004). Micelle–vesicle transition of oleyldimethylamine oxide in water. Colloids Surf B Biointerfaces 38:131–138.

Nishiyama N, Kataoka K. (2001). Preparation and characterization of size-controlled polymeric micelle containing *cis*-dichlorodiammineplatinum(II) in the core. J Control Release 74:83–94.

Ollivon M, Lesieur S, Grabielle-Madelmont C, Paternostre M. (2000). Vesicle reconstitution from lipid–detergent mixed micelles. Biochim Biophys Acta 1508:34–50.

Rijcken CJ, Snel CJ, Schiffelers RM, van Nostrum CF, Hennink WE. (2007). Hydrolysable core-crosslinked thermosensitive polymeric micelles: synthesis, characterisation and *in vivo* studies. Biomaterials 28:5581–5593.

Sautou-Miranda V, Brigas F, Vanheerswynghels S, Chopineau J. (1999). Compatibility of paclitaxel in 5% glucose solution with ECOFLAC low-density polyethylene containers-stability under different storage conditions. Int J Pharm 178:77–82.

Sezgin Z, Yüksel N, Baykara T. (2006). Preparation and characterization of polymeric micelles for solubilization of poorly soluble anticancer drugs. Eur J Pharm Biopharm 64:261–268.

Simões SI, Marques CM, Cruz ME, Cevc G, Martins MB. (2004). The effect of cholate on solubilisation and permeability of simple and protein-loaded phosphatidylcholine/sodium cholate mixed aggregates designed to mediate transdermal delivery of macromolecules. Eur J Pharm Biopharm 58:509–519.

Simões SI, Tapadas JM, Marques CM, Cruz ME, Martins MB, Cevc G. (2005). Permeabilisation and solubilisation of soybean phosphatidylcholine bilayer vesicles, as membrane models, by polysorbate, Tween 80. Eur J Pharm Sci 26:307–317.

Singla AK, Garg A, Aggarwal D. (2002). Paclitaxel and its formulations. Int J Pharm 235:179–192.

Stuart MC, Boekema EJ. (2007). Two distinct mechanisms of vesicle-to-micelle and micelle-to-vesicle transition are mediated by the packing parameter of phospholipid–detergent systems. Biochim Biophys Acta 1768:2681–2689.

Szebeni J, Muggia FM, Alving CR. (1998). Complement activation by Cremophor EL as a possible contributor to hypersensitivity to paclitaxel: an *in vitro* study. J Natl Cancer Inst 90:300–306.

Sznitowska M, Klunder M, Placzek M. (2008). Paclitaxel solubility in aqueous dispersions and mixed micellar solutions of lecithin. Chem Pharm Bull 56:70–74.

van Tellingen O, Beijnen JH, Verweij J, Scherrenburg EJ, Nooijen WJ, Sparreboom A. (1999). Rapid esterase-sensitive breakdown of polysorbate 80 and its impact on the plasma pharmacokinetics of docetaxel and metabolites in mice. Clin Cancer Res 5:2918–2924.

van Zuylen L, Verweij J, Sparreboom A. (2001). Role of formulation vehicles in taxane pharmacology. Invest New Drugs 19:125–141.

Wang J, Mongayt D, Torchilin VP. (2005). Polymeric micelles for delivery of poorly soluble drugs: preparation and anticancer activity *in vitro* of paclitaxel incorporated into mixed micelles based on poly(ethylene glycol)–lipid conjugate and positively charged lipids. J Drug Target 13:73–80.

Wang W, Wang YJ, Wang DQ. (2008). Dual effects of Tween 80 on protein stability. Int J Pharm 347:31–38.

Wu L, Tang C, Yin C. (2010). Folate-mediated solid–liquid lipid nanoparticles for paclitaxel-coated poly(ethylene glycol). Drug Dev Ind Pharm 36:439–448.

Yang TF, Chen CN, Chen MC, Lai CH, Liang HF, Sung HW. (2007). Shell-crosslinked Pluronic L121 micelles as a drug delivery vehicle. Biomaterials 28:725–734.

Yi Y, Yoon HJ, Kim BO, Shim M, Kim SO, Hwang SJ, Seo MH. (2007). A mixed polymeric micellar formulation of itraconazole: characteristics, toxicity and pharmacokinetics. J Control Release 117:59–67.

Yin H, Lei S, Zhu S, Huang J, Ye J. (2006). Micelle-to-vesicle transition induced by organic additives in cationic surfactant systems. Chemistry 12:2825–2835.

Yuan F, Dellian M, Fukumura D, Leunig M, Berk DA, Torchilin VP, Jain RK. (1995). Vascular permeability in a human tumor xenograft: molecular size dependence and cutoff size. Cancer Res 55:3752–3756.

Zhang C, Qu G, Sun Y, Wu X, Yao Z, Guo Q, Ding Q, Yuan S, Shen Z, Ping Q, Zhou H. (2008). Pharmacokinetics, biodistribution, efficacy and safety of *N*-octyl-*O*-sulfate chitosan micelles loaded with paclitaxel. Biomaterials 29:1233–1241.

Zhang JA, Anyarambhatla G, Ma L, Ugwu S, Xuan T, Sardone T, Ahmad I. (2005). Development and characterization of a novel Cremophor EL free liposome-based paclitaxel (LEP-ETU) formulation. Eur J Pharm Biopharm 59:177–187.