

RESEARCH ARTICLE

Preparation, characterization, and pharmacokinetics of liposomal docetaxel for oral administration

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Abstract A docetaxel (DTX) liposomal formulation composed of egg phosphatidylcholine, sodium deoxycholate, and stearylamine was developed. Eudragit (0.5%) was coated to deliver the drug to the region between the distal small intestine and the colon. Lyophilized trehalose and mannitol were used as cryoprotectants because they preserve the particle integrity and good appearance. In vitro release studies showed that the amount of drug released from the coated liposomes was low in solution 1, which simulated the pH condition of the stomach. Especially during the average gastric emptying time, the amount of drug released decreased when Eudragit was added. The plasma DTX concentration was evaluated in pharmacokinetic studies. The plasma drug concentration after intravenous (i.v.) administration decreased rapidly within 120 min. Free DTX formulated using Tween 80 and the lyophilized Eudragit-coated liposomal formulation were compared after oral administration. The oral liposomal formulation had a longer half-life ($t_{1/2}$) and three-fold higher oral bioavailability. Thus, lyophilized Eudragit-coated liposomal DTX could be a promising therapy for various solid tumors to improve patient convenience and quality of life.

Keywords Docetaxel · Eudragit · Oral liposome · Pharmacokinetic · TEM

Introduction

Docetaxel (DTX) is a semisynthetic analog of the taxoid family widely used as an antineoplastic agent to treat breast, non-small cell lung, head and neck, and ovarian cancers (Huizing et al. 1995; Clarke and Rivory 1999; Lyseng-Williamson and Fenton 2005). DTX potently inhibits microtubule depolymerization by binding to tubulin and promoting its assembly. Therefore, this drug blocks cells in the late G₂/M phase of the cell cycle and induces apoptosis (Schiff et al. 1979; Ringel and Horwitz 1991). Despite its high potency, there are many limitations to its clinical application. Because DTX has poor aqueous solubility, it is formulated in Tween 80 (polysorbate 80)/ethanol/saline solutions (Taxotere[®]; Sanofi-Aventis, NJ, USA), and administered as 1-h intravenous (i.v.) infusions (Engels et al. 2005; Gao et al. 2008). DTX is known to cause serious or fatal toxicities such as neutropenia, peripheral neuropathy, and hypersensitivity reactions. It requires premedication with corticosteroids, antihistamines, and long infusion times. This causes additional overnight hospital stays and further inconvenience to patients. Polysorbate 80 is responsible for these toxic effects and also interferes with permeability-glycoprotein (P-gp), the multidrug efflux pump, thereby affecting the pharmacokinetics (Weiss et al. 1990; Rowinsky 1997).

Numerous oral chemotherapeutic agents are currently used clinically or are under development (DeMario and Ratain 1998). Oral drug formulations have been developed to enhance patient convenience and quality of life and for pharmacoeconomic advantages. In addition, they facilitate

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protracted drug administration for chronic regimens (Kruijtzter et al. 2002). Nevertheless, most cytotoxic drugs are administered by i.v. or subcutaneous injection because of their low or variable oral bioavailability. For drugs with narrow therapeutic indexes, high variability in bioavailability may cause toxicities and reduced efficacy. Orally administered DTX has low bioavailability (< 10% as a monotherapy) (Kuppens et al. 2005) because of its insolubility, interaction with permeability-glycoprotein (P-gp), and first-pass extraction by cytochrome P450 (Wils et al. 1994). Therefore, alternative formulations for DTX need to be developed for possible oral delivery.

To improve the pharmacokinetic profile and anticancer effects of DTX, a safer and better tolerated pharmaceutical vehicle than polysorbate 80 should be developed. Many dosage forms have been suggested, for instance microemulsions (Yin et al. 2009), cyclodextrin (Grosse et al. 1998), DTX-polymer conjugates (Khandare et al. 2006; Liu et al. 2007; Esmaeili et al. 2008), and nanoparticles (des Rieux et al. 2006; Choi and Han 2018). However, these formulations have several disadvantages such as limited solubility, poor physicochemical versatility, and they require surfactants or organic solvents (Ma and Williams 2018). Liposomes are considered ideal drug delivery systems with decreased toxicities and increased efficacy compared to other delivery carriers (Choi et al. 2013; Shin et al. 2014). They are composed of phosphatidylcholine lipid bilayers and can encapsulate both hydrophilic and hydrophobic drugs. In addition, liposomes have several significant advantages such as the potential for diverse compositional and structural configurations, high biocompatibility, and ability to recognize cells (Lasic 1998; Voskuhl and Ravoo 2008). Although liposomes have been widely used for parenteral delivery, their potential for oral delivery has also been explored. Oral liposomes may provide high potential for solubilization and protection from degradation by gastrointestinal (GI) fluids to the entrapped drug (Kato et al. 1993; Cai et al. 2010). Most significantly, the biocompatibility of lipid bilayers and their comparatively small size enhance oral absorption through the GI mucosa (Aungst 1993). Because it is commonly accepted that particles with a size of 10–1000 nm and, particularly, below 200 nm, can be absorbed through epithelial membranes (Russell-Jones 2001). However, this is still not enough to achieve sufficient plasma levels after oral administration and appropriate physicochemical properties. Therefore, their intestinal absorption and physical stability need to be improved.

A strategy to improve the oral absorption is to avoid the harsh condition of the stomach including the higher enzymatic activities and low pH. Therefore, the colon, with lower proteolytic activity, is considered an attractive site for drug delivery because of its excellent responsiveness to

mucoadhesive and biodegradable polymers. It also provides longer residence time and has a milder pH condition (Sinha et al. 2007). Thus, surface modification or coating with colon-targeted polymers is a favorable strategy for delivering poorly absorbed drugs such as DTX. It has previously been reported that among various coating polymers such as chitosan, Carbopol, and Eudragit, coating with Eudragit resulted in the highest entrapment and mucoadhesiveness (Khan et al. 2000; Karn et al. 2011). Eudragit L100 and S100 are anionic polymers that are soluble in solutions above pH 6.0 and 7.0, respectively. The GI environment of individuals is variable, and an appropriate combination of polymers enables drugs to be released in the region of the distal small intestine up to the colon.

Lyophilization is an effective method to overcome most of the physical and chemical instabilities of liquid dosage forms including leakage of entrapped drug, liposomal aggregation, and bilayer fusion. It protects the liposomes from degradation owing to the ability to prevent hydrolysis during storage (van Winden et al. 1997). However, in the process of lyophilization, the integrity and encapsulation efficiency of liposomes could be compromised (Gulati et al. 1998). Thus, the addition of an appropriate cryoprotectant is necessary to obtain optimal freeze-dried products. Trehalose is known to maintain the glass transition temperature (T_g) of the mixture and forms hydrogen bonds with phospholipids, protecting the membrane (Patist and Zoerb 2005). Mannitol has a bulking effect and supports a good cake-shape appearance (Telang et al. 2003). Desirable lyophilized liposomal formulations were obtained when trehalose and mannitol were combined in the free-drying process (Guan et al. 2011).

In this study, a pharmaceutically applicable oral liposomal formulation was developed to improve patient compliance and convenience and reduce toxicities. Lyophilization was performed using various lipid compositions, coating materials, and cryoprotectants, and the size, zeta potential, and encapsulation efficiency of the liposomes were evaluated. Moreover, a pharmacokinetic study was also performed to confirm the therapeutic potential of the formulation as an oral drug delivery system.

Materials and methods

Materials

Sodium deoxycholate (SDC), stearylamine, D-(+)-trehalose dehydrate, D-mannitol, potassium phosphate monobasic, DTX, chloroform (CHCl_3), and Sephadex G-75 were purchased from Sigma-Aldrich Chemical Co., Ltd. (St. Louis, MO, USA). Soybean phosphatidylcholine (SPC)

and L- α -phosphatidylcholine (EPC) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Eudragit S100 and L100 were purchased from Evonik (Essen, Germany). Acetonitrile and methanol (both high-performance liquid chromatography [HPLC] grade) were purchased from Duksan Co., Ltd., (Asan, Korea). All other reagents were of analytical grade and used without further purification.

Preparation of DTX-loaded liposomes

As shown in Fig. 1, liposomes composed of 39.0 mmol EPC and 1 mg DTX were dissolved in 1 mL chloroform with stearylamine comprising 20 mol% total lipid. After evaporating the organic solvent under nitrogen gas at room temperature using a rotary evaporator (Laborota 4000, Heidolph, Italia), the dry lipid film was suspended in 1 mL of phosphate-buffered saline (PBS; 10 mM, pH 7.4) containing SDC (7.5 mg). The mixture was sonicated (Laboratory Supplies Co., Inc., NY, USA) for 30 s in cold water (4 °C). The un-encapsulated components were removed from the liposome suspension using gel chromatography with a Sephadex G-75 column (Sigma-Aldrich Chemical Co., Ltd., St. Louis, MO, USA) at the final step of each liposome preparation.

Preparation of Eudragit-coated liposomes

The liposomes were coated using procedures previously described by Karn et al. with modifications (Karn et al. 2011). Because the GI pH is highly variable among individuals, only one kind of Eudragit is not sufficient for coating the liposomes. Thus, dispersions of Eudragit L100 and S100 were prepared separately and mixed at a ratio of 4:1 (Khan et al. 2000). Then, solutions of Eudragit L100/S100 of various concentrations (0, 0.025, 0.05, 0.1, 0.125, 0.25, 0.5, 1, and 2% (w/v) in PBS were added drop-wise to an equal volume of the liposomal suspension under magnetic stirring at room temperature for 1 h.

Preparation of lyophilized liposomes

Lyophilized liposomes were prepared using procedures described by Nounou et al. 2005 and Chaudhury et al. (2012). Briefly, 20% trehalose (v/w) and 10% mannitol (v/w) were added as cryoprotectants to equal volumes of Eudragit-coated DTX liposome suspension. The liposome samples were frozen at -80 °C for 3 days (Deep Freezer, Operon, Kimpo, Korea) and then lyophilized using a freeze-dryer (Operon, Kimpo, Korea) for 24 h under vacuum. The lyophilized samples were stored in a refrigerator at 4 °C for further characterization and analysis. The lyophilized cakes were immediately rehydrated with distilled water at room temperature. The reconstituted materials were vortexed at room temperature for adequate dispersion.

Determination of size distribution and zeta potential

The size distribution and zeta potential of the liposomes were measured using a dynamic laser-light scattering (DLS) system (DLS, NICOMP 380ZLS, Inc., CA, USA) using a He-Ne laser light source. Each sample was analyzed at room temperature at a 90° scattering angle.

Encapsulation efficiency of DTX liposomes

The amount of DTX encapsulated in the liposome samples was analyzed using high-performance liquid chromatography (HPLC) using procedures described by Marchettini et al. (2002) with modifications. The HPLC system consisted of a Capcellpak C18 UG120 column (150 \times 4.6 mm I.D., 5 μ m) and Waters 486 tunable absorbance detector (Waters, MA, USA) adjusted to 227 nm. The mobile phase was composed of phosphate buffer (KH₂PO₄, 30 mM, pH 3):acetonitrile (53:47, v/v). The flow rate of the mobile phase was 1.3 mL min⁻¹. The chromatograms were analyzed using the Millennium 32 program (Waters, MA, USA) (Rouini et al. 1998). To measure the DTX concentration in the liposomes, each sample (250 μ L) was diluted

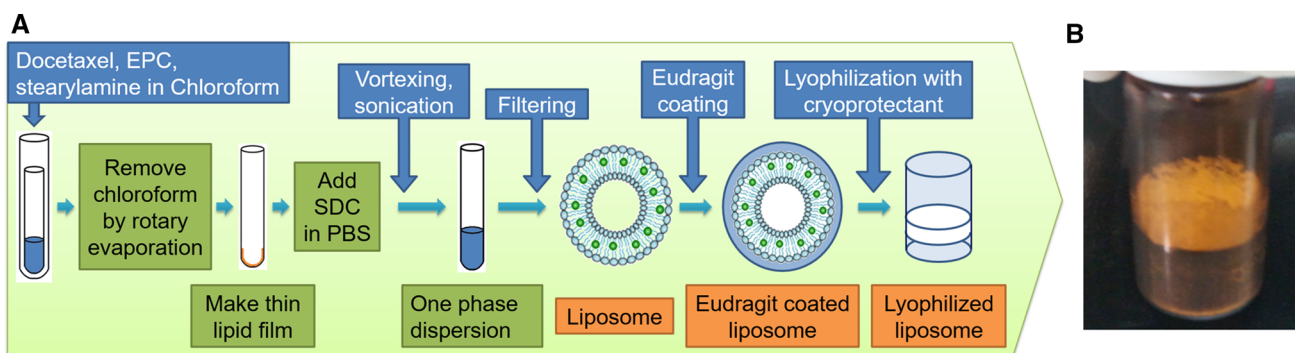


Fig. 1 a Preparation of lyophilized Eudragit-coated liposomal docetaxel DTX. b Morphology of lyophilized liposomal product

with an adequate volume of acetonitrile for extraction and vortexed thoroughly. After centrifugation (11,000 rpm, 20 min), the supernatant was transferred to another tube and evaporated under a gentle stream of nitrogen gas. The residue was resuspended in 250 μL mobile phase. These resuspensions were filtered through 0.45 μm syringe filters, and 20 μL of each sample was injected into the HPLC system. The amount of drug encapsulated in the liposomes was calculated from known concentrations of DTX using a standard curve. The encapsulation efficiency and loading efficiency of DTX (%) were calculated using the following formula:

$$\text{Encapsulation efficiency of DTX (\%)} = \frac{\text{Amount of DTX in liposome sample}}{\text{Initial loading amount of DTX}} \times 100,$$

$$\text{Lading efficiency of DTX (\%)} = \frac{\text{Amount of DTX in liposome sample}}{\text{Amount of polymer}} \times 100.$$

Energy-filtering transmission electron microscopy (EF-TEM) examination

The morphologies of the liposomes before coating, before lyophilization, and after rehydration were observed using an energy-filtering transmission electron microscope (EF-TEM, EM 912 Ω , Carl Zeiss, Germany). The lyophilized liposomes were diluted with distilled water, and 20 μL samples were placed on formvar-carbon-coated Cu grids. Liposomes were negatively stained with 2% uranyl acetate and gently blotted with a filter paper, followed by drying at room temperature. The stained samples were observed using an EF-TEM at 120 kV. Three grids were prepared, and the images of each sample were obtained.

In vitro DTX release from DTX liposomes

In vitro DTX release from uncoated and Eudragit-coated liposomes in different pH, media were determined. According to the Korean Pharmacopoeia IX (Dissolution Test), Solutions 1 (2.0 g sodium chloride in 7.0 mL hydrochloric acid and 1000 mL water, pH 1.2) and 2 (a mixture of phosphate buffer solution [pH 6.8] and water) were used to simulate the pH conditions of the stomach and small intestine, respectively. The liposomal suspension (2 mL) in dialysis cassette (Pierce Slide-A-Lyzer[®] dialysis cassette with a molecular weight (MW) cutoff of 10 K, Thermo Scientific, IL, USA) was placed into 400 mL of release medium containing 2% Tween 80 (w/v) at 37 $^{\circ}\text{C}$ and adequately agitated. After 0.5, 1, 2, 4, 6, 8, 12, and 24 h, aliquots of the dissolution media (0.5 mL) were

collected from the flask. The concentration of released drug was determined using HPLC.

In vivo study

All animal experiments were approved by the SMU-Institutional Animal Care and Use Committee (IACUC) of the Sookmyung Women's University, Korea. Male Sprague–Dawley (SD) rats (7-week-old, 230–250 g) purchased from Samtako Bio Korea Inc. (Osan, Korea) were maintained in isolated cages under a controlled temperature of approximately 23 $^{\circ}\text{C}$ with a 12-h light/dark cycle and provided food and water ad libitum. The rats were divided into three groups and were fasted overnight before i.v. and oral administration. The left femoral artery of each rat was cannulated with a polyethylene tube filled with heparin solution for serial blood sampling. DTX (1 mg mL⁻¹) was dissolved in polysorbate 80 (Tween 80)/ethanol/saline [20:13:67 (v/v/v); Taxotere[®]] for i.v. and oral administration. The liposomal DTX suspension for oral administration was prepared by reconstituting the lyophilized dry cake with distilled water. The DTX suspension was administered directly into the stomach of rats via a technique called oral gavage. In this procedure a stainless steel bulb tipped gavage needle is attached to a syringe and used to deliver the compound into the stomach. The blood samples were obtained from the femoral arteries of the rats at 1, 5, 15, 30, 60, 120, 240, 360, 480, and 720 min after i.v. injection of DTX (10 mg kg⁻¹), and 15, 30, 45, 60, 90, 120, 240, 360, 480, 720, and 1440 min after oral administration of DTX (20 mg kg⁻¹) and liposomal DTX (10 mg kg⁻¹). The plasma samples were obtained by centrifuging each blood sample at 3000 rpm for 10 min (Micro 17R centrifuge; Hanil Science Industrial Co., Ltd., Incheon, Korea) and immediately refrigerated at - 80 $^{\circ}\text{C}$ until the analysis.

Liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis

A 10 μL aliquot of the internal standard solution (IS, paclitaxel, 1 $\mu\text{g mL}^{-1}$ in methanol) was added to 200 μL plasma. After adding acetonitrile for extraction, samples were vortexed and centrifuged at 13,000 rpm for 10 min. The supernatant (300 μL) was transferred into a fresh tube and mixed with *tert*-butyl methyl ether (TBME, 500 μL) for liquid–liquid extraction (Lee et al. 1999). After vortexing and centrifugation, the upper, ether layer was collected in a fresh tube and evaporated under a gentle stream of nitrogen gas. The residue was reconstituted with 30% acetonitrile (100 μL) and a 10 μL aliquot was injected into the LC–MS/MS system (AB Sciex Model API 3000 quadrupole LC–MS/MS spectrometer, Applied Biosystems, Concord, Canada) equipped with a Turboionspray

source and operated in the positive ion multiple reaction monitoring (MRM) mode. The LC–MS/MS mobile phase was composed of 5 mM ammonium acetate run at a flow rate of 1 mL min⁻¹.

Pharmacokinetic analysis

Various pharmacokinetic parameters of DTX after i.v. and oral administration were calculated using a two- and one-compartment models, respectively. The time-plasma concentration profiles obtained from each PK study were curve-fitted using Sigmaplot software (version 10.0). After i.v. administration of DTX, the following pharmacokinetic parameters were estimated: elimination rate constant (k_{10} , min⁻¹), the transfer rate constant from peripheral to central compartment (k_{21} , min⁻¹), the transfer rate constant from central to peripheral compartment (k_{12} , min⁻¹), and the plasma half-lives at the α and β phases ($t_{1/2\alpha}$ and $t_{1/2\beta}$, respectively, min). The area under the plasma concentration–time curve (AUC, $\mu\text{g min mL}^{-1}$), area under the moment curve (AUMC, $\mu\text{g min}^2 \text{mL}^{-1}$), mean residence time (MRT, min), total plasma clearance (CL_t , mL min⁻¹), steady-state volume of distribution (V_{dss} , mL kg⁻¹), and V_d of the central compartment (V_1 , mL) were also calculated. After oral administration of DTX, the highest concentration (C_{max} , $\mu\text{g mL}^{-1}$), the time to reach the C_{max} (T_{max} , min), and the absolute bioavailability (BA, %) were calculated using standard methods.

Statistical analysis

All results except the pharmacokinetic data are expressed as the mean \pm standard deviation (SD). Pharmacokinetic parameters are expressed as the mean values \pm standard error (SE). Statistical analyses were performed using the Student's *t* test and statistical significance was accepted at $P < 0.05$ (95% confidence level) or < 0.01 (99% confidence level).

Results

Selection of phospholipid type

Phospholipids are suitable for use as formulation excipients for poorly water-soluble drugs because of their amphiphilic property. They have several advantages for oral drug

delivery, such as improving bioavailability, changing release profile of substances, and protecting active agents (Fricker et al. 2010). SPC and EPC are commonly used phospholipids for drug formulation. Therefore, these phospholipids were tested for the liposomal formulation of DTX. As shown in Table 1, particle sizes of the SPC and EPC liposomes were 82.3 ± 11.1 and 59.3 ± 3.4 nm, respectively. No significant differences were observed in the zeta potential of both formulations. The encapsulation efficiency of SPC and EPC liposomes was 18.8 and 33.6%, respectively. The EPC formulation of DTX showed better liposomal properties, such as increased encapsulation efficiency and decreased particle size, than the SPC formulation. Therefore, EPC was selected to prepare the DTX liposomes.

Selection of optimum Eudragit combination

Eudragit L100 and S100 are anionic polymers synthesized from methacrylic acid and methacrylic acid methyl ester. Therefore, positively charged liposomes containing stearylamine were prepared and coated with Eudragit by electrostatic interaction. Figure 2 shows the relationship between the Eudragit concentration in the coating solution and physical properties of the coated liposomes. The uncoated liposomes had a diameter in the range of 55–70 nm with positive surface charge. Coating of the liposome surface was proved by the inversion of the zeta potential. Increasing the Eudragit concentration in coating solution beyond 0.5% did not further decrease the zeta potential. Therefore, the 0.5% Eudragit coating solution appeared sufficient to cover all the liposomes. The particle size also changed according to the Eudragit concentration of the coating solution. The analyzed particle size of the liposomes coated with 0.025–0.1% Eudragit coating solution was extremely high (> 500 nm). This phenomenon was presumably attributable to the formation of large agglomerates. The size of liposomes coated with 0.5% Eudragit coating solution was substantially larger than that of the uncoated liposomes. Based on the size and zeta potential results, the 0.5% Eudragit combination was used in all further studies.

Lyophilization

The commonly used cryoprotectants mannitol and trehalose were chosen for lyophilization because of their good

Table 1 Composition and properties of soybean phosphatidylcholine (SPC) and L- α -phosphatidylcholine (EPC) liposomes

Phospholipid type	Phospholipid concentration (mM)	Particle size (nm)	Zeta potential (mV)	Encapsulation efficiency (%)
SPC	38.7	82.3 ± 11.1	22.5 ± 2.1	18.7 ± 0.3
EPC	39.0	59.3 ± 3.4	19.3 ± 1.6	33.6 ± 3.9

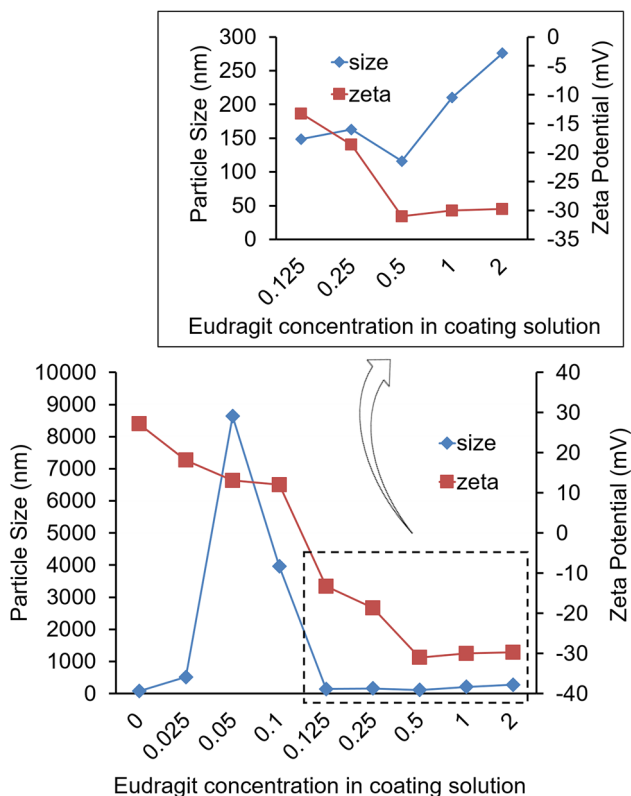


Fig. 2 Concentration of Eudragit combination and changes of particle size and zeta potential of Eudragit-coated liposomes at concentration of 0–2%. Magnified view of concentration range of 0.125–2% is highlighted in inset box

bulking and particle protecting effects, respectively. Eudragit-coated DTX liposomes were lyophilized with various concentration of the cryoprotectants. The effect of different concentration of cryoprotectants on the liposomes

Table 2 Relationship between concentration of cryoprotectants and properties of lyophilized liposomes

Conc. of cryoprotectant (% w/v)	Particle size (nm)	Encapsulation efficiency (%)
Trehalose 0%	2663 ± 104	22.5 ± 4.5
Trehalose 10%	725 ± 74	22.1 ± 9.3
Trehalose 20%	358 ± 3	24.0 ± 1.9
Trehalose 30%	266 ± 48	23.2 ± 0.8
Trehalose 20% plus mannitol 10%	205 ± 37	31.9 ± 3.3

Table 3 Characterization of various liposomal docetaxel (DTX)

	Liposomal DTX	Eudragit-coated liposomal DTX	Lyophilized liposomal DTX
Particle size (nm)	59.3 ± 3.4	116.4 ± 5.9	204.9 ± 36.8
Zeta potential (mV)	19.3 ± 1.6	- 31.1 ± 0.6	- 22.9 ± 4.0
Encapsulation efficiency (%)	33.6 ± 3.9		31.9 ± 3.3
Loading efficiency (%)	0.112 ± 0.013		0.106 ± 0.011

Eudragit concentration for liposomal coating: 0.5%

is shown in Table 2. The mean diameter of lyophilized liposomes became smaller with higher amounts of trehalose in the formulations. There was no significant difference in encapsulation efficiency (approximately 23%) with increasing concentrations of trehalose. When 20% trehalose and 10% mannitol were used, the lyophilized products had the smallest particle size of approximately 205 nm and the highest encapsulation efficiency (31.9%).

Encapsulation efficiency of DTX in lyophilized liposomes

The amount of DTX in the lyophilized liposomes was measured using HPLC. The calculated encapsulation efficiency of DTX in the lyophilized liposomes was $31.9 \pm 3.3\%$, which was slightly changed compared to that of the unlyophilized DTX liposomes ($33.6 \pm 3.9\%$). However, this change seemed negligible (Table 3). The particle size and zeta potential values after reconstitution of the lyophilized liposomes were 204.9 ± 36.8 nm and $- 22.9 \pm 4.0$ mV, respectively.

EF-TEM examination

Figure 3 shows the TEM photographs of DTX liposomes before coating, before lyophilization, and after rehydration. They were discrete particles with sharp boundaries and near-spherical morphology. The mean particle size of liposomes as shown by the TEM images was consistent with the results measured using DLS.

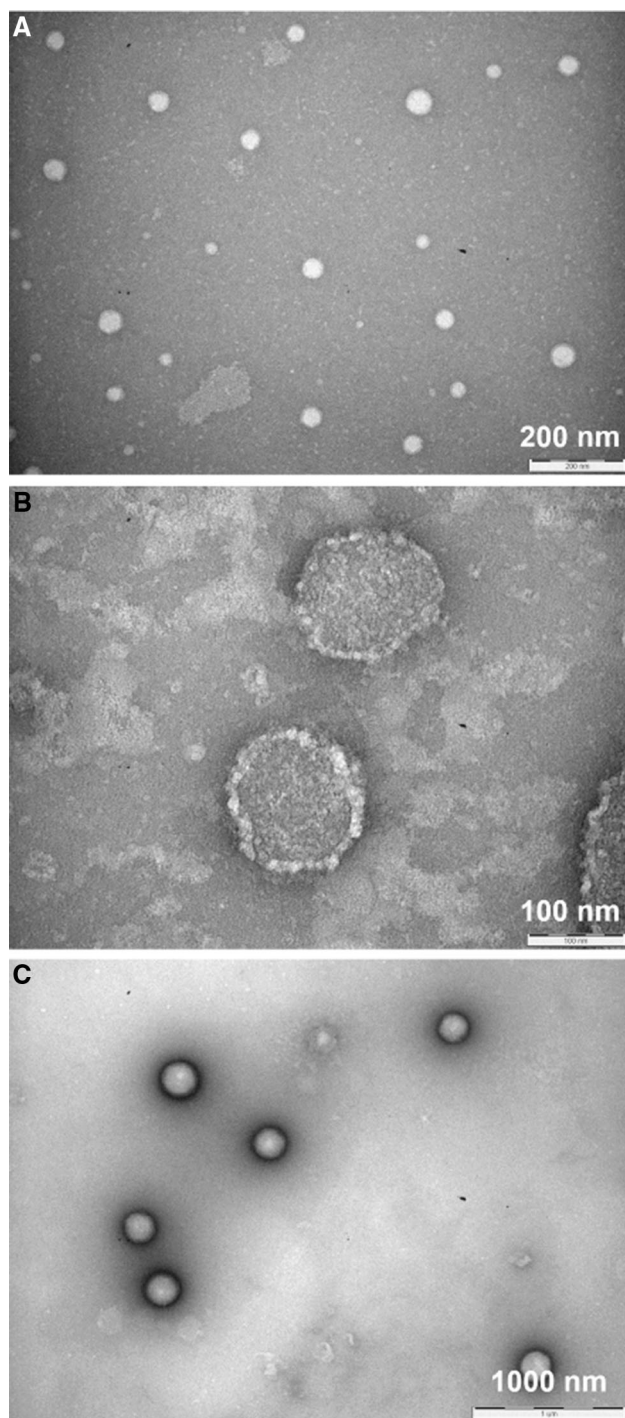


Fig. 3 Energy-filtering transmission electron microscopy (EF-TEM) images of various liposomal formulations of docetaxel (DTX). **a** Uncoated liposomal DTX (scale bar = 200 nm), **b** Eudragit-coated DTX liposomes (scale bar = 100 nm), and **c** lyophilized Eudragit-coated DTX liposomes (scale bar = 1000 nm)

In vitro release test of liposomal DTX

Figure 4 shows the drug release profiles of uncoated and coated liposomes in two types of release media. In Solution

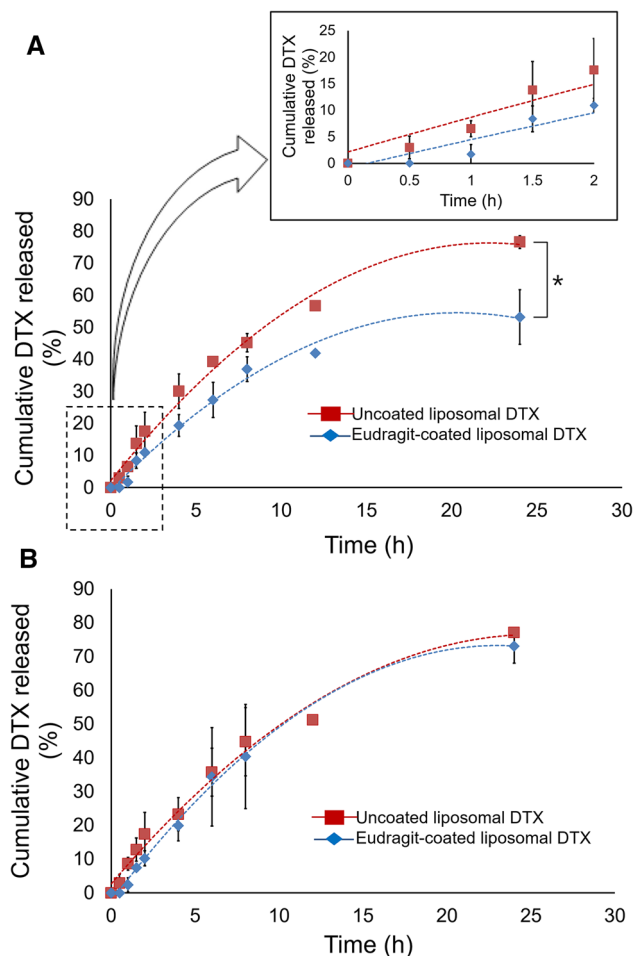


Fig. 4 Drug release profiles of uncoated liposomes and Eudragit-coated liposomes at pH **a** 1.2 and **b** 6.8. Drug released over 2 h, corresponding to average gastric emptying times at pH 1.2 is highlighted in inset box. Data are mean \pm standard deviation (SD) of three experiments (* $P < 0.05$)

1 at pH 1.2, the amount of drug released from the Eudragit-coated liposomes was lower than that from the uncoated liposomes. It is reported that a cup of water leaves the stomach in < 30 –60 min (O'Sullivan et al. 1987). Many factors could affect average gastric emptying time (Okabe et al. 2015), so we expected drug solution to leave the stomach within the period of average 120 min. Over a period representing the average gastric emptying time, 17% of the drug was released from the uncoated liposomes, which was in contrast to the 10% released from Eudragit-coated liposomes over the same period. No significant difference in release property was observed between the formulations in Solution 2 at pH 6.8, which showed that approximately 70–80% DTX was released within 24 h in the release media.

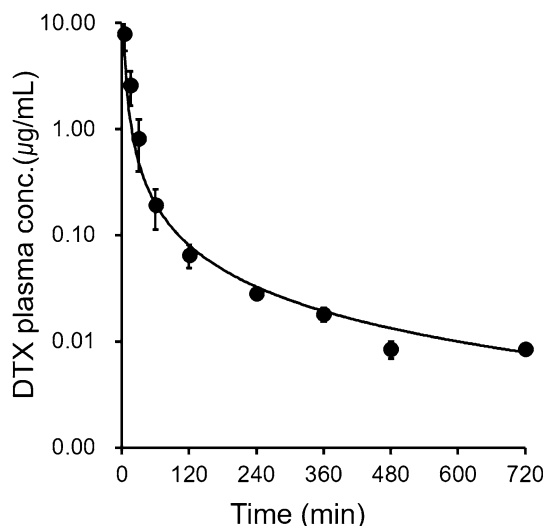


Fig. 5 Plasma docetaxel (DTX) concentration–time profile after intravenous (i.v.) administration of free DTX at a dose of 10 mg kg^{-1} in rats

Table 4 Pharmacokinetic parameters after intravenous (i.v.) administration of 10 mg kg^{-1} free docetaxel (DTX) in rats

Parameter	Mean \pm SE
A ($\mu\text{g mL}^{-1}$)	17.2 ± 3.72
B ($\mu\text{g mL}^{-1}$)	0.126 ± 0.0351
α (min^{-1})	0.115 ± 0.00991
β (min^{-1})	0.00518 ± 0.000735
k_{12} (min^{-1})	0.0153 ± 0.00394
k_{21} (min^{-1})	0.00594 ± 0.000695
k_{10} (min^{-1})	0.0987 ± 0.00783
$t_{1/2\alpha}$ (min)	6.18 ± 0.592
$t_{1/2\beta}$ (min)	143 ± 24.3
AUC ($\mu\text{g min mL}^{-1}$)	183 ± 48.7
MRT (min)	39.7 ± 10.9
V_{dss} (mL kg^{-1})	3294 ± 1799
AUMC ($\mu\text{g min}^2 \text{mL}^{-1}$)	6117 ± 937
CL_t (mL min^{-1})	68.7 ± 20.6
V_1 (mL)	687 ± 193

Pharmacokinetic studies

Figure 5 shows the plasma DTX concentration–time profiles after i.v. injection into the femoral vein of SD rats. The pharmacokinetic parameters measured after i.v. administration of a single dose of 10 mg kg^{-1} free DTX are summarized in Table 4. After i.v. administration of free DTX, the plasma drug concentration rapidly decreased within 120 min (α phase), followed by a slower decrease (β phase). The mean plasma $t_{1/2}$ values at the α and β phases

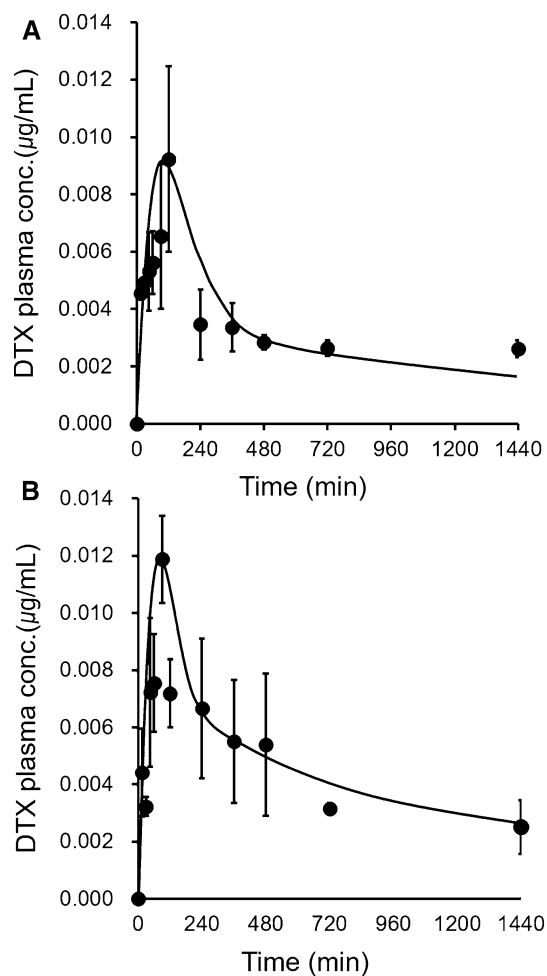


Fig. 6 Plasma docetaxel (DTX) concentration–time profiles after oral administration at doses of **a** 20 mg kg^{-1} DTX and **b** 10 mg kg^{-1} Eudragit-coated liposomal DTX in rats

were 6.18 and 143 min, respectively, and the AUC was $183 \mu\text{g min mL}^{-1}$. The free drug formulated in Tween 80 and Eudragit-coated liposomal formulation were orally administered at doses of 20 and 10 mg kg^{-1} , respectively. The concentration of DTX in the plasma samples after oral injection was measured over time (Fig. 6). The resulting pharmacokinetic parameters are listed in Table 5. After oral administration, the C_{max} and T_{max} of the 20 mg kg^{-1} DTX suspension were $0.011 \mu\text{g mL}^{-1}$ and 110 min, respectively. The corresponding values for the 10 mg kg^{-1} dose of liposomal DTX were $0.009 \mu\text{g mL}^{-1}$ and 90 min, respectively. The $t_{1/2}$ values of each formulation were 567 and 818 min, respectively. The BA of DTX after oral liposomal administration was three times higher than that after oral suspension administration.

Table 5 Pharmacokinetic parameters after oral administration of 20 mg kg⁻¹ free docetaxel (DTX) and 10 mg kg⁻¹ Eudragit-coated liposomal DTX

Parameter	DTX (20 mg kg ⁻¹ , mean ± SE)	Eudragit-coated liposomal DTX (10 mg kg ⁻¹ , mean ± SE)
T _{max} (min)	110 ± 10.0	90.0 ± 9.49
C _{max} (μg mL ⁻¹)	0.0112 ± 0.00193	0.00981 ± 0.00169
K _a (min ⁻¹)	0.0609 ± 0.0257	0.0349 ± 0.0165
K (min ⁻¹)	0.00168 ± 0.000726	0.000995 ± 0.000181
t _{1/2} (min)	567 ± 181	818 ± 182
AUC (μg min mL ⁻¹)	6.98 ± 0.846	10.8 ± 2.39
V _β (mL kg ⁻¹)	44,745 ± 14,275	64,605 ± 14,381
BA (%)	1.91 ± 0.232	5.92 ± 1.31

Discussion

DTX is one of the most important chemotherapeutic agents with high potency against many solid tumors. However, its poor solubility in water and critical side effects are the most common obstacles to its clinical application. DTX is commercially formulated in Tween 80 (polysorbate 80) and ethanol. Polysorbate 80 causes severe toxicities, hypersensitivities, and the requirement of premedication and hospitalization. To overcome these obstacles, a novel Eudragit-coated lyophilized DTX liposome, an alternative formulation to Taxotere[®], was developed. While developing such liposomes, selection of phospholipids is important because they can determine liposomal properties. SPC and EPC were tested for the preparation of liposomes, and we found that liposomes composed of EPC showed a smaller particle size and better encapsulation efficiency than those formulated with SPC (Table 1). The mean diameter and zeta potential of uncoated DTX liposomes were approximately 60 nm and 20 mV, respectively (Table 3). The positive charge of the uncoated liposomes was presumed to be due to the stearylamine used in the preparation. Two types of Eudragit, Eudragit L100 and S100, were used for the coated liposomes to target the desired release site. Eudragit has pH-dependent solubility and mucoadhesive properties, which protect the entrapped drug from the acidic environment of the stomach and improve the oral DTX absorption. Therefore, combining the two polymers could overcome the high GI pH variability of individuals. By measuring the particle size, the appropriate concentration for Eudragit in the coating solution was determined, and it sufficiently covered the surface of the liposomes. The size of liposomes could be increased after coating of materials, and increased size varies depend of many factors (Karn et al. 2011; Barea et al. 2012; Hua 2014). Even though size of liposome was somewhat increased, Eudragit-coated liposomes showed appropriate drug release

profiles, assuming that there's no stability issue on the liposomes. Furthermore, the adequate covering was indirectly proved by the maintenance of zeta potential of the product at the same value (Fig. 2). Stability issues can be overcome by lyophilization with proper cryoprotectants and reconstitution immediately before use; 20% trehalose and 10% mannitol were selected for lyophilization based on the average size and encapsulation efficiency (Table 2). The lyophilized product particles were cake-shaped, compact, and the size change was relatively small (Fig. 1b and Table 3). The morphology of the formulations was observed using an EF-TEM. The discrete particle and spherical structure of the liposomes were clearly visible (Fig. 3). The in vitro release study proved that the pH-dependent solubility of Eudragit-coated liposomes induced drug release in the small intestine up to the colon (Fig. 4). The Eudragit coating decreased drug release during the simulated average gastric emptying time. In the pharmacokinetic study, the free drug formulated in Tween 80 and the liposomal formulation were administered orally at doses of 20 and 10 mg kg⁻¹, respectively (Fig. 6). The t_{1/2} of the oral liposomes was higher than that of the oral suspension, and their AUC values were not significantly different despite the different doses. Thus, the developed liposomal DTX formulation showed a three-fold higher oral BA in rats than that of the oral free DTX suspension (Table 5). There are several advantages of this liposome system compared to other liposome systems. First, this liposomes can be stored in refrigerator for long period of time after lyophilization. Second, no materials except water is required for lyophilized cake to form liposomes. Third, this liposome needs no device to inject because it is designed as oral dosage form. After oral administration, as mentioned above, Eudragit makes liposome to release its payload in the region of the distal small intestine up to the colon.

In conclusion, the lyophilized Eudragit-coated liposomal delivery system could be regarded as a promising strategy for oral administration of DTX. Its small particle size and site-specific delivery in the GI tract facilitated its permeation of the intestinal barrier and enhanced systemic delivery of the entrapped drug. Additionally, our designed formulation could overcome the hypersensitive reaction of existing DTX formulations such as Taxotere[®] by the removal of polysorbate 80.

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Compliance with ethical standards

Conflict of interest All the authors declare that they have no conflict of interest.

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