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Stable and efficient delivery of docetaxel by micelle-encapsulation using a tripodal cyclotriphosphazene amphiphile

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ABSTRACT

Docetaxel micelle-encapsulated by a tripodal cyclotriphosphazene amphiphilile $[NP(PEG750)(GlyPheLeu)_2Et]_3$ (CP750) exhibited outstanding drug-loaded micelle stability in aqueous solution compared with the polymeric micelles assembled from linear block copolymers. Furthermore, docetaxel micelle-encapsulated by CP750 is obtainable in solvent free powder form, which is immediately soluble in any aqueous media including saline and PBS and very stable to photo-degradation even in the room light at room temperature. Although docetaxel micelle-encapsulated by CP750 did not display highly improved pharmacokinetic profile compared with Taxotere® currently in clinical use, its *in vivo* xenograft trials exhibited excellent antitumor efficacy by showing complete tumor regression against the breast cancer cells (MDA-MB-231) at a lower dose of 5 mg/kg and better efficacy against gastric cancer cells (MKN-28) compared with Taxotere®. Furthermore, according to the comparative acute toxicity study, toxicities associated with Taxotere® may be remarkably reduced by micelle-encapsulation of docetaxel using CP750, which afforded a much higher LD_{50} value of 75 mg/kg compared with 28 mg/kg of docetaxel in Taxotere®. Thus docetaxel micelle-encapsulated by CP750 has entered the stage of preclinical studies.

1. Introduction

Docetaxel, a semi-synthetic analogue of paclitaxel, is currently one of the most important chemotherapeutic agents, since it is clinically very efficacious against a variety of tumors including breast, ovarian, non-small cell lung, prostate, and gastric cancers (Hennenfent and Govindan, 2006; Bissery et al., 1995; Van Oosterom and Schrijvers, 1995). Docetaxel is known to be more potent than paclitaxel, but its photo-instability (Dev et al., 2006; Kumar et al., 2007) causes more inconveniences in drug storage and patient treatment compared with paclitaxel.

Similarly to paclitaxel that is solubilized using a surfactant Cremophore EL and ethanol, docetaxel also has a low water solubility (6–7 μg/ml) and is solubilized in aqueous solution by formulation using a surfactant polysorbate 80 (Tween 80) and a 13% ethanol solution (Nuijen et al., 2001). Docetaxel in such a formulation named "Taxotere®" is known to cause several adverse effects such as fluid retention, neurotoxicity, and neutropenia due to the agent itself and hypersensitivity due to the solvent system (Van Zuylen et al., 2001; Persohn et al., 2005; Gelmon, 1994).

One of the most promising approaches to overcome such side effects associated with small molecular drugs is to use polymeric drug delivery systems, which is called 'polymer therapy' (Haag and Kratz, 2006; Kabanov and Okano, 2003). In contrast to the low molecular weight surfactant micelles such as polysorbate 80 with a high CMC value (>100 mg/L), polymeric micelles with lower CMC values (10–100 mg/L) form stable micelles, which can afford to improve not only the physicochemical properties such as water solubility but also pharmacokinetics and tumor selectivity of small molecular drugs.

In general, there are two different ways for application of polymeric drug delivery systems to cancer therapy (Sohn and Jun, 2009). One is simply to use a drug carrier polymer for physical encapsulation of a known anticancer drug by using polymeric micelles or nanoparticles, or by homogeneous dispersion of a drug in a biodegradable hydrogel or solid matrix. Another method is to conjugate drug molecules by covalent bonding to a carrier polymer

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directly or using a biodegradable spacer, producing a new prodrug. However, the chemical conjugation of drug molecules to a carrier polymer usually accompanies significant change in physicochemical properties of the drug and/or carrier molecules, and consequently, it is not easy to design a polymeric conjugate drug satisfying all the required properties. In particular, photo-sensitive docetaxel is very difficult to conjugate to conventional organic polymers with its intact structure. On the other hand, it is easier to physically encapsulate docetaxel using polymeric micelles.

There are several studies recently reported on docetaxel formulations based on polymeric micelles. The polymeric micelles attempted are based on the linear amphiphilic diblock copolymers composed of poly(ethylene glycol) (PEG) or poly(N-vinylpyrrolidone) (PVP) as a hydrophilic segment and poly(D,L-lactide) (PLA), poly(ε-caprolactone) (PLC), poly(styrene oxide) (PSO) or poly(butylene oxide) (PBO) as a hydrophobic segment. Representative block copolymers reported on the docetaxel formulation are PEG-b-PLA (Shin et al., 2009), PVP-b-PLA, (Gaucher et al., 2010; Le Garrec et al., 2005), MPEG750-b-CL (Carstens et al., 2008), PEO-b-PSO and PEO-b-PBO (Mahmoud et al., 2007), and MPEG-PLA/Pluronic copolymer micelles (Mu et al., 2010).

However, all these linear block copolymers exposed commonly a few critical problems for practical applications to docetaxel formulation. First of all, although these polymeric micelles have lower CMC values than the low molecular weight surfactant micelles as above-mentioned, most of docetaxel-loaded polymeric micelles are not stable enough to hold the drug component in the micelle core and drug precipitates mostly within 24 h after drug loading. The reason was not clearly explained. Furthermore, the *in vivo* antitumor activity of docetaxel formulated using such polymeric micelles seems not to be very much improved in spite of its remarkably increased maximum tolerated dose. Therefore, it is urgent to explore a new type of micelles stable enough to hold docetaxel for practical duration and affording higher antitumor efficacy.

We have recently reported novel cyclotriphosphazene micelles of a general formula **[N=P(X)(Y)]$_3$** prepared by stepwise substitutions of cyclic chlorophosphazene [N=PCl$_2$]$_3$ with equimolar hydrophilic (**X**) and hydrophobic (**Y**) nucleophiles in *cis*-nongeminal way (Lee et al., 2000; Jun et al., 2006). In particular, these tripodal amphiphiles with a methoxy poly(ethylene glycol) (MPEG) as a hydrophilic group and a linear oligopeptide as a hydrophobic group self-assemble in aqueous solution into very stable spherical micelles with a mean diameter of 7.4–13.9 nm (Toti et al., 2007). We have shown in our previous work that micelle-encapsulation of a hydrophobic platinum(II) anticancer agent using an amphiphilic cyclotriphosphazene can greatly improve not only tumor targeting properties but also important pharmacokinetic parameters such as plasma half-life ($t_{1/2}$) and the AUC value (Jadhav et al., 2010). However, it was found later that the micelle-encapsulated platinum(II) drug exhibited no plausible *in vivo* antitumor efficacy in the nude mouse xenograft model probably due to its unfavorable releasing kinetics.

Surprisingly, we have recently found that docetaxel micelle-encapsulated by the same amphiphilic cyclotriphosphazene (CP750) as used for micelle-encapsulation of the platinum(II) drug exhibits contrasting results by displaying outstanding *in vivo* antitumor efficacy with remarkably reduced acute toxicity, although its pharmacokinetic parameters are not remarkably improved compared with Taxotere®. Further important is that, in contrast to the polymeric micelles assembled from the linear amphiphilic block copolymers that are not able to hold docetaxel in their micelle core, the present cyclotriphosphazene micelles are extraordinarily stable enough to hold docetaxel in the micelle core with no precipitation and remarkably enhanced photo-stability. Herein we report preparation and properties of docetaxel micelle-encapsulated by a tripodal cyclotriphosphazene amphiphile.

2. Materials and methods

2.1. Materials

Docetaxel was supplied by Hanmi Research Center, Korea. The present amphiphilic cyclotriphosphazene, [NP(MPEG750)(GlyPheLeu)$_2$Et]$_3$ (CP750) used as a micellar drug carrier for encapsulation of docetaxel was prepared according to the authors' procedure (Lee et al., 2000; Jun et al., 2006). Briefly, three chlorine atoms of hexachlorocyclotriphosphazene are substituted initially with the methoxy poly(ethylene glycol) at a temperature lower than −20 °C to obtain *cis*-nongeminal conformation and then followed by substitution of the rest chlorine atoms with the equimolar hexapeptide ethyl ester.

2.2. Instruments and measurements

^{1}H NMR measurements were made with a Bruker 250 spectrometer operating at 250 MHz in the Fourier transform mode. Proton-decoupled ^{31}P NMR spectra were measured with the Varian Unity INOVA-500 spectrometer operating at 500 MHz using triphenyl phosphate as an external standard. Particle size measurements were performed using a Malvern Nano-ZS model using a He–Ne laser (633 nm), with data analysis by the auto-measure software version 6.20 (Malvern, UK). The critical micelle concentration (CMC) was measured by a fluorescence probe technique using pyrene (Inoue et al., 1998).

2.3. Micelle-encapsulation of docetaxel

Required amounts of docetaxel (5–20 mg) and the cyclotriphosphazene, CP750 (200–400 mg) are completely dissolved in 5 ml of absolute ethanol to obtain a clear solution, and then ethanol was slowly evaporated by rotary evaporator to dryness. The residue was subjected to vacuum-dry for 0.5–1.0 h to remove any trace amount of solvent molecules and finally dissolved in distilled water. The aqueous solution is filtered through a 0.45 μm syringe filter and then freeze-dried to obtain a micelle-encapsulated docetaxel in powder form, which is immediately soluble in any aqueous media including saline and PBS. Such a typical solvent-free formulation contains 10% docetaxel and 90% CP750, although docetaxel can be loaded into the carrier up to 25%. Docetaxel can be solubilized as much as 5% in aqueous solution using the 10% formulation, but 0.1% aqueous solution of docetaxel was used for pharmacokinetic and other animal studies.

2.4. Animals

Healthy male Sprague-Dawley rats (7–8 weeks old) and BALB/C nude mice (5 weeks old, 15–20 g) were supplied by Orient Bio Inc., Korea. Male ICR mice, aged 6 weeks and weighing 20–22 g, were purchased from G-Bio Inc., Korea. All the animals were housed in cages maintained at 23 ± 3 °C and a relative humidity of 50 ± 5% with a 12 h light/dark cycle for 5–7 days before experiments. Water and commercial laboratory complete food for animals were available *ad libitum*. All the animal experiments were approved by the Institutional Animal Ethics Committee of Ewha Womans University, Korea.

2.5. Pharmacokinetic study

The male Sprague-Dawley rats were cannulated using polyethylene tubing (PE-60) for blood sampling and were fasted overnight with free access to water before the experiments. The rats were assigned to one of the two groups. Taxotere® or docetaxel micelle-encapsulated by CP750 were injected into the femoral vein of rats

at a dose of 5 mg/kg. Blood samples (0.25 mL) were collected via the carotid artery before injection (0 h), and at 0.033, 0.083, 0.167, 0.33, 0.5, 0.75, 1, 2, 4, and 6 h after intravenous injection. Blood samples were centrifuged at 11,000 rpm for 15 min, and the plasma was taken and stored at −20 °C until HPLC analysis. Pharmacokinetic parameters were estimated from plasma concentration–time curve using WinNonlin software (Pharsight Co., Mountain View, CA, USA). The pharmacokinetic parameters estimated from the data are as follows; the initial plasma concentration (C_0), the area under the plasma concentration–time curve from 0 h to the last sampling time (AUC_{last}), the area under the curve from 0 h to infinity (AUC_{Inf}), elimination half-life ($t_{1/2}$), apparent volume of distribution (V_z), and total clearance (Cl).

2.6. In vitro cytotoxicity

In vitro cytotoxicity of docetaxel micelle-encapsulated by CP750 and Taxotere® for comparison was assayed using a modified procedure of a sulforhodamine B (SRB) staining method (Skehan et al., 1990) against eight selected human tumor cell lines of MCF-7 (breast), SK-OV3 (ovary), A549 (lung), SNU638 (stomach), HCT-15 (colon), PC-3 (prostate), MES-SA (cervical) and A-431 (epidermoid).

2.7. Nude mouse xenograft trial

The *in vivo* antitumor activity of docetaxel micelle-encapsulated by CP750 and Taxotere® for comparison was assayed against human breast cancer cells (MDA-MB-231) and human gastric cancer cells (MKN-28) using BALB/C nude mice (5 weeks old, 15–20 g). Cells cultured in a solution of RPMI 1640 containing 10% fetal bovine serum (cell culture media) at 37 °C under 5% CO_2 atmosphere were treated with 0.25% Trypsin-ethylenediaminetetraacetic acid (EDTA). The incubated tumor cells were centrifuged in the cell culture media for 5.5 min. After centrifugation, the cell culture media were removed and RPMI 1640 without 10% FBS was added into tumor cells. The single floated tumor cells (1×10^7) in 100 μl of RPMI 1640 were subcutaneously injected into the back side of the right flank of each mouse using a 1 ml syringe within 15 min. When the tumor was grown up to reach about 100–150 mm^3 in size, the test drugs were administered by intravenous injection via a tail vein on day 1, 5, and 9 in two dosages of 5 and 15 mg/kg for our micelle-encapsulated docetaxel but one dosage of 15 mg/kg for Taxotere®, which is in the optimum dose range of Taxotere® in xenograft models (Bissery et al., 1991; Kraus et al., 2003). The body weight and tumor volume of the mice were measured every 2–3 days. The tumor volume was calculated with an equation of $[A/2 \times (B/2)^2] \times 4\pi/3$ where A is the long length and B is the short length of the tumor.

2.8. Acute toxicity study

The experiments using male ICR mice to determine the lethal dose (LD_{50}) of docetaxel micelle-encapsulated by CP750 or Taxotere® for comparison were designed in accordance with the method provided by the OECD guideline 423, but the doses were modified based on our preliminary toxicity test for Taxotere®. Prior to dosing, food but not water was withheld for 4 h. Different concentrations of the samples (5, 30, 50 and 100 mg/kg of Taxotere® and 5, 50, 300, 500, 1000, and 2000 mg/kg of docetaxel micelle-encapsulated by CP750) were intravenously administered to each group of three mice. A group of three mice, receiving identical volume of 0.9% saline was served as a control. After injection, the changes in body weight and the survival rate were carefully and daily recorded over a period of 14 days. The survival rate of the mice was calculated as (number of live mice/total number of mice tested) × 100%. The LD_{50} values of the test samples were calculated according to the OECD guideline 425.

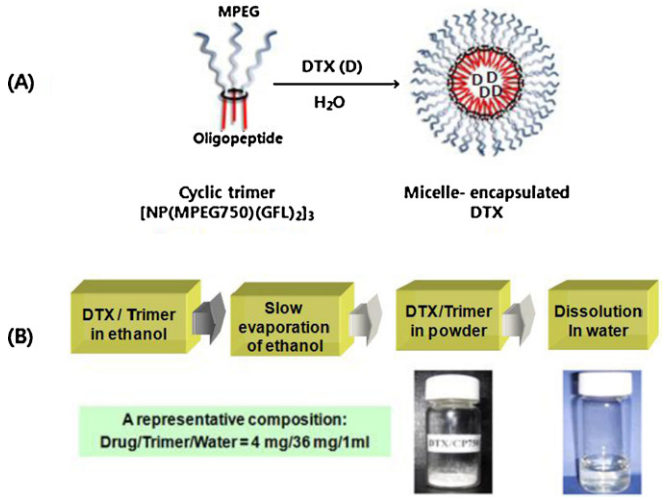

Fig. 1. The conceptual diagram (A) and a schematic procedure (B) for micelle-encapsulation of docetaxel (DTX) using a tripodal cyclotriphosphazene amphiphile $[NP(MPEG750)(GlyPheLeu)_2Et]_3$ (CP750) in aqueous solution.

3. Results and discussion

3.1. Micelle-encapsulated docetaxel and its physicochemical properties

Docetaxel was easily micelle-encapsulated simply by slow evaporation of the ethanol solution containing desired amounts of docetaxel and the carrier CP750. Surprisingly, we have found that the same micellar drug carrier CP750 can give rise to different pharmacokinetic and pharmacodynamic results depending on the drug incorporated in its micelle core, as will be demonstrated in the following sections, although it is known that the interaction between the drug molecules and the hydrophobic environment of the micelle core is an important factor to determine the properties of the micelle-encapsulated drug (Torchilin, 2001; Rapoport, 2007).

The conceptual diagram (A) and a schematic procedure (B) for micelle-encapsulation of docetaxel using CP750 are displayed in Fig. 1. First of all, it should be pointed out here that docetaxel micelle-encapsulated by CP750 is obtained as a photo-stable powdery form (Fig. 1B), which will be discussed in more detail later in this section. Docetaxel could be loaded up to approximately 25% (w/w) of the cyclotriphosphazene micelle, but 10% docetaxel loaded micelle solution was used for most of animal studies. The powdery micellar docetaxel is immediately soluble in any aqueous media including saline and PBS, and the resultant solution was very stable without significant change in micelle particle size even after more than a thousand times dilution to μM concentrations of the micellar carrier in both pure water and saline.

One of the most important advantages of the present formulation of docetaxel micelle-encapsulated by CP750 is the excellent stability of the docetaxel-loaded micelles in contrast to the polymeric micelles composed of linear block copolymers, which are unable to hold the entrapped docetaxel in the micelle core with durable stability. As above-mentioned, docetaxel entrapped by the polymeric micelles assembled from the linear block copolymers precipitates mostly within 24 h in aqueous solution (Shin et al., 2009; Carstens et al., 2008). However, docetaxel micelle-encapsulated by CP750 in aqueous solution did not precipitate even after three months in the room light at ambient temperature, although the purity of docetaxel entrapped in the

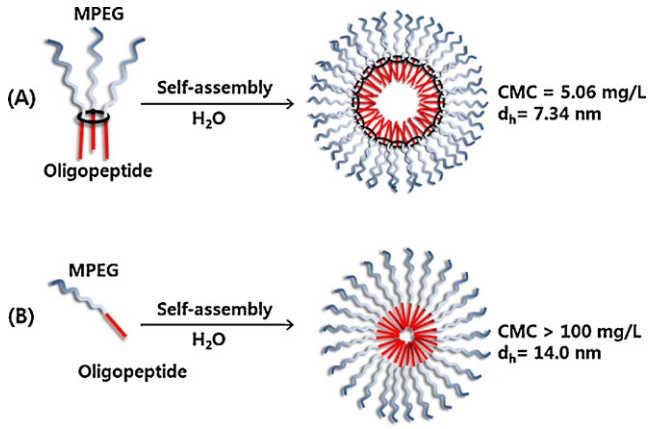

Fig. 2. Micelles self-assembled from the tripodal (A) and linear amphiphiles (B) bearing the same PEG750 as a hydrophilic group and the same hexapeptide, GlyPheLeuGlyPheLeuEt as a hydrophobic group.

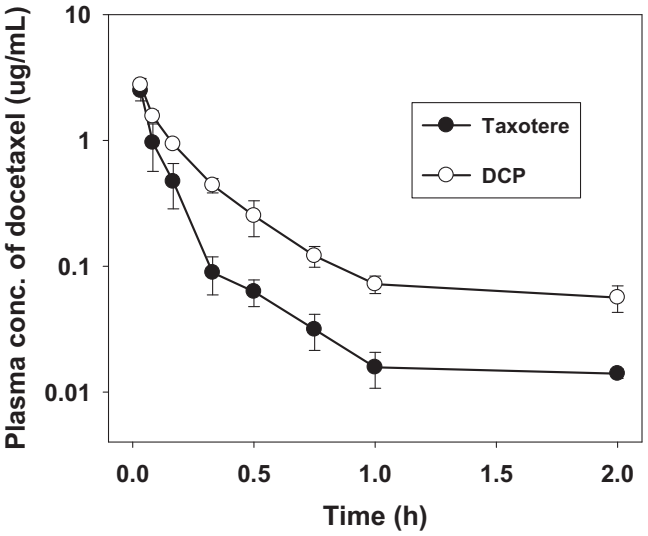

Fig. 3. Docetaxel plasma concentration versus time profiles of Taxotere® and docetaxel micelle-encapsulated by CP750 (DCP) (n = 4–7).

micelle core was lowered due to partial photo-degradation in aqueous solution (Fig. S1 of Supplementary data).

The reason for instability of the docetaxel-loaded micelles assembled from linear diblock copolymers was not clearly commented in the literature, but it may be presumed that the linear diblock copolymers cannot assemble into tightly packed spherical micelles due to their long chained hydrophobic segments, resulting in a relatively long diameter (20–80 nm) and a fairly high CMC value (10–100 mg/L). The stability of empty micelles is solely dependent on its CMC value, but the stability of drug-loaded micelles must be affected by the interaction between the drug molecules and the micelle core environment. In particular, the hydrophobic interaction between docetaxel molecules and the diffused hydrophobic core of the polymeric micelles seems not strong enough to hold the drug molecules in the micelle core.

In contrast to the long linear block copolymers forming polymeric micelles with a diffused hydrophobic core, the present tripodal amphiphile unimer bears three hydrophobic oligopeptide groups in the same direction (Fig. 2A), which are subjected to strong intra- and intermolecular hydrophobic interactions to assemble into closely packed spherical micelles with a mean diameter of 7–9 nm and a low CMC value. The CMC value of empty CP750 measured by a pyrene fluorescence technique was 5.06 mg/L in distilled water, which was not significantly changed by 10% docetaxel loading. Similarly, the mean particle size of docetaxel-loaded CP750 was not significantly changed from that of CP750 unloaded with docetaxel. Such an extraordinary stability of the micelles self-assembled from the tripodal cyclotriphosphazene amphiphile may further be confirmed by direct comparison with that of the amphiphilic linear block copolymer composed of the same PEG750 and hexapeptide (Fig. 2B) as employed for the preparation of CP750. This linear block copolymer was reported to self-assemble into loosely packed micelles with a mean diameter of 14.0 nm and a CMC value of over 100 mg/L (Xu et al., 2007).

Another great advantage of the present docetaxel formulation using our tripodal cyclotriphosphazene amphiphile is that its micelle-encapsulated docetaxel is obtained as a powdery form, which is very stable to photo-degradation of docetaxel, as above-mentioned. Taxotere® currently in clinical use is supplied as a set of two separate vials, one containing docetaxel in Tween 80 and another 13% ethanol solution because of photo-instability of docetaxel in solution. Consequently, serious inconveniences are accompanied in both drug storage and clinical use. However, no photo-degradation of docetaxel formulated as powder form by micelle-encapsulation using CP750 was detected even after 6 months exposure in the room light at room temperature (Table S1 of Supplementary data). Furthermore, it was found that photo-stability of docetaxel in aqueous solution was remarkably improved by micelle-encapsulation using CP750. For instance, the purity of 0.1% docetaxel solution in distilled water (docetaxel/CP750/H_2O = 1 mg/9 mg/1 ml) in a colorless vial exposed in the room light at room temperature for a month changed from 0.1% to approximately 0.076%, while the purity of the same solution kept in refrigerator (0 °C) did not nearly changed (Table S2 of Supplementary data).

3.2. Pharmacokinetics

In order to compare the pharmacokinetic behaviors of docetaxel micelle-encapsulated by CP750 and Taxotere® as a reference, we have performed pharmacokinetic studies using male Sprague-Dawley rats by the standard protocol (Song et al., 2005). The plasma concentration of docetaxel versus time profile is illustrated in Fig. 3 and relevant pharmacokinetic parameters estimated are listed in Table 1.

The pharmacokinetic profiles of the micelle-encapsulated docetaxel by CP750 and Taxotere® displayed in Fig. 3 appear to be similar except for some delayed elimination phase and higher concentration for our micelle-encapsulated docetaxel compared with Taxotere®. The plasma elimination half-life ($t_{1/2}$) and the area under the curve (AUC_{last}) of our micelle-encapsulated docetaxel increased only approximately 43% and 66%, respectively, compared with those of Taxotere®. Such results are in contrast to

Table 1
Pharmacokinetic parameters of docetaxel encapsulated by CP750 after a single IV injection at the dose of 5 mg/kg to male rats (n = 4–7).

PK parameters[a]	Taxotere®	DCP[b]
C_0 (μg/mL)	5.32 ± 1.58	4.07 ± 0.92
AUC_{last} (μg h/mL)	0.360 ± 0.116	0.598 ± 0.050
AUC_{Inf} (μg h/mL)	0.399 ± 0.193	0.664 ± 0.123
$t_{1/2}$ (h)	0.496 ± 1.11	0.711 ± 0.796
V_z (L)	1.24 ± 2.21	1.67 ± 1.55
Cl (L/h)	3.38 ± 1.00	1.86 ± 0.281

[a] C_0, initial plasma concentration; AUC_{last}, the area under the plasma concentration–time curve from 0 h to the last sampling time; AUC_{Inf}, the area under curve from 0 h to infinity; $t_{1/2}$, elimination half-life; Cl, total clearance; V_z, apparent volume of distribution.
[b] DCP: docetaxel micelle-encapsulated by CP750.

Table 2
In vitro cytotoxicity of docetaxel micelle-encapsulated by CP750.

Tumor cell lines	*In vitro* cytotoxicity (IC_{50}, nM)	
	Taxotere®	DCP[a]
HCT-15 (colon)	34.1 ± 0.08	54.7 ± 17.2
A549 (lung)	5.83 ± 1.85	8.81 ± 1.41
PC3 (prostate)	9.80 ± 2.09	12.6 ± 1.22
SNU638 (stomach)	0.748 ± 0.09	3.65 ± 0.83
SK-OV-3 (ovary)	9.21 ± 0.59	14.4 ± 1.42
A431 (epidermoid)	4.90 ± 2.33	10.8 ± 2.73
MCF-7 (breast)	4.24 ± 0.52	8.74 ± 0.40
MES-SA (cervical)	31.1 ± 10.2	26.8 ± 8.97

[a] DCP: docetaxel encapsulated by CP750.

the remarkably increased elimination half-life (6 times) and AUC value (10 times) of the hydrophobic platinum anticancer agent, *cis*-(dicyclohexylamine)Pt$(NO_3)_2$ micelle-encapsulated by the same CP750 compared with those of free platinum drug (Jadhav et al., 2010). However, surprisingly, this micelle-encapsulated platinum drug exhibited no plausible antitumor efficacy in the nude mouse xenograft trial (unpublished result). On the other hand, docetaxel micelle-encapsulated by CP750 exhibited excellent antitumor efficacy in the nude mouse xenograft model as will be shown later, although its pharmacokinetic parameters were not remarkably improved as above-mentioned.

The reason why the pharmacokinetic parameters of our micelle-encapsulated docetaxel are not remarkably improved may be probably due to the inherent properties of the hydrophobic taxane drug molecules, which are known to be subjected to very rapid clearance by hepatic metabolism and biliary excretion (Clarke and Rivory, 1999) as also shown in the table. In fact, previous studies have also reported that docetaxel encapsulated into polymeric micelles assembled from linear block copolymers did not exhibit remarkably enhanced pharmacokinetic parameters compared with Taxotere® formulated using the surfactant micelle polysorbate 80 (Gaucher et al., 2010; Mu et al., 2010). However, it may be pointed out that the improvements of the above-mentioned plasma elimination half-life and the AUC values of our micelle-encapsulated docetaxel are significant, which will be clearly shown in the following sections of *in vivo* drug efficacy and acute toxicity studies.

3.3. *In vitro cytotoxicity*

In order to compare the spectrum of antitumor activity of docetaxel micelle-encapsulated by CP750 with that of Taxotere®, *in vitro* cytotoxicity was assayed using a modified SRB method (Skehan et al., 1990) against selected human tumor cells, and the results are listed in Table 2. There seems no significant change in the spectrum of antitumor activity depending on the formulation of docetaxel against the tested cell lines, although the present micelle-encapsulated docetaxel exhibits generally a little lower cytotoxicity probably due to slower release of docetaxel molecules from much more hydrophobic micelle core of CP750. It is interesting to note in the table that the present micelle-encapsulated docetaxel displays excellent cytotoxicity not only against female human tumor cells except for cervical MES-SA but also against non-small cell lung A549, prostate PC3 and stomach SNU638 which are least responsive to chemotherapy.

3.4. *In vivo xenograft trials*

We have performed xenograft trials of the present docetaxel micelle-encapsulated by CP750 (DCP) and Taxotere® as a reference against two different human cancer cells, a breast cancer cell line (MDA-MB-231) and a gastric cancer cell line (MKN-28). The results of the xenograft trial showing *in vivo* efficacy against the breast cancer cells are illustrated in Fig. 4. All the tumor volumes of the mice treated with Taxotere® and docetaxel micelle-encapsulated by CP750 (DCP) are decreasing right after initial injection and converging to zero tumor volume from 3 to 4 weeks since the initial injection. After 35 days observation, all the survived mice were sacrificed for autopsy to confirm complete disappearance of tumor tissue.

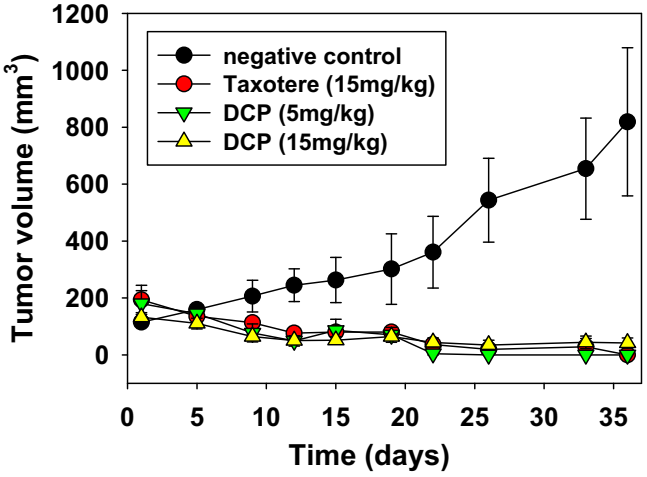

Fig. 4. Results of nude mouse xenograft trials to evaluate *in vivo* antitumor efficacy of docetaxel micelle-encapsulated by CP750 (DCP) and Taxotere® against the breast cancer cells (MDA-MB-231).

Thus Taxotere® exhibited excellent antitumor efficacy by showing complete tumor regression at its optimal dose of 15 mg/kg (Bissery et al., 1991) without any death of 5 mice. The present micellar docetaxel has also shown exactly the same antitumor efficacy as Taxotere® with complete tumor regression at the dose of 5 mg/kg without any death of 5 mice, but one mouse was dead at the higher dose of 15 mg/kg of our micellar docetaxel due to drug toxicity (Fig. S2 of Supplementary data), which means that the present docetaxel micelle-encapsulated by CP750 is dose dependent. As a result, the optimal dose of our micellar docetaxel is presumed to be 5 mg/kg for the treatment of the breast cancer cells. Although some severe weight loss was observed right after the drug treatment (Day 1–Day 9) for both groups treated with a dose of 15 mg/kg of Taxotere® and docetaxel micelle-encapsulated by CP750 (DCP), only a minor weight loss was observed for the group treated with 5 mg/kg of DCP probably due to reduced toxicity at its optimal dosage as above-mentioned (Fig. S3 of Supplementary data).

The results of the xenograft trial against the gastric cancer cells are illustrated in Fig. 5. Similarly to the results of the xenograft trial against the breast cancer cells, all the tumor volumes of the mice treated with Taxotere® and our micellar docetaxel are also decreasing and converging to zero tumor volume after 4 weeks since the initial drug injection, but a little different responses were observed against the gastric cancer cells. First of all, Taxotere® exhibited excellent tumor efficacy by showing complete tumor regression for three of the five mice treated but one mouse was dead due to toxicity (Fig. S4 of Supplementary data). On the other hand, no mortality was observed for the mice treated with our micellar docetaxel, although severe weight loss was observed after drug injections (Fig. S5 of Supplementary data), and tumor was completely disappeared from two of the five mice treated at a dose of 5 mg/kg and four of five mice at a dose of 15 mg/kg. Such results imply that docetaxel micelle-encapsulated by CP750 is more efficacious and less toxic than Taxotere® at the dose of 15 mg/kg against the gastric cancer cells. Therefore, it may be presumed that docetaxel micelle-encapsulated by CP750 is more potent than Taxotere®, although

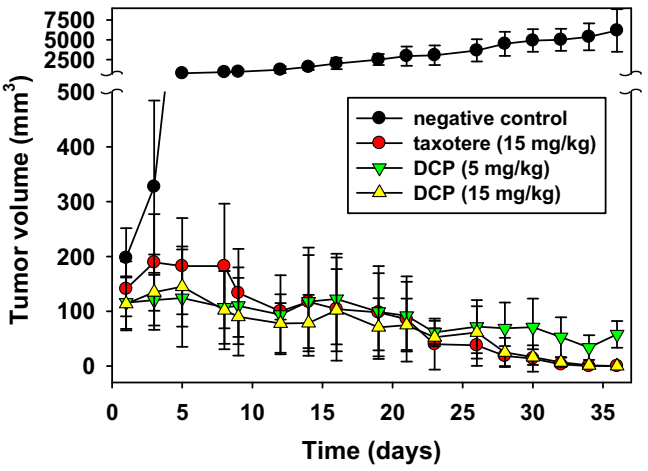

Fig. 5. Results of nude mouse xenograft trials to evaluate *in vivo* antitumor efficacy of docetaxel micelle-encapsulated by CP750 (DCP) and Taxotere® against the gastric cancer cells (MKN-28).

the optimal dose may be a little different depending on the kind of tumor cells.

3.5. Acute toxicity

Animals were dosed by intravenous injection at 5, 30, 50 and 100 mg/kg of Taxotere® (TX) and 5, 50, 300, 500, 1000, and 2000 mg/kg of docetaxel micelle-encapsulated by CP750 (DCP) containing 10% docetaxel to obtain their LD_{50} values and evaluate its acute toxicity. After exposure, the mortality and changes in body weight of the mice were carefully and daily recorded for a total of 14 days. The survival rates of each group consisting of 3 mice treated at different dosages are displayed in Fig. 6.

In case of Taxotere®, all three mice survived at its lowest dosage of 5 mg/kg, and only one of 3 mice survived at 30 mg/kg, but all the mice died at higher dosages. According to the OECD guideline, the LD_{50} value of Taxotere® was calculated to be approximately 28 mg/kg. In case of docetaxel micelle-encapsulated by CP750, no mortality was observed up to the dose of 500 mg/kg, but all the mice treated with 1000 and 2000 mg/kg died within several minutes after injection. The LD_{50} value of docetaxel micelle-encapsulated by CP750 was determined to be about 750 mg/kg, which corresponds to 75 mg/kg based on docetaxel. This result suggests that the acute toxicity of our micelle-encapsulated docetaxel by CP750 is reduced to approximately one third of acute toxicity of docetaxel in Taxotere®.

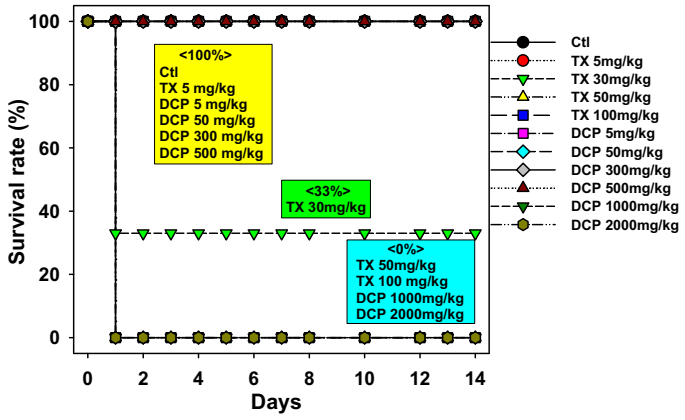

Fig. 6. Survival rate of the mice intravenously treated with different doses of Taxotere® (TX) or docetaxel micelle-encapsulated by CP750 (DCP).

4. Conclusions

It has been demonstrated in this study that the same micellar drug carrier can give rise to pharmacokinetically and pharmacodynamically contrasting results depending the kind of drug incorporated in its micelle core due to the hydrophobic interaction between drug molecules and the micelle core. In contrast to the hydrophobic platinum anticancer agent micelle-encapsulated using CP750, docetaxel micelle-encapsulated using the same CP750 exhibited excellent *in vivo* efficacy, although its plasma half-life and AUC value were not remarkably improved. Nude mouse xenograft trials have shown complete tumor regression against the breast cancer cells (MDA-MB-231) at a lower dose of 5 mg/kg and better efficacy against gastric cancer cells (MNK-28) at the same dose of 15 mg/kg compared with Taxotere®. However, the more important aspect of the present tripodal cyclotriphosphazene micelles is that two most critical problems associated with docetaxel formulation using conventional linear block copolymer micelles, that is, docetaxel-loaded micelle stability and inherent photo-instability of docetaxel in solution could be overcome by using the present tripodal cyclotriphosphazene micelles CP750. Furthermore, according to the comparative acute toxicity study, toxicity associated with Taxotere® may be remarkably reduced by micelle-encapsulation of docetaxel using CP750, which afforded a much higher LD_{50} value of 75 mg/kg compared with 28 mg/kg of docetaxel in Taxotere®. Thus docetaxel micelle-encapsulated by CP750 has entered the stage of preclinical studies.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijpharm.2011.10.052.

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