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Preparation of camptothecin-loaded polymeric micelles and evaluation of their incorporation and circulation stability

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Abstract

To improve its aqueous solubility and stability in biological fluid, CPT was physically loaded in polymeric micelles. Polymeric micelles were composed of various poly(ethylene glycol)–poly(aspartate ester) block copolymers (PEG-P(Asp(R))). The incorporation and circulation stability of CPT micelles were evaluated by measuring the CPT in micelle using gel-permeation chromatography and by CPT concentration measurement after intravenous injection using HPLC, respectively, in terms of chemical structure of block copolymers. The stability of CPT-loaded micelles in vivo depended on the amount of benzyl esters, and length of PEG in the polymers to a greater degree than it did in vitro. A stable formulation of CPT-loaded micelles was obtained using PEG-P(Asp) with PEG of 5000 (MW), 27 Asp units, and 57–75% benzyl esterification of Asp residue. This CPT-loaded micelles showed about a 17-fold lower blood clearance value than unstable micelles. The CPT-loaded micelles are potentially delivered to tumor sites owing to an extended circulation in the blood stream.

Keywords: Camptothecin; Polymeric micelles; Long circulating; In vivo stability

1. Introduction

Camptothecin (CPT) is a naturally occurring cytotoxic alkaloid isolated from the Chinese plant *Camptotheca accuminata* (Wall et al., 1966). CPT and some of its analogs have shown a broad spectrum of antitumor activity against many solid tumors in xenografts (Giovanella et al., 1989, 1991). CPT inhibits the enzyme DNA topoisomerase I, initially by noncovalent binding and subsequently by stabilization of the complex through a nucleophilic attack by the enzyme at the acyl position of the CPT lactone ring (Hertzberg et al., 1989). In early clinical trials, CPT was formulated as a water-soluble CPT-Na^+ (Moertel et al., 1972). However, it was later reported that the lactone E-ring is important for cytotoxicity and that the open-ring carboxylated CPT-Na^+ is inactive. The lactone of CPT is converted to carboxylate in a pH-dependent equilibrium (Fig. 1) (Fassberg and Stella, 1992). To overcome the solubility and stability problems of CPT, several approaches have been investigated. Water-soluble CPT analogs have been prepared but the majority of them were less potent in assays both in vitro and in vivo than the parent drug (Wall and Wani, 1995). Therefore, the development of adequate drug carriers is gaining increasing attention. These include methods such as conjugation to polymers (Zamai et al., 2003; Singer et al., 2001), intercalation into liposomes (Burke et al., 1992; Cortesi et al., 1997), solubilization in microemulsions (Cortesi et al., 1997), formation of inclusion complexes with cyclodextrins (Kang et al., 2002) and entrapment in microspheres (Shenderova et al., 1999; Tong et al., 2003). However, concerning long circulation carriers of CPT in blood stream, there was not enough information.

Drug carriers with longer retention time in the blood stream can be delivered to solid tumors site by a passive

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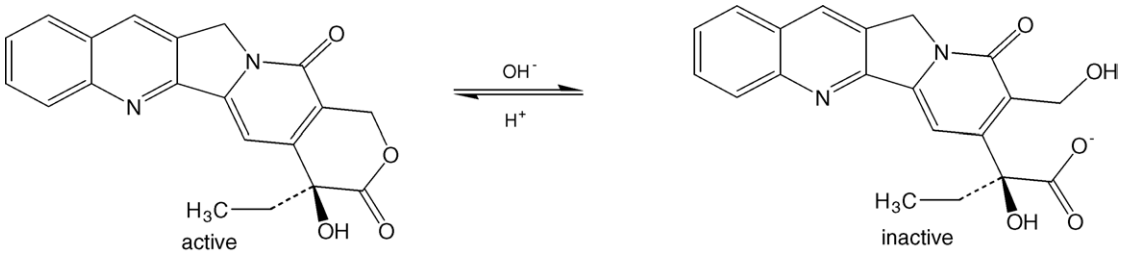

Fig. 1. The structure of camptothecin and equilibrium reaction between the active form and inactive form.

targeting mechanism based on the enhanced permeability and retention effect (EPR effect) (Matsumura and Maeda, 1986; Maeda, 2000; Maeda et al., 2000). Recently, antitumor drug targeting using polymeric micelle carrier systems was achieved with doxorubicin (adriamycin, ADR) using poly(ethylene glycol)–poly(aspartate derivative) block copolymer PEG-P(Asp(R)) (Yokoyama et al., 1987, 1990, 1991, 1998). As a result of selective delivery to tumor site by the EPR effect, dramatically enhanced antitumor effects were obtained in vivo (Yokoyama et al., 1999). In this system, ADR was chemically conjugated to the aspartic acid residue of the block copolymer as hydrophobic species for micelle formation and enhance the physical incorporation of ADR in the inner core. However, the chemically conjugated ADR did not play a role in the expression of anticancer activity (Yokoyama et al., 1999, 1998). Only the physically incorporated ADR expressed selective anticancer activity by being recruited to solid tumor sites.

In previous studies, we reported polymeric micelle system for incorporation of CPT, in which CPT incorporation efficiency and CPT-loaded micelles stability were improved by modification of a hydrophobic segment of the PEG-P(Asp) (Yokoyama et al., 2004; Opanasopit et al., 2004). Chemical structure of block copolymers would largely influence to the stability of polymeric micelles in blood stream. In this study, the in vivo circulation stability of CPT-loaded micelles was evaluated in terms of copolymer composition (PEG length, Asp unit number, ester groups, and esterification degree) and feeding ratio of the drug to copolymer, and compared with incorporation stability of CPT. CPT-loaded micelles with a stable copolymer composition enhanced the circulation time of CPT in the blood stream, which will contribute to recruitment to solid tumor sites.

2. Materials and methods

2.1. Materials

Poly(ethylene glycol)–poly(β-benzyl L-aspartate) block copolymer (PEG–PBLA) was synthesized as described previously (Yokoyama et al., 1992). (s)-(+)-CPT was purchased from Aldrich Chem. Co. (Milw., WI, USA). 1,8-Diazabicyclo[5,4,0]7-undecene (DBU), high performance liquid chromatography (HPLC) grade acetonitrile and triethylamine acetate were purchased from Wako Pure Chemicals, (Tokyo, Japan). *N*,*N*-dimethylformamide (DMF) was dried over a molecular sieve (4A), and distilled under reduced pressure. Other chemicals were of reagent grade.

2.2. Synthesis of diblock copolymers (PEG–PBLA and its derivatives)

Poly(ethylene glycol)–poly(β-benzyl L-aspartate) block copolymer (PEG–PBLA) was synthesized by ring-opening polymerization of benzyl L-aspartate *N*-carboxy anhydride from a terminal primary amino group of α-methyl-ω-amino-poly(oxyethylene), and poly(ethylene glycol)–poly(aspartic acid) block copolymer (PEG-P(Asp)) was obtained by alkaline hydrolysis of PEG–PBLA as reported previously (Yokoyama et al., 1992). Briefly, PEG–PBLA was dispersed in a measured volume of 0.5N NaOH that contained 1.5 mol. equivalents of NaOH to the benzyl aspartate residue of PEG–PBLA. With stirring at room temperature, the solution became homogeneous in approximately 15 min. Then, 6N HCl was added (10 mol. equivalents of HCl to the benzyl aspartate residue) to the solution, and this solution was dialyzed against 0.1N HCl, followed by distilled water using a SpectraPor®-6 dialysis membrane (MWCO: 1000). PEG-P(Asp) block copolymer was obtained by freeze-drying the dialyzed solution.

Esterification of the aspartic acid residues was achieved through nucleophilic substitution of the carboxyl group with a halogen compound using 1,8-diazabicyclo [5,4,0] 7-undecene (DBU) as a catalyst, as reported previously (Opanasopit et al., 2004). PEG-P(Asp) block copolymer was dissolved in DMF and added to a halogen compound or a mixture of two halogen compounds (benzyl bromide, *n*-butyl bromide, or lauryl bromide) and DBU. The reaction mixture was stirred at 50 °C for ca. 16 h. Then, it was poured into a 10-fold volume excess of diethyl ether, and the precipitated polymer was collected by filtration, followed by washing with diethyl ether and drying. In order to remove DBU from the polymer products, polymers were dissolved in DMSO and added to 6N HCl that was much excess equivalents to the aspartic acid residue of the block copolymer. Then, this solution was dialyzed against distilled water, and freeze-dried.

Ester contents of the block copolymers were determined in ^{1}H NMR spectra by comparing the methylene protons of the hydrophilic PEG block and protons of the hydrophobic moieties of the poly(aspartate) block.

2.3. Preparation of CPT-loaded micelles and CPT solution

CPT was incorporated into polymeric micelles by an evaporation method as reported previously (Opanasopit et al., 2004). Briefly, CPT (0.05, 0.1, 0.2, or 0.4 (CPT/polymer, w/w)) was

dissolved in a mixture of chloroform (1 ml per 1 mg CPT) and acetonitrile (0.67 ml per 1 mg CPT), and added to 5 mg of block copolymer. The solvent was removed by evaporation in a nitrogen gas flow. Then, 3 ml of distilled water was added and sonicated for 2 min using a probe type sonicator model VC 100 (Sonics & Materials Inc., Newtown, Connecticut, USA) equipped with a standard 6 mm probe in a cycle of sonication for 0.5 s and standby for 0.5 s at 80 °C. The solution obtained was centrifuged at 1400 × *g* for 10 min. Subsequently, the supernatant was collected and filtered through a 1 μm pore-sized nylon filter (Puradisc 25NYL, 6751-2510, Whatman, Clifton, New Jersey, USA). CPT-loaded polymeric micelles were kept in a freezer at −20 °C until animal experiments.

The CPT solution was prepared by dissolving CPT (13 mg) in 50 ml of polyethylene glycol 400, propylene glycol and polysorbate 80 (40:50:2, volume ratio) (Yang et al., 1999).

2.4. Determination of CPT content and particle size of micelles

CPT-loaded micelles were dissolved in a mixture of DMSO:H_2O (9:1). The amount of CPT incorporated into polymeric micelles was determined by UV–vis absorption at 365 nm. The incorporation efficiency was calculated as the percentage share of the initial drug used in the preparation for incorporation into the micelles. The mean particle diameters were determined using a dynamic light scattering particle size analyzer (DLS-7000, Otsuka Electronics, Osaka, Japan) at 25 °C by diluting dispersion to an appropriate volume with water.

2.5. Incorporation stability of CPT-loaded micelles evaluated by GPC

The incorporation stability of CPT-loaded micelles was evaluated by gel-permeation chromatography (GPC) as described previously (Yokoyama et al., 1994). GPC was carried out using a Tosoh HPLC system SC-8010 equipped with a Tosoh TSKgel G3000PW_{XL} column. Distilled water was used as the eluent at a flow rate of 1 ml/min at 40 °C. Sample solutions (50 μl) were injected into the column. The detection was performed by measuring absorption at 351 nm for CPT using a Tosoh UV-8010 detector and a refractive index (RI) detector for polymers. A micelle peak was observed at the gel-exclusion volume. GPC with UV detection allowed us to evaluate the nature of the polymeric micelles obtained and the degree of drug incorporation. The peak area detected by UV absorption represents the amount of CPT loaded into the micelles. Therefore, the ratio of the micelle peak area/CPT concentration of the injected sample [CPT] was evaluated as the incorporation stability of CPT-loaded micelles. The small values of the peak area/[CPT] means that most of the CPT was adsorbed to the GPC column by hydrophobic interactions due to unstable packaging of CPT in the micelles. When this ratio was large, CPT was more stably incorporated into micelles.

2.6. Measurement of CPT concentration in plasma

CPT-loaded micelles were intravenously (i.v.) administered to male ddY mice (weighing 18–20 g, Tokyo Laboratory Animal Science Co., Ltd., Tokyo, Japan) via lateral tail veins at a dose of 2.5 mg/kg. For each sampling point, three mice were injected with CPT-loaded micelles. At various time points after the administration, approximately 1 ml of blood was withdrawn using a heparinized syringe and centrifuged at 15,300 × *g* for 4 min to obtain the plasma. Immediately after that, 0.15 M aqueous phosphoric acid was added to the plasma and mixed vigorously (Onishi et al., 2003). CPT was extracted with chloroform: methanol (4:1 volume ratio). After centrifugation of the mixture at 15,300 × *g* for 4 min, 25 μl of the chloroform: methanol layer was directly injected into the HPLC system to determine the concentration of CPT. This operation gave the total concentration of free and incorporated CPT in micelles.

The HPLC analysis was performed at room temperature. A Shimadzu LC-10AT (Shimadzu Corp., Japan) apparatus equipped with a Shimadzu RF-$10A_{XL}$ fluorescence detector in which the excitation and emission wavelength was set at 369 and 426 nm, respectively, was used. A Tosoh TSK-gel ODS-80Ts column (150 mm × 4.6 mm i.d.) was also used. The mobile phase was composed of 23:77 (v/v) acetonitrile–triethylamine acetate buffer (1% (v/v) adjusted to pH 5.5 with glacial acetic acid), and the flow rate was set at 1 ml/min (Warner and Burke, 1997). The areas under the concentration curve (from 0 to 24 h; AUC) were calculated using the trapezoid method.

2.7. Statistical analysis

The results were analyzed statistically using the Student's *t*-test. When comparisons between groups yielded a value for $P < 0.05$, the difference between those groups was considered significant.

3. Results and discussion

3.1. Characterization

Polymeric micelles with a particle diameter of less than 100 nm and a PEG-coated surface have been found to well avoid entrapment by the reticuloendothelial system (RES) and to well leak in diseased areas with highly permeable blood vessels, resulting in passive targeting to the diseased sites (Yokoyama et al., 1993; Kwon et al., 1994); this is known as the EPR effect. In order to sufficiently acquire this EPR effect, we examined the effect of various polymers on the CPT incorporation stability and evaluated the pharmacokinetic profile of CPT-loaded micelles.

Chemical structure of poly(ethylene glycol)–poly(aspartate ester) block copolymer (PEG-P(Asp(R))) is shown in Fig. 2. The block copolymers are coded by the chain lengths of both blocks, the name of the hydrophobic group, and the degree of esterification as summarized in Table 1. For example, 5-27 Bz44 represents a block copolymer composed of a PEG block of molecular weight 5000 and a P(Asp) block possessing 27 units of aspartic acid, in which 44%

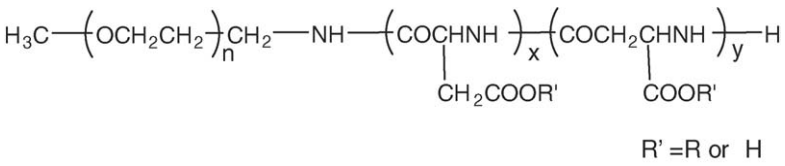

Fig. 2. Chemical structure of poly(ethylene glycol)–poly(aspartate ester) block copolymer (PEG-P(Asp(R))).

Table 1
Poly(ethylene glycol)–poly(aspartate ester) diblock copolymers (PEG-P(Asp(R)))

Code	PEG (MW)	Asp[a] ester ($x+y$ unit)	Ester (R)	Esterification (%)
5-27 Bz44	5000	27.1	Benzyl	44
5-27 Bz57	5000	27.1	Benzyl	57
5-27 Bz75	5000	27.1	Benzyl	75
5-52 Bz74	5000	52.0	Benzyl	74
12-25 Bz71	12000	27.1	Benzyl	71
12-50 Bz63	12000	50.0	Benzyl	63
5-27 *n*-Bu6 + Bz57	5000	27.1	*n*-Butyl + benzyl	6 + 57
5-27 Lau5 + Bz58	5000	27.1	Lauryl + benzyl	5 + 58

[a] Asp: aspartate.

of the aspartic acid residues are esterified with a benzyl group.

3.2. Ester groups of polymers

To determine the structural requirements for stability, polymers with three kinds of ester groups were synthesized. The incorporation of CPT into polymeric micelles was successfully achieved by the evaporation method which provided high CPT yields (Opanasopit et al., 2004). Table 2 shows the effect of ester groups (benzyl and mixture (benzyl + lauryl, benzyl + *n*-butyl)) on the entrapment efficiency, incorporation stability evaluated by GPC and the % injected dose in plasma after 4 h. When the ratio of micelles peak area/CPT concentration [CPT] was large, the CPT incorporated into the micelles was more stable. CPT micelles of these three ester groups showed similar stability in vitro, but not in vivo. Benzyl polymeric micelles suggested a longer circulation time than *n*-butyl and lauryl ones in spite that the mixture had a more hydrophobic inner core than benzyl. This implies that not only hydrophobicity but also physical factors such as rigidity and π–π interactions of the inner core-forming block contributed to the incorporation, since the lauryl ester (C12) is more hydrophobic than the benzyl ester (C7).

Table 3
Effect of PEG length (5000 or 12,000) and Asp unit (27 or 50) on stability of CPT-loaded micelles at a feeding ratio (CPT/polymer) of 0.4 (w/w)

Code	Peak area/[CPT][a]	% Injected dose in plasma after 4 h[b]
5-27 Bz75	46.5	9.3 ± 1.8
12-25 Bz71	26.5	2.6 ± 0.6
5-52 Bz74	86.1	6.7 ± 0.5
12-50 Bz63	21.9	2.2 ± 0.4
CPT-solution[c]	–[d]	0.04 ± 0.03

[a] Incorporation stability; peak area, evaluated by GPC, was divided by CPT concentration in micelles [CPT] ($n = 1$–2).
[b] Results at a dose of 2.5 mg/kg are given as the mean ± S.D. ($n = 3$).
[c] CPT was dissolved in polyethylene glycol 400:propylene glycol: Tween 80 = 40:58:2 (volume ratio).
[d] Not done.

PEG–PBLA can be handled as benzyl-100% because its aspartic acid residues were fully benzylated. However, in the stability assay using GPC, PEG–PBLA micelles provided much lower incorporation stability than the benzyl-57% block copolymer. This indicates that the conformation and/or configuration of the hydrophobic inner core-forming polymer block contributes to a stable incorporation by providing the appropriate space for CPT.

3.3. PEG length and Asp unit of polymers

To determine the contribution of PEG length and Asp unit to the micelles' stability, four polymeric micelles of Bz with different combinations of PEG length (5000 or 12,000) and Asp units (27 and 50) were prepared (Table 3). Compared with the CPT solution as a control, at both 27 and 50 Asp units, CPT micelles of PEG 5000 showed a longer circulation than those of PEG 12,000 in vivo.

An increase in the chain length of a hydrophobic block at a given chain length of a hydrophilic block causes a decrease in the critical micelle concentration (CMC) (Leibler et al., 1983). In contrast, the ADR-loaded PEG-P(Asp(ADR)) micelles, pos-

Table 2
Effect of ester group of polymer 5-27 (PEG 5000-Asp unit 27) on stability of CPT-loaded micelles at a feeding ratio (CPT/polymer) of 0.1 (w/w)

Code	Esterification (%) of diblock copolymer	Entrapment efficiency (%)[a]	Peak area/[CPT][b]	% Injected dose in plasma after 4 h[c]
5-27 *n*-Bu6 + Bz57	*n*-Bu 6 + Bz57	37.8	58.1	0.15 ± 0.08
5-27 Lau5 + Bz58	Lauryl 5 + Bz58	47.5	48.3	0.07 ± 0.03
5-27 Bz57	Bz57	88.6	55.3	7.6 ± 0.8
PEG–PBLA	Bz100	67.7	1.5	–[d]

[a] $n = 1$–2.
[b] Incorporation stability; peak area, evaluated by GPC, was divided by CPT concentration in micelles [CPT] ($n = 1$–2).
[c] Results at a dose of 2.5 mg/kg are given as the mean ± S.D. ($n = 3$).
[d] Not done.

sessing a longer hydrophilic PEG chain and a shorter hydrophobic P(Asp(ADR)) chain circulated longer in blood (Kwon et al., 1993, 1994), accumulated more in tumors (Kwon et al., 1994), and showed greater antitumor activity (Yokoyama et al., 1993). This is a reversed relationship estimated from the CMC phenomenon of block copolymers. This implies that dynamic stability of the polymeric micelles that is defined with a dissociation constant of the micelle structure is more important in vivo than static micelle stability that is defined with CMC values. In this study, at a similar number of the Asp units, the micelles possessing PEG 5000 was found to be more stable than those possessing PEG 12,000 in blood circulation. This fact was opposite to the ADR case. More detailed study is required to elucidate the relationship between in vivo stability and compositions of polymeric micelles by more quantitatively evaluating strength and nature (e.g., degree of contribution of π–π interaction) of interactions utilized for micelle formation and drug incorporation.

3.4. Benzyl ester content in micelles

For the ADR-loaded polymeric micelle system, a larger amount of the chemically conjugated ADR (63 mol% with respect to the aspartic acid residue of the block copolymer) provided more stable circulation in blood of the physically entrapped ADR that exhibited targeted anti-tumor activity than a smaller amount case (41 mol%). In the present study, to determine the contribution of esterification to micelle stability, three kinds of 5-27 micelles with different amounts of Bz were prepared. The mean particle sizes of 5-27 Bz44, Bz57 and Bz75 were 275.8, 182.7 and 196.1 nm, respectively. The effect of esterification of 5-27 Bz on the stability of CPT-loaded micelles at a feeding ratio (CPT/polymer) of 0.4 (w/w) was examined by GPC and by measuring the % injected dose in plasma after 4 h (Fig. 3). When the benzyl ester content was increased from 44 to 75%, the stability of polymeric micelles was similar in vitro, but CPT-loaded micelles of 5-27 Bz44 showed poor circulation stability. CPT-loaded micelles were able to maintain stability in vivo on esterification of more than 57% of the polymer. As showing Table 2, PEG–PBLA (Bz100%) micelles were not stable in in vitro. Therefore, stable micelle formulation was obtained when the esterification ratio of Bz was appropriate (57–75%). This finding corresponded well that CPT release rate from the micelles for PEG–PBLA or 5-27 Bz44 was faster than that for 5-27 Bz75, when incubated in PBS at 37 °C (Opanasopit et al., 2004). The result suggested that the contribution of π–π interaction between aromatic groups of CPT molecules could be maintained, when the degree of esterification of Bz was more than 57%.

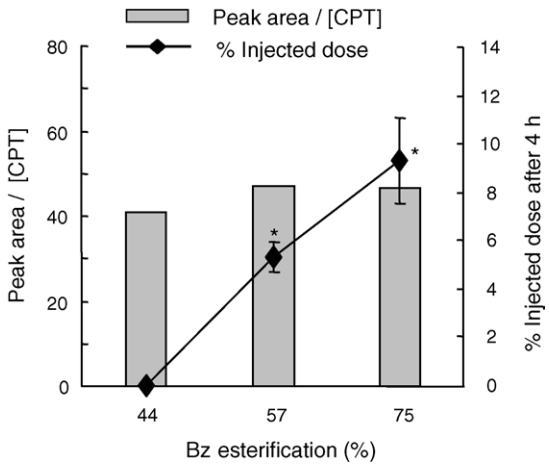

Fig. 3. Effect of esterification of 5-27 Bz on stability of CPT-loaded micelles at a feeding ratio (CPT/polymer) of 0.4 (w/w). The ratio of peak area/[CPT] indicated the incorporation stability of CPT-loaded micelles. Percentage injected dose represents the mean ± S.D., $n = 3$. $^{*}P < 0.05$; compared with 5-27 Bz44.

3.5. Feeding ratio of CPT/polymer in micelle

To investigate the influence of feeding ratio on the micelle characteristics, CPT-loaded polymeric micelles were prepared at the different feeding ratio (0.05, 0.1, 0.2 and 0.4 (w/w)). Fig. 4 shows the entrapment efficiency, mean particle size, and the incorporation and circulation stability of CPT-loaded 5-27 Bz57 polymeric micelles, respectively. Regardless of feeding

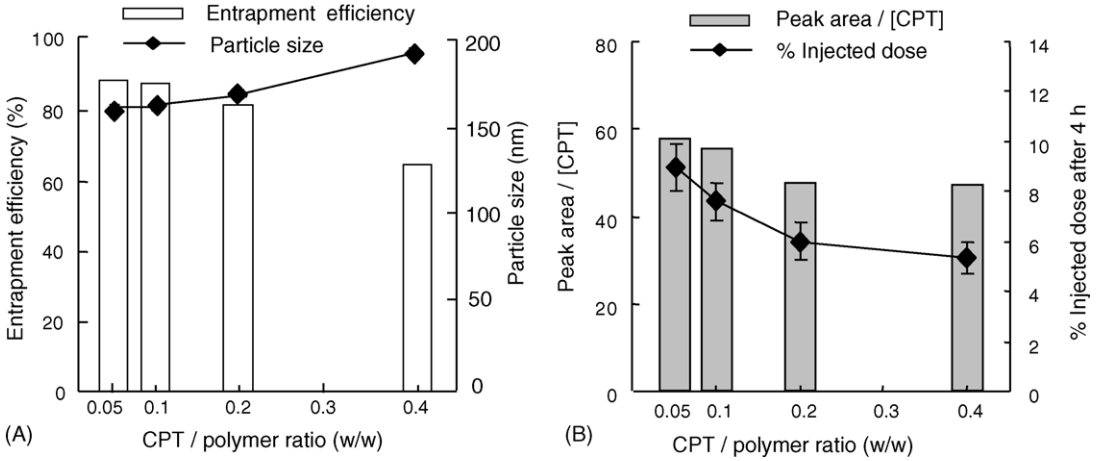

Fig. 4. Effect of feeding ratio (CPT/5-27 Bz57 polymer, w/w) on entrapment efficiency, particle size (A), and the incorporation (peak area/[CPT]) and circulation stability of CPT-loaded micelles (B). Particle size and % injected dose represent the mean ± S.D., $n = 3$.

ratio of CPT/polymer, obtained CPT-loaded micelles showed similar particle size (150–200 nm) and stability of in vitro and in vivo (5–10% injected dose after 4 h). CPT-loaded 5-27 Bz57 micelles were stable in vivo even if the amount of CPT in the polymer was increased. In the case of ADR-loaded polymeric micelles, 21 w/w% ADR was physically incorporated whereas intact ADR, having antitumor activity, accounted for only 5 w/w% (Yokoyama et al., 1999). The feeding ratio of 0.4 (w/w) CPT/polymers corresponds to more than 20 w/w% of CPT in obtained micelles, where CPT was incorporated in the active lactone form (>95%). Therefore, this system will be able to deliver a massive amount of intact drug to the targeted site.

3.6. Plasma concentration–time profiles

As a stable formulation of CPT-loaded micelle was obtained using the polymer with 57–75% Bz esterification at a feeding ratio (CPT/polymer) of 0.4 (w/w), the plasma pharmacokinetics of the CPT-loaded 5-27 Bz63 polymeric micelles was compared with unstable formulations such as 5-27 Bz44 and CPT solution (Fig. 5). CPT-loaded 5-27 Bz63 and 5-27 Bz44 micelles at a feeding ratio (CPT/polymer) of 0.4 (w/w) showed 275.8 ± 14.8 and 276.5 ± 24.8 nm in size, respectively. As expected from Fig. 3, a long-circulation was obvious for the CPT in 5-27 Bz63 compared to 5-27 Bz44 and CPT solution. This finding corresponded with the result of CPT release from polymeric micelles in vitro, showing the slower CPT release from 5-27 Bz75 than 5-27 Bz44 in PBS at 37 °C (Opanasopit et al., 2004). Hydrophilic PEG chains exposed to the aqueous surroundings may prevent the adsorption of blood proteins onto the micelles' surface and from being cleared through RES. In spite of similar particle size, the stable polymeric micelle (Bz63) showed about a 17-fold lower clearance value than the unstable one (Bz44) (Table 4). This finding suggested that the stable incorporation of CPT into micelles by the hydrophobic interaction of intact CPT with inner core of polymeric micelles, e.g., π–π interactions of the benzyl ester, may be important in the circulation stability.

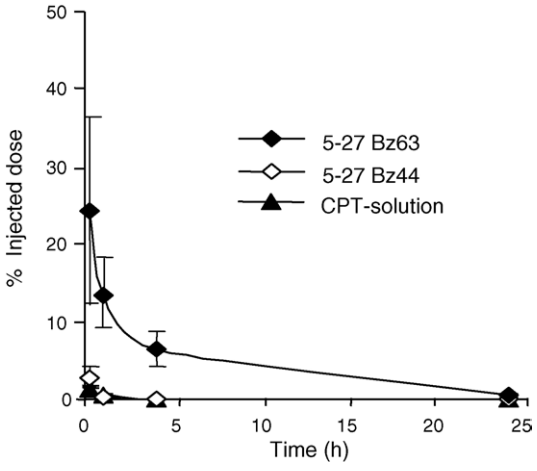

Fig. 5. Plasma concentration–time curves of the CPT-loaded 5-27 Bz (44 or 63) micelles and CPT solution following i.v. administration at a dose of 2.5 mg CPT/kg in ddY mice. The feeding ratio (CPT/polymer) was 0.4 (w/w). Results are given as the mean ± S.D., $n = 3$.

Table 4
Pharmacokinetic parameters after i.v. administration of CPT-loaded 5-27 Bz (44 or 63) micelles in mice at a dose of 2.5 mg/kg

Code	AUC (μg h ml^{-1})	Clearance (ml h^{-1} g^{-1})	
5-27 Bz63	47.0 ± 2.4	0.053 ± 0.003	**
5-27 Bz44	2.7 ± 0.02	0.91 ± 0.007	

The feeding ratio (CPT/polymer) was 0.4 (w/w). AUC: area under the concentration–time curve from 0 to 24 h. Results are given as the mean±S.D. ($n = 3$).
** $P < 0.01$

4. Conclusion

The stable formulation of CPT-loaded micelles in vivo strongly depended on the amount of benzyl esters and length of the PEG of polymers, more so than in vitro. A stable formulation of CPT-loaded micelles was obtained using PEG-P(Asp) with a PEG of 5000 (MW), 27 Asp units, and 57–75% benzyl esterification. The CPT-loaded micelles are potentially delivered to tumor sites owing to an extended circulation in the blood stream.

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