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Journal of Controlled Release 112 (2006) 329–332
journal of controlled release
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Enhanced antitumor effect of camptothecin loaded in long-circulating polymeric micelles

Kumi Kawano [a], Masato Watanabe [a], Tatsuhiro Yamamoto [b], Masayuki Yokoyama [b], Praneet Opanasopit [c], Teruo Okano [c], Yoshie Maitani [a,*]

[a] Institute of Medicinal Chemistry, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan
[b] Kanagawa Academy of Science and Technology, KSP East 404, Sakado 3-2-1, Takatsu-ku, Kawasaki-shi, Kanagawa 213-0012, Japan
[c] Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Kawada-cho 8-1, Shinjuku-ku, Tokyo 162-8666, Japan

Received 18 October 2005; accepted 20 March 2006
Available online 27 March 2006

Abstract

A water-insoluble antitumor agent, camptothecin (CPT) was successfully incorporated into polymeric micelles formed from poly(ethylene glycol)-poly(benzyl aspartate) block copolymers (CPT-loaded polymeric micelles). Antitumor effects and biodistribution of CPT-loaded micelles were evaluated in mice subcutaneously transplanted by colon 26 tumor cells. Tumor growth was significantly inhibited after a single i.v. injection of CPT-loaded polymeric micelles at doses of either 15 or 30 mg/kg. Efficacy of a single high-dose injection was comparable to low dose multiple injections. CPT loaded in polymeric micelles showed prolonged blood circulation and higher accumulation in tumors compared with CPT in solution. Polymeric micelle systems offer a stable and effective platform for cancer chemotherapy with CPT.

Keywords: Camptothecin; Polymeric micelle; Antitumor effect; Biodistribution; Colon 26 tumor

1. Introduction

In cancer chemotherapy, the usage of anticancer drugs has been limited by their toxic side-effects in normal organs. Anticancer drug carriers, such as liposomes, microspheres and polymeric systems, have been developed to target and improve their efficacy toward malignant cells, and to reduce toxicity. Long-circulating carriers with nanoscopic dimensions in the bloodstream can passively deliver chemotherapeutic agents to tumor sites via an enhanced permeability and retention effect (EPR effect) [1,2].

Polymeric micelles are prepared from block copolymers possessing both hydrophilic and hydrophobic chains, and they have received much attention in drug delivery research. Their innate characteristics for drug targeting include solubilization of hydrophobic molecules, small particle size, high structural stability, extended drug release, and prevention of rapid clearance by the reticuloendothelial system. Anticancer drug targeting using polymeric micelles was first employed in enhancing the in vivo anticancer activity of doxorubicin [3,4]. Such systems have now been applied to other anticancer drugs, such as paclitaxel [5], cisplatin [6], methotrexate [7] and KRN 5500 [8].

Camptothecin (CPT), a plant alkaloid extracted from *Camptotheca acuminate*, acts as a potent antitumor agent by inhibiting the nuclear enzyme topoisomerase I. CPT inhibits the growth of a wide range of tumors [9,10]. However, the major drawbacks of the drug have always been water insolubility and lactone instability. The lactone ring in CPT plays an important role in the drug's biological activity but it exists in a pH-dependent equilibrium with an open ring carboxylate form (Fig. 1(A)). The lactone ring opens at physiological pH or above, making this drug much less active and highly toxic (such as myelosuppression, haemorrhagic cystitis and diarrhea), and precludes its clinical use.

In our previous study, CPT was successfully incorporated in poly(ethylene glycol)-poly(L-aspartate ester) block copolymer micelles with high incorporation efficiency by optimizing its preparation method and copolymer structure [11,12]. Polymers exhibiting 60–70% benzyl esterification of the aspartate chain yielded micelles that were stable in blood plasma [13]. In this report, the antitumor effects and biodistribution of CPT-loaded polymeric micelles were evaluated in mice bearing colon

* Corresponding author. Tel./fax: +81 3 5498 5048.
E-mail address: yoshie@hoshi.ac.jp (Y. Maitani).

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doi:10.1016/j.jconrel.2006.03.012

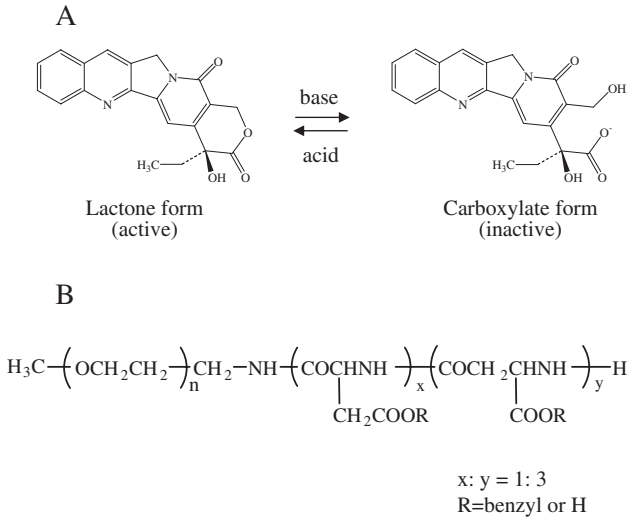

Fig. 1. pH-dependent equilibrium of camptothecin (A) and chemical structure of block copolymer (PEG-P(Asp(Bz-70))) (B).

26 solid tumors. Polymeric micelles loaded with CPT prolonged its blood circulation time and enhanced its antitumor effect due to tumor accumulation by the EPR effect.

2. Materials and methods

2.1. Preparation of CPT-loaded polymeric micelles

Poly(ethylene glycol)-poly(benzyl aspartate-70) block copolymer (PEG-P(Asp(Bz-70))) was synthesized by benzylesterification of poly(ethylene glycol)-poly(aspartic acid) as described previously [11]. PEG-P(Asp(Bz-70)) was composed of the poly(ethylene glycol) (PEG) block of molecular weight of 5000 determined by gel-permeation chromatography, and the p(Asp) block possessing 25 units of the aspartic acid residues on average determined by ^{1}H NMR spectroscopy. Seventy percent of the aspartic acid residue was esterified with benzyl group determined by ^{1}H NMR spectroscopy (Fig. 1(B)). From these three values, the molecular weight of PEG-P(Asp(Bz-70)) was calculated to be 9700. (s)-(+)-Camptothecin (CPT, Aldrich Chem. Co.) was incorporated into polymeric micelles by an evaporation method as reported previously [12], using 2 mg of CPT and 5 mg of PEG-P(Asp(Bz-70)). CPT incorporation efficiency in micelle to the drug in preparation was 63%. The average particle size was 191.8 ± 12.7 nm, measured by dynamic light scattering particle size analyzer (ELS-800, Otsuka Electronics, Osaka, Japan).

2.2. Antitumor activity

Antitumor activity of CPT-loaded polymeric micelles was evaluated with mouse bearing colon adenocarcinoma 26. The animal experiments were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals of Hoshi University. Colon 26 cells (1×10^4 cells/0.1 ml) were transplanted into CDF_1 female mice (5 weeks old, Sankyo Labo Service Corporation, Tokyo, Japan) subcutaneously, and drug injection was started when tumor volume reached approximately 100 mm^3. Drug was injected into a tail vein once or three times at a three day interval. 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) [14]. Tumor volume and body weight were measured for individual animals. Tumor volume was calculated as follows; volume $= \pi/6 \times LW^2$, where L is the long diameter and W is the short diameter. Percentage of tumor growth inhibition (T/C%) was calculated from relative tumor volume at day 8, following the equation: T/C% = 100 × (mean relative tumor volume of treated group) / (mean relative tumor volume of control group).

2.3. CPT biodistribution in tumor bearing mice

CPT biodistribution was evaluated in CDF_1 female mice (5 weeks old) subcutaneously transplanted by colon 26 cells (1×10^4 cells/0.1 ml) after tumor volume reached approximately 100 mm^3. CPT-loaded micelles and CPT solution were intravenously administered via lateral tail veins at a dose of 2.5 mg/kg. Twenty-four hours after injection, blood was collected with heparinized syringe and centrifuged to obtain the plasma. The tumor and major tissues were excised and homogenized in phosphate buffered saline (pH 7.4). For the determination of CPT, an aliquot of plasma or the tissue homogenate was acidified with the aqueous phosphoric acid (0.15 M) and then CPT was extracted with chloroform:methanol (4:1 volume ratio). After centrifugation of the mixture, 25 μl of the chloroform:methanol layer was directly analyzed by the HPLC system (Shimadzu Corp., Japan), using a Tosoh TSK-gel ODS-80Ts column (150 × 4.6 mm I.D., Tosoh Corp., Japan) and a fluorescence detector (excitation: 369 nm, emission: 426 nm). 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) at a flow rate 1.0 ml/min [15]. Standard curve with concentrations ranging from 25 ng/ml to 1.0 μg/ml of the drug exhibited good linearity with a correlation coefficient of 0.999.

3. Results and discussion

3.1. Antitumor effect of CPT-loaded polymeric micelles in colon 26 solid tumors

Antitumor effect of CPT-loaded polymeric micelles was evaluated in mice bearing colon 26 solid tumors (Fig. 2). In a preliminary study, murine weight loss was over 20% (three days after injection) and it took two days to recover from a single i.v. administration of 40 mg/kg CPT-loaded polymeric micelles to normal CDF_1 mouse (data not shown). This result suggested that 30 mg/kg would be the maximum dose for murine cancer treatment. The dose of CPT solution (1.5 mg/kg) was decided by its solubility (0.26 mg/ml) in the solvent used and tolerable body weight loss for treatment. Treatment with CPT solution showed tumor growth inhibition (T/C%) of 49.6% at day 8, whereas polymeric micelles treated at either 15 or 30 mg/kg of CPT were 27.5% and 18.5%, respectively (Fig. 2(A)). Furthermore, these polymeric preparations significantly inhibited tumor growth at

day 8 compared with control ($P<0.01$) without significant adverse effects, such as weight loss ($P>0.05$) (Fig. 2(B)).

When the total CPT dose was fixed at 30 mg/kg, a single i.v. injection of CPT-loaded polymeric micelles exhibited comparable inhibition of tumor growth to a triple injection at a dose of 10 mg/kg/day (T/C% of 42.1%) (Fig. 2 (C)). Cytotoxicity of CPT and other topoisomerase I inhibitors is S-phase specific and in vivo studies have suggested that multiple administration of CPT-derivatives were effective against tumors [16]. CPT conjugated with poly(L-glutamic acid) (PG-CPT) or *N*-(2-hydroxypropyl) methacrylamide (HPMA-CPT) needed frequent administration in order to be effective, even though enhanced accumulation was observed in the tumor [17–19]. Furthermore, at equivalent drug levels, repeated administration (40 mg/kg × 4, 4 day interval) of PG-CPT was more efficacious than a single bolus (160 mg/kg) [17]. This discrepancy may be due to a difference in the rate of release of free CPT from the polymeric carriers. Although CPT has been shown to release slowly from PG-CPT and HPMA-CPT [17,19], CPT-loaded polymeric micelles quickly shed nearly half of its load within 24 h, despite retardation by highly benzyl esterified polymer [12]. These findings suggest that the rapid bioavailability of free CPT from the polymeric micelles, relative to other polymeric carriers, may indeed improve antitumor activity subsequent to the passive accumulation of polymeric carriers in the tumor tissue.

3.2. Tumor accumulation of CPT-loaded polymeric micelles

The biodistribution profiles of CPT-loaded polymeric micelles and CPT solution were determined 24 h after i.v. injection of 2.5 mg/kg into mice bearing colon 26 tumor (Fig. 3). Blood

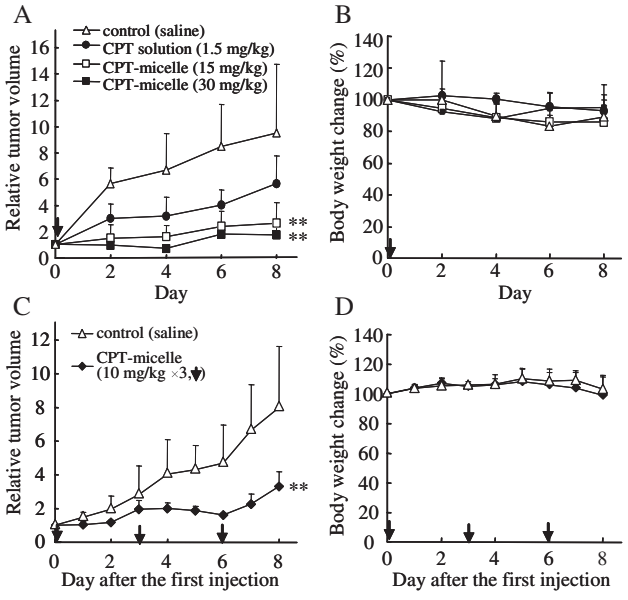

Fig. 2. Antitumor activity (A, C) and body weight change (B, D) after a single (A, B) and a triple (C, D) injection of CPT-loaded polymeric micelles in mice bearing colon 26 tumor. Arrows indicate the day of drug injections. Tumor volumes are plotted in ratios to the initial volume at day 0. Each value represents the mean ± S.D. ($n=4$–5). **: $P<0.01$, compared with control at day 8 (Scheffe's *F*-test).

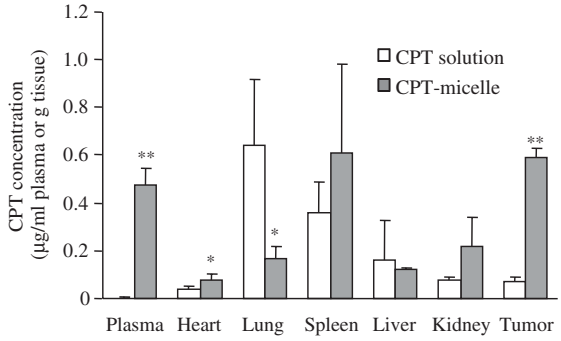

Fig. 3. CPT biodistribution in mice bearing colon 26 tumor 24 h after i.v. injection of CPT-loaded polymeric micelles and CPT solution at a dose of 2.5 mg/kg. Each value represents the mean ± S.D. ($n=3$). *: $P<0.05$, *: $P<0.01$, compared with CPT solution (Students' *t*-test).

plasma levels of CPT-loaded micelles (1.1% of injected dose) were approximately 150 times higher than CPT solution. Tumor accumulation of CPT-loaded polymeric micelles (approximately 1.3% of injected dose per g tissue) was nearly 8 times higher than CPT solution. Elevated pulmonary CPT levels by CPT solution may be due to embolization of lung capillaries arising from drug precipitation [14].

The pharmacokinetic (area under the plasma concentration–time curve, AUC) profile of CPT-loaded polymeric micelles was approximately 17 times higher than CPT solution when administered at a dose of 2.5 mg/kg in ddY mice [13]. Drug carriers with a prolonged circulation time are able to increase their accumulation in tumor tissues by the EPR effect and, consequently, improve antitumor activity. Furthermore, polymeric micelles could maintain CPT lactone form even in the presence of serum [12]. These characteristics suggest that polymeric micelles possess the ability to deliver large amounts of CPT, in its most active lactone form, to the tumor site by passive targeting with a long-circulating carrier.

4. Conclusion

Polymeric micelles increased the antitumor effects of camptothecin (CPT) in mice subcutaneously transplanted with a colon 26 tumor. The observed therapeutic efficacy of micelles is probably related to the extended circulation time. Passive accumulation of this preparation in tumor sites induced a similar level of significant tumor regression, whether from a single bolus of 30 mg/kg CPT or three repeated doses of 10 mg/kg. Polymeric micelle systems offer a stable and effective platform for cancer chemotherapy with camptothecin.

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