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Research paper

Preparation and antitumor characteristics of PLA/(PEG-PPG-PEG) nanoparticles loaded with camptothecin

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Abstract

Camptothecin (CPT)-loaded nanoparticles were prepared using poly(DL-lactic acid) (PLA) and poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) copolymer (PEG-PPG-PEG), and examined for particle characteristics, in vitro release, pharmacokinetics and efficacy. The preparative condition, in which the ratio of PLA/PEG-PPG-PEG/CPT was 35/35/4 (w/w/w) and organic solvent (dichloromethane) was evaporated from the emulsion at 18 °C, gave the nanoparticles with the diameter of approximately 230 nm, fairly high drug content (ca. 1.6% (w/w)) and stable entrapment of the drug, which were used for in vivo studies. After i.v. administration to normal rats, the nanoparticles showed slightly smaller *AUC* but much larger *MRT* as compared with CPT solution, and delivered the drug greatly to the surrounding tissues, in particular to the liver. When antitumor effect was examined by i.v. administration to mice bearing sarcoma 180 (S-180) solid tumor, the nanoparticles showed a significant suppression of tumor growth without body weight loss, and their effect was better than that of CPT solution. The PLA/PEG-PPG-PEG nanoparticles were considered potentially useful to enhance the efficacy of CPT, to which the high drug retention in the body and gradual drug release appeared to be importantly related.

Keywords: PLA/(PEG-PPG-PEG) nanoparticles; Camptothecin; In vitro release; Pharmacokinetics; Antitumor effect

1. Introduction

Camptothecin (CPT), a plant alkaloid isolated from *Camptotheca acuminata*, has been reported to be highly potent in vitro against various tumors by inhibiting the activity of DNA topoisomerase I [1–4]; however, it is not used clinically due to its poor water solubility and to the in vivo low efficacy and severe toxic side effects of its conventional dosage forms [3]. Therefore, its analogues have been produced actively in an attempt to improve CPT characteristics. One of the analogues, irinotecan hydrochloride (CPT-11), exhibits a broad and high antitumor effect in vivo, and is clinically available [5,6]. CPT-11 is regarded as a prodrug of 7-ethyl-10-hydroxycamptothecin (SN-38), being a semi-synthetic derivative of CPT [7–9]. CPT-11 is more than thousand times less effective in vitro than SN-38 and CPT, but is more effective in vivo, which is associated with their pharmacokinetic properties. Namely, SN-38 is eliminated more rapidly from the systemic circulation than CPT-11, while CPT-11 can supply SN-38 at an effective level for a long period [5,6]. Furthermore, SN-38 is more toxic than CPT-11.

As CPT and SN-38 are time-dependently effective [10,11], their prolonged supply is very useful to obtain a higher efficacy. In the previous study [12], CPT-11-loaded nanoparticles were prepared using poly(DL-lactic acid) (PLA) and poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) copolymer (PEG-PPG-PEG) in order to improve the efficacy of CPT-11. The CPT-11-loaded nanoparticles showed longer systemic retention of CPT-11 and greater effect against a solid

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tumor as compared with CPT-11 solution. One reason for the improved efficacy was considered to be enhanced permeability and retention (EPR) effect [13]; that is, nanoparticles with a diameter of some dozen – 400 nm and hydrophilic molecules on the surface tend to exhibit long plasma residence and accumulate more easily into diseased parts such as a solid tumor due to the more permeable blood capillaries and lack of lymphatic drainage, leading to better efficacy against a solid tumor. In addition, we reported that CPT-11-loaded PLA/PEG-PPG-PEG nanoparticles enhanced the in vivo effect of CPT-11 against murine M5076 liver metastasis [14]. This was probably because the nanoparticles enhanced the contact of the drug with the tumor cells. Furthermore, improved efficacy of CPT-11 was also reported in CPT-11-loaded liposomes displaying systemically long retention [15]. However, in those systems loaded with CPT-11, a fairly high dose of CPT-11 or frequent administration was required to obtain a high efficacy, and the improvement was not dramatic [12,14]. This is considered to be because CPT-11 itself is a prodrug of SN-38 and not an active agent. That is, targeting or prolonged supply of active agents such as SN-38 or CPT is considered critical to high enhancement of efficacy. In fact, recently, modification or encapsulation of active agents like CPT, not CPT-11, has been attempted actively; that is, liposomes [16], nanocrystalline suspensions [4], solid lipid nanoparticles [17], polymeric nanoparticles [18] and polymer-drug conjugates [19–21] have been produced to improve antitumor characteristics of CPT or related agents. It was demonstrated that these dosage forms were useful for the enhancement of active agents like CPT. In this study, preparation of PLA/PEG-PPG-PEG nanoparticles loaded with CPT, not CPT-11, was attempted, and the resultant nanoparticles were evaluated based on the in vitro characteristics, pharmacokinetic behaviors and antitumor effects.

2. Materials and methods

2.1. Materials

Poly(DL-lactic acid) (PLA) (MW 10,000) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) copolymer with MW 8400 and 80% (w/w) ethylene glycol (PEG-PPG-PEG) was obtained from Aldrich Chemical Company, Inc. (Milwaukee, USA). Camptothecin (CPT) was purchased from Sigma Chemical Co. (St. Louis, USA). All other chemicals were of reagent grade.

2.2. Animals and tumor

Male Wistar rats (6 weeks-old, 200 g) and male ddY mice (6–7 weeks-old, 30–35 g) were purchased from Tokyo Laboratory Animals Science Co. Ltd. (Tokyo, Japan), and bred on the breeding diet MF (Oriental Yeast, Tokyo, Japan) with water ad libitum at 23 ± 1 °C and a relative humidity of $60 \pm 5\%$. They were used for the experiments soon after purchase. The experimental protocol was approved by the Committee on Animal Research of Hoshi University, Japan. The animal experiments were performed in compliance with the Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University, Japan.

Sarcoma 180 (S-180) cells were kindly supplied by Cell Resource Center for Biomedical Research of Tohoku University (Japan) and used as tumor cells. The tumor cells were maintained by intraperitoneal transplantation of 1×10^6 cells suspended in Hanks' balanced solution (0.1 ml) per mouse. In antitumor experiments, S-180 cells (1×10^6 cells) suspended in Hanks' balanced solution (0.1 ml) were inoculated subcutaneously to each mouse at its axillary region.

2.3. Preparation of PLA/(PEG-PPG-PEG) nanoparticles loaded with CPT

Nanoparticles were prepared by O/W emulsification and subsequent evaporation of organic solvent. Two different methods (Methods 1 and 2) were used. Method 1: PLA (35 or 50 mg), PEG-PPG-PEG (21 or 30 mg) and CPT (1, 2 or 4 mg) were dissolved in 8 ml of dichloromethane, and 50 ml of water was added. The mixture was mixed vigorously with a vortex mixer for 30 s, and sonicated at 45 kHz (100 W) for 5 min to obtain the O/W emulsion. These operations were conducted at 25–30 °C. Then, the mixture was stirred at 25–30 °C for 2 h, and condensed with a rotary evaporator under reduced pressure at 35 °C for 1 h. The resultant suspension was separated by gel permeation chromatography (GPC) with a Sephadex G-50 column (2.6 cm in diameter × 15 cm in length) using water as the elution solvent to yield the nanoparticles. Method 2: PLA (35 mg), PEG-PPG-PEG (21 or 35 mg) and CPT (4 mg) were dissolved in 12 ml of dichloromethane. To the resultant solution, 50 ml of water of 37 °C was added, mixed vigorously with a vortex mixer for 30 s, and sonicated at 45 kHz (100 W) for 15 min to obtain the O/W emulsion. The emulsion was stirred at 18 °C for 2 h, and condensed with a rotary evaporator under reduced pressure at 35 °C for 1 h. Finally, GPC was performed for the resultant suspension in the same manner as above to obtain the nanoparticles. Table 1 summarizes preparative conditions for various nanoparticles.

2.4. HPLC assay

High performance liquid chromatography (HPLC) was used for the assay of CPT in the samples. A Shimadzu LC-6 A equipped with a Shimadzu RF-10AXL fluorescence detector (ex. 355 nm, em. 515 nm) and Shimdazu C-R7A plus chromatopac was used. A Waters Nova-Pack C18 (4 μm) column (4.6 mm in diameter × 150 mm in length) was used at 35 °C in a column oven. A mixture of methanol and water (11:9, v/v) with the pH adjusted to 5