Biomedicine & Pharmacotherapy 80 (2016) 162–172

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Improved tumor targeting and antitumor activity of camptothecin loaded solid lipid nanoparticles by preinjection of blank solid lipid nanoparticles

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ARTICLE INFO

Article history:
Received 13 February 2016
Received in revised form 17 March 2016
Accepted 17 March 2016

Keywords:
Camptothecin
Solid lipid nanoparticles
Reticuloendothelial system blocking
Tumor targeting
Pharmacokinetics
Antitumor activity

ABSTRACT

This study aimed to enhance the in vivo antitumor effects of camptothecin (CPT), a strong antitumor agent whose delivery is limited by poor aqueous solubility and instability of the active lactone form. CPT was loaded into sterically stabilized, solid lipid nanoparticles (CPT-SLNs) formulated for intravenous administration. The influence of preinjected blank SLNs on the tumor targeting, pharmacokinetics and antitumor activity of CPT-SLNs was investigated. The CPT-SLNs composed of trilaurin-based lipid matrix containing poloxamer188 and pegylated phospholipid as stabilizers were prepared by hot homogenization method and evaluated for in vitro characteristics and in vivo performance. The CPT-SLNs showed an in vitro long-term sustained release pattern and effectively protected the CPT lactone form from hydrolysis under physiological conditions. Notable tumor targeting and tumor growth inhibition were observed after intravenous administration of CPT-SLNs to mice with subcutaneous transplants of CT26 carcinoma cells. In pharmacokinetic studies in rats, CPT-SLNs markedly elevated plasma CPT level and prolonged blood circulation compared to free CPT. Nonetheless, high uptake of CPT-SLNs by reticuloendothelial system (RES)-rich tissues resulted in limited tumor targeting of CPT-SLNs and plasma CPT levels. Preinjection of blank SLNs before administration of CPT-SLNs to tumor-bearing mice substantially reduced the accumulation of CPT-SLNs in RES organs. This led to significantly enhanced tumor targeting, improved pharmacokinetic parameters and increased antitumor efficacy of CPT-SLNs. These results suggested that the in vivo antitumor effects of CPT-SLNs could be further enhanced by preinjection of blank SLNs. Therefore, CPT-SLNs with preinjected blank SLNs could be a potential approach for stable and effective CPT-based cancer therapy.

1. Introduction

Camptothecin (CPT) is a potent broad-spectrum anticancer drug that inhibits topoisomerase I during the S-phase of cell cycle [1–3]. CPT is poorly water soluble and its active lactone form rapidly hydrolyzes to the inactive carboxylate form under physiological conditions [4,5], limiting the delivery and clinical application of CPT in cancer therapy [6,7]. CPT also requires a prolonged schedule of multiple, low-dose administrations to show antitumor efficacy in humans [8]. Among various delivery approaches to improve the solubility and lactone ring stability of CPT, nanoparticulate delivery systems that incorporate CPT have been extensively investigated for their drug-loading capacity, controlled release and tumor-targeting ability [7,9,10]. In particular, solid lipid nanoparticles (SLNs) consisted of physiological lipids and biocompatible stabilizers have received considerable attention as injectable and targetable nanosized CPT carriers that have low cytotoxicity relative to polymeric nanoparticles [9,11,12]. In the previous researches with CPT-loaded SLNs, encapsulation of CPT into the lipid core matrix of SLNs solubilized the hydrophobic CPT molecules and protected them from rapid hydrolysis before their sustained release [13–15]. The nanosize and lipophilic nature of CPT-loaded SLNs resulted in their prolonged circulation in blood, which led to CPT accumulation in tumor tissues via the enhanced permeation and retention (EPR) effects and consequently improved the antitumor activity of CPT [13,16,17].

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http://dx.doi.org/10.1016/j.biopha.2016.03.018
0753-3322/

Nanoparticles in the bloodstream are usually recognized as foreign substances and quickly removed from blood by the reticuloendothelial system (RES) of macrophages, particularly liver Kupffer cells [11,18,19]. The rapid and extensive uptake of nanoparticles by the RES is problematic for the systemic delivery of drugs to non-RES tumors or tissues, although RES accumulation is useful for treating tumors or diseases in which RES-containing cells are the target [11,18,19]. Attempts to reduce the phagocytic uptake of nanoparticles and increase their blood circulation time have modified the nanoparticle size, surface hydrophobicity or charge [11,18,19]. The surface of nanoparticles has often been modified with hydrophilic polymers such as polyethylene glycol (PEG) or poloxamer [18,20–24]. The decreased uptake by the RES of surface modified (stealth) nanoparticles encapsulating antitumor agents can enhance the targeting of non-RES tumor tissues by leaky vascularization of longer-circulating nanoparticles [18,22,24]. However, even for stealth nanoparticles, considerable uptake by the RES occurs after long circulation times, limiting their ability to effectively target tumors [13,25,26]. Some PEGylated liposomes, polymeric nanoparticles, or SLNs also induce accelerated blood clearance and lose their long-circulating characteristics upon repeated administration [27–30]. Thus, other strategies besides surface modification are needed to reduce the RES clearance of nanoparticles carrying antitumor drugs to maximize tumor targeting and to improve antitumor efficacy with minimal adverse effects [10,31].

Rapid uptake of liposomes or lipid microemulsions intravenously administered to animals by the RES can be effectively inhibited by blocking the RES with blank colloidal carriers or RES blocking agents such as dextran sulfate, aminomannose-cholesterol, or latex particles [32–38]. Temporary, reversible RES blocking may cause the remainder of the injected drug-carrying particles in circulation to increase their accumulation at non-RES target sites; this procedure is referred to as inverse targeting [32,34–36,39]. Recently, inverse targeting was demonstrated by Liu et al. [40] by pretreating with intralipid that significantly decreased initial RES uptake of superparamagnetic iron-oxide nanoparticles. For applications with SLNs, the same principle could be applied to suppress RES function without permanent or irreversible impairment by predosing safe blocking agents. The initial RES accumulation of antitumor drug-loaded SLNs intravenously administered without pretreatment of blocking agents might have toxic or adverse effects in non-tumor RES-rich tissues or organs such as the liver or spleen [32,38]. It could be expected that blank, drug-free SLNs might be effective as nontoxic, transient RES blocking agents if they were composed of a biocompatible and biodegradable lipid matrix that could be readily cleared from the blood into the RES [32,38,39].

In this study, we postulated that the phagocytic clearance of CPT-loaded SLNs by the RES would be effectively reduced by a transient RES blocking with preinjection of blank SLNs. We further hypothesized that the RES blocking by blank SLNs would result in prolonged blood circulation of CPT-loaded SLNs with enhanced tumor targeting. To investigate these hypotheses, we studied the influence of preinjected blank SLNs on in vivo tissue distribution, tumor targeting, pharmacokinetics and antitumor activity of sterically stabilized CPT-loaded SLNs (CPT-SLNs) designed using physiologically safe components. The stealth CPT-SLNs were employed for a combination effect of RES avoidance with preinjection of blank SLNs. CPT-SLNs were physicochemically characterized and examined for in vitro cytotoxicity, release and stability. In vivo performance studies on the CPT-SLNs were performed in the presence and absence of preinjected blank SLNs.

2. Materials and methods

2.1. Materials

CPT, trilaurin (TL), poloxamer188, *n*-hexyl-*p*-hydroxybenzoate and 3-(4,5-dimethylthazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Egg yolk phosphatidylcholine (ePC) and distearoyl-phosphatidyl-ethanolamine-*n*-poly(ethylene glycol)$_{2000}$ (PEG_{2000}-PE) were from Avanti Polar Lipid Inc. (Alabaster, AL, USA). The purity degree of all lipids was over 99%. All solvents were of high performance liquid chromatography (HPLC) grade and other chemicals were of highest reagent grade.

2.2. Animals and cell lines

Male BALB/c mice (6–8 weeks old, 20–25 g) and male Sprague-Dawley (SD) rats (8 weeks old, 280–300 g) were purchased from Orient Bio (Sungnam, Korea). All animal care and procedures were conducted in accordance with the ARRIVE guidelines, and the animal study protocol was approved (Approval No. 2014-047) by the Institutional Animal Care and Use Committee on the Sungsim Campus of the Catholic University of Korea (Bucheon, Korea). The mice and rats were housed in an air-conditioned room with free access to water and fasted for 12 h before drug administration. The CT26 human colon carcinoma cell line was obtained from Korean Cell Line Bank (Seoul, Korea). Roswell Park Memorial Institute Medium (RPMI 1640), pH 7.4 phosphate-buffered saline (PBS), fetal bovine serum (FBS) and other materials for cell culture were from Gibco Life Technologies (Rockville, MD, USA).

2.3. Preparation of CPT-SLNs

CPT-SLNs were prepared by hot melt homogenization method, as described previously [41,42]. CPT (0.1 g) was dispensed into a mixture of TL (0.9 g), ePC (0.25 g), DSPE-PEG_{2000} (0.25 g) and poloxamer188 (1.0 g) melted in a pear-shaped glass tube maintained at 65 °C in a bath-type Branson 3210R-DTH ultrasonic sonicator (Danbury, CT, USA), and homogenously mixed by sonicating for 1 h to completely dissolve CPT. Glycerin (2.5 g) as an isotonic agent was dissolved in 95 mL of distilled water and preheated to 65 °C. The entire water phase was added to the tube containing the lipid phase. The resultant mixture was sonicated for 3 h to yield a hot, milky, crude emulsion. The emulsion was homogenized for 10 cycles at 65–70 °C with 100 MPa using an Avestin Emulsiflex® EF-B3 high pressure homogenizer (Ottawa, ON, Canada) wired with Barnstead Thermolyne® heating tape (Dubuque, IA, USA). The fine emulsion was rapidly dipped into liquid nitrogen and thawed in a water-bath at room temperature to make a CPT-SLN suspension, followed by mixing with the cryoprotectants, mannitol (0.5 g) and dextrose (0.25 g). The final suspension was immediately cooled at −70 °C, and lyophilized at −25 °C under a vacuum (5 mm Hg) for 48 h using an Ilshin Bondiro freeze-dryer (Yangju, Korea). The soft, crumbly lyophilate was gently pulverized with a rubber spatula below 40% relative humidity. Lyophilized CPT-SLNs were easily reconstituted in distilled water. Blank SLNs were prepared in the absence of CPT only by employing the same components and procedures as those used for preparing CPT-SLNs.

2.4. Characterization of CPT-SLNs

The morphology of CPT-SLNs dispersed in water was examined using a Carl Zeiss LIBRA 120 energy-filtered transmission electron