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Selective tissue distribution and long circulation endowed by paclitaxel loaded PEGylated poly(ε-caprolactone-co-L-lactide) micelles leading to improved anti-tumor effects and low systematic toxicity

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ABSTRACT

High tumor targeting and sustained drug concentration are key points for successful anti-tumor therapy, however, it is a challenging task. In this work, a novel micelle formulation of paclitaxel (PTX) has been prepared for the purpose of prolonging the blood circulation time as well as improving the accumulation of the drug within the tumor tissue. PEGylated P(CL-co-LLA) (poly(ε-caprolactone-co-L-lactide)) micelles containing PTX were prepared by solid dispersion–sonication method with a higher drug-loading efficiency and encapsulation ratio (28.4% and 94.7%, respectively). Pharmacokinetic study revealed that the drug-loading micelles exhibited a higher AUC values and a prolonged residence time of drug in the blood circulation than those of PTX injection. As demonstrated by tissue distribution and anti-tumor study in S180 tumor-bearing mice, the PEG-P(CL-co-LLA)/PTX micelles displayed modified tissue distribution of PTX and increased accumulation of PTX in tumor, therefore, resulted in anti-tumor effects enhancement and drug concentration in the normal tissues reduction. Furthermore, the preliminary safety tests were performed by measuring the body weight, histopathology, blood cell counts and clinical chemistry parameters, and the results showed no subacute toxicity to hematological system, major organs or tissues in mice. Taken together, our valuation shows that PEG-P(CL-co-LLA) micelles is a potential drug delivery system of PTX for the effective treatment of the tumor and systematic toxicity reduction, thus, the micellar formulation can provide a useful alternative dosage form for i.v. administration of PTX.
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1. Introduction

Paclitaxel (PTX), one of the most successful anticancer drugs, is the first class of microtubule stabilizing agents, and has been demonstrated significant antitumor activity in clinical trials against a broad range of solid tumors, especially against non-small-cell lung cancer, metastatic breast cancer and refractory ovarian cancer (Kim et al., 2001; Singla et al., 2002). However, because of low therapeutic index and poor aqueous solubility of approximately 1 μg/mL of PTX, the generally used commercial preparation of PTX is Taxol, a concentrated solution containing 6 mg PTX/mL of Cremophor EL (polyoxyl 35 castor oil) and dehydrated alcohol (1:1, v/v), which is diluted 5–20-fold in normal saline or dextrose solution before administration (Kim et al., 2001). Unfortunately, serious side effects have been reported, such as hypersensitivity, neurotoxicity, nephrotoxicity, endothelial and vascular muscles causing vasodilatation, labored breathing and lethargy attributable to intravenous administration of the current Cremophor EL-based formulation (Weiss et al., 1990). For this reason, the extensive clinical application of this drug is extremely limited (Singla et al., 2002).

Due to these problems, there is a need for the development of alternate formulation of PTX with good aqueous solubility, targeting delivery to tumor tissues and the ability to reduce side effects. Accordingly, a number of alternative formulations were investigated for solubilization of PTX, including nanoparticles, liposomes, microspheres, PTX-polymer conjugates, dendritic polymers, implants, water-soluble prodrugs, *etc.* (Kim et al., 2001; Singla et al., 2002; Lee et al., 2003; Zhu et al., 2010; Gong et al., 2012a,b). These vehicles employed have shown much promise to replace the Cremophor EL-based vehicle for PTX delivery. Among these approaches, copolymeric micelles have drawn much attention due to their great flexibility in tuning drug solubility, micelle size, targeted delivery and stability (Yoncheva et al., 2012).

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In the last decade, biodegradable polymers such as poly(caprolactone) (PCL) and poly(lactide) (PLA) are widely studied for the preparation of nanoparticles. Particularly, PEGylated amphiphilic polymers such as PEG–PCL (Wang et al., 2011; Gong et al., 2013) and PEG–PLA (Venkatraman et al., 2005; Li et al., 2010; Xia et al., 2012) have attracted attention in drug delivery. In these block copolymers, PEGblock forms the outer shell of nanoparticles whereas PCL or PLA, due their hydrophobic nature, forms nanoparticle core. PCL is hydrophobic biodegradable polyester which provides good encapsulation efficiency to lipophilic drugs *via* hydrophobic interactions. However, it exhibits very slow degradation because of its hydrophobic and crystalline nature (Li et al., 2000). In addition, lipophilic drugs often get trapped in the hydrophobic core of the nanoparticles and achieve no or limited release in the later time intervals. Therefore, there is a need of optimized block copolymers that can provide continued drug release. Literature reported that crystallinity of PCL can be modulated by conjugating with PLA segment (Ge et al., 2000; Chen et al., 2003). PLA is a rather brittle polymer with a low degradation rate, and compounding with PCL is frequently employed to improve mechanical properties (Renouf-Glauser et al., 2005; Richter et al., 2010a,b). What's more, copolymerization of CL and LLA can take advantage of the degradability of PLA and the permeability to drugs of PCL. The drug release can be optimized by adjusting the ratio of CL to LA and may be further optimized by changing the molecular weight of the copolymer. Considering these facts, we copolymerized CL and LLA in the presence of methoxypoly(ethylene glycol) (MPEG) as initiator which resulted in copolymers with lower crystallinity, higher water solubility and higher compatibility with PTX (Li et al., 2013).

In our previous work, new amphiphilic block copolymers, methoxypoly(ethylene glycol)-b-poly(ε-caprolactone-co-L-lactide) [PEG-P(CL-co-LLA)] (Li et al., 2013) copolymers were first designed and synthesized to obtain excellent paclitaxel micellar carriers. The obtained copolymers were characterized and their water solubility, critical micelle concentration (CMC) and crystallinity were investigated in detail. In this study, PEG-P(CL-co-LLA) micelle were used as a biodegradable drug carrier for encapsulation of PTX. We prepared stealth micelles loaded with PTX. The *in vitro* cytotoxicity, *in vivo* pharmacokinetic behaviors, tissue distribution and *in vivo* anti-tumor effect of the micelles were investigated and compared with the PTX injection to exploit the possibility of prolonging the blood circulation time, passive targeting and sustained drug release in tumor site. Furthermore, the safety of PEG-P(CL-co-LLA)/PTX micelles following intravenous injection was assessed using healthy mice.

2. Materials and methods

2.1. Materials

Methoxypoly(ethylene glycol)-b-poly(ε-caprolactone-co-L-lactide) [MPEG-P(CL-co-LLA)] (Mn = 10,280 (GPC), composition (wt%) MPEG:ε-CL:LLA = 44:15:41) copolymer was synthesized in our lab as described previously (Li et al., 2013). Paclitaxel (analytical grade, Yunnan Ziyun Biotechnology Co., Ltd., China). PTX injection (Anzatax Injection Concentrate, 30 mg/5 mL) was produced by Beijing Shiqiao Biological Pharmaceutical Co., Ltd. (Beijing, China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and dimethyl sulfoxide (DMSO) were obtained from Sigma Co., Ltd. (USA). Penicillin–streptomycin, folate-free RPMI-1640 medium (R1145), fetal bovine serum (FBS), 0.25% (w/v) trypsin–0.03% (w/v) EDTA solution and Phosphate buffer solution (PBS) were purchased from Gibco BRL (Gaithersburg, MD, USA). Kunming mice transplanted with S180 cells in the intraperitoneal cavity was kindly donated by Shanghai Institute of Pharmaceutical Industry. Water was purified by distillation, deionization, and reverse osmosis (Milli-Q plus). All reagents were analytical grades and used without further purification.

Breast cancer cell line MCF-7, human ovarian carcinoma Skov-3 cells and Mice sarcoma 180 (S180) cells were kindly donated by the Department of Pharmacology, Shandong University. The cell lines were grown using 75 cm^3 flasks in a humidified 5% CO_2/95% atmosphere incubator at 37 °C, in RPMI-1640 medium, supplemented with 10% fetal bovine medium (FBS) and 100 U/mL penicillin and 100 μg/mL streptomycin. Cells grown to confluence were subcultured every other day after trypsinized with 0.25% trypsin–EDTA and diluted (1/3) in fresh growth medium.

2.2. Preparation of PTX-loaded PEG-P(CL-co-LLA) micelles

Paclitaxel-loaded PEG-P(CL-co-LLA) micelles were prepared by a solid dispersion method (Wang et al., 2011). In brief, 30 mg of PTX was mixed with 70 mg of PEG-P(CL-co-LLA) copolymer in 3 mL of acetone, followed by sonication at room temperature for 30 min. Then, the solution was evaporated on a rotary evaporator under reduced pressure at 60 °C to obtain a homogenous coevaporation PTX/copolymer matrix. After cooling down, 10 mL of water was added into the resulting matrix and the solution was immediately sonicated three times using a probe-type sonifier (Soniprep 150, Sanyo) under the ice bath condition. The pulse was turned off for 2 s with the interval of 4 s to inhibit increase in temperature. Then, the mixture was centrifuged at 4000 rpm for 15 min to remove the unloaded drugs and the supernatant was passed through membrane filter (pore size: 0.45 μm, Millipore), followed by lyophilization or stored at 4 °C for use.

2.3. Evaluation of particle size and zeta potential

The average particle size and size distribution of PTX-loaded micelles were determined using a Zetasizer Nano ZS (Malvern Instruments Ltd., UK). The polydispersity index range was comprised between 0 and 1. Zeta potentials of micelles were measured with Zetasizer NanoZS/ZEN3600 (Malvern Instruments, Herrenberg, Germany). The concentration of micelles was kept constant at 3 mg/mL. Analyses were typically performed at a temperature of 25 °C. Each sample was determined in triplicate.

2.4. Transmission electron microscopy (TEM) observation

The morphology and size of micelles were observed using a transmission electron microscope (TEM) (JEM-2100F, JEOL, Japan). A drop of sample solution (3 mg/mL) was placed onto a 300-mesh copper grid coated with carbon. After 2 min, the grid was tapped with a filter paper to remove surface water, followed by air drying and negatively stained with 2% phosphotungstic acid for 30 s. The grid was dried at room temperature and then observed by TEM.

2.5. Determination of drug-loading parameters

200 μL of PTX-loaded micelles were first dissolved in 10 mL of acetonitrile. Followed by sonication for 10 min, the solution was centrifuged at 10,000 rpm for 10 min and the supernatant was filtered with a 0.2 μm syringe filter and analyzed its concentration using high-performance liquid chromatography (HPLC) equipped with a LC-10ADvp pump, a SPD-10Avp UV–vis detector (Waters, USA). Sample solution was injected at least three times at a volume of 20 μL into a Dikma-ODS C18 column (150 mm × 4.60 mm, 5 μm) preceded by a C_{18} guard column (Dikma, China). The mobile phase was a mixture of water and acetonitrile in the volume ratio of

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