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Deoxycholic acid-modified chitooligosaccharide/mPEG-PDLLA mixed micelles loaded with paclitaxel for enhanced antitumor efficacy

Chengjun Jiang [a,1], Hangxiang Wang [b,1,*], Xiaomin Zhang [c], Zhibin Sun [c], Feng Wang [d], Jun Cheng [b], Haiyang Xie [b], Bo Yu [c,*], Lin Zhou [b,*]

[a] Department of Chemical and Biological Engineering, Zhejiang University of Science & Technology, Hangzhou, Zhejiang, PR China
[b] First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, Zhejiang, PR China
[c] Hangzhou PushiKang Biotechnology Co., Ltd., Zhejiang, PR China
[d] College of Life and Environmental Science, Shanghai Normal University, Shanghai, PR China

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ABSTRACT

Poly(ethylene glycol) (PEG) as a block in polymeric micelles can prolong circulation life and reduce systemic clearance but decrease the cellular uptake. To overcome this limitation, a mixed micelle composed of deoxycholic acid-modified chitooligosaccharide (COS-DOCA) and methoxy poly(ethylene glycol)-polylactide copolymer (mPEG-PDLLA) was designed to load paclitaxel (PTX). The PTX-loaded mixed micelles was prepared by nanoprecipitation method with high drug-loading efficiency of 8.03% and encapsulation efficiency of 97.09% as well as small size (~40 nm) and narrow size distribution. COS-DOCA/mPEG-PDLLA mixed micelles exhibited the sustained release property. Due to the positive charge and bioadhesive property of COS-DOCA, the cellular uptake of PTX in mixed micelles was higher in cancer cells but lower in macrophage cells compared to the mPEG-PDLLA micelles. The systemic toxicity of PTX in mixed micelles was much lower than Taxol using zebrafish as a toxicological model. Furthermore, the PTX-loaded COS-DOCA/mPEG-PDLLA mixed micelles can prolong the blood circulation time of PTX and enhance the antitumor efficacy in A549 lung xenograft model. Our findings indicate that COS-DOCA/mPEG-PDLLA mixed micelles could be a potential vehicle for enhanced delivery of anticancer drugs.

1. Introduction

Paclitaxel (PTX) is one of the most important first-line chemotherapeutic agents against a wide range of cancers (Rowinsky and Donehower, 1995). However, due to the poor aqueous solubility, its clinical application is limited. To improve the aqueous solubility of PTX, the commercial formulation Taxol® contains a high concentration of Cremophor EL, which leads to serious side effects, such as hypersensitivity, neurotoxicity and nephrotoxicity (Hennenfent and Govindan, 2006; Sharma et al., 1995; Weiss et al., 1990). With the goal of replacing Cremophor EL and improving delivery efficacy, various drug delivery systems have been developed, including nanoparticles (Wang et al., 2013; Win and Feng, 2006), liposomes (Koudelka and Turanek, 2012; Yoshizawa et al., 2011; Zhang et al., 2005), micelles (Gaucher et al., 2010; Kim et al., 2001a) and other formulations (Liu et al., 2010; Mora-Huertas et al., 2010).

Polymeric micelles formed by amphiphilic block copolymers have been extensively studied as an attractive nanocarrier system (Gong et al., 2012; Torchilin, 2007). Polymeric micelles may improve drug solubilization, control drug release, reduce non-specific uptake by reticuloendothelial system (RES), and increase tumor targeting by the enhanced permeability and retention (EPR) effect (Gaucher et al., 2010; Gong et al., 2012; Maeda, 2012). Because of their low critical micelle concentration, amphiphilic copolymer can self assemble to form nanosized aggregates that possess a core-shell structure. Poorly soluble drugs can be effectively incorporated into the hydrophobic core. Furthermore, the micelle corona formed by hydrophilic polymer blocks such as poly(ethylene glycol) (PEG) provides longevity in vivo by reducing their opsonization and clearance by RES (Chen et al., 2008; Fontana et al., 2001; Miller et al., 2012). Methoxy PEG-polylactide copolymer (mPEG-PDLLA) is a typical amphiphilic block copolymer, which is widely used to encapsulate anticancer drugs with a mesoscopic size range about 20–50 nm (Chen et al., 2008; Kim et al., 2001a). However, PEG can interfere with interactions between polymeric micellar nanocarrier systems and target cells, thus negatively influencing the therapeutic outcomes.

* Corresponding author. Tel.: +86 571 88981338.
E-mail address: wanghx@zju.edu.cn (H. Wang).
[1] These authors contributed equally to this work.

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Recently, a large number of studies have been focused on mixed polymeric micelles that can combine the prominent advantages of different types of single polymeric micelles (Kulthe et al., 2011; Mu et al., 2010; Saxena and Hussain, 2012; Zhang et al., 2011). The loading content and stability of drug in mixed micelles can be significantly improved compared with single copolymer micelles. More importantly, the release rate and function of micelles can be desirable to modify by forming mixed micelles (Harmon et al., 2011; Wang et al., 2005). For example, Wei et al. have reported the Pluronic mixed micelles composed of Pluronic P123 and F127 to encapsulate PTX. Compared to Taxol, the mixed polymeric micelles effectively enhanced the in vitro cytotoxicity of PTX and greatly increased the blood circulation time. It demonstrated the enhancement of the antitumor efficacy in A-549 lung tumor model (Zhang et al., 2011).

Chitooligosaccharide (COS) is an abundant natural polysaccharide composed of randomly distributed D-glucosamine and *N*-acetyl-D-glucosamine units (Garcia-Fuentes and Alonso, 2012; Park et al., 2010). The unique characteristics such as biodegradability, biocompatibility, hydrosolubility and positive charge make it ideal as nanometric drug delivery materials (Hu et al., 2002; Hyung Park et al., 2006; Kim et al., 2001b). Moreover, COS has widely been used as a coating material to tailor the surface charge and bioadhesive property in pharmaceutical and biomedical fields (Kim and Rajapakse, 2005). Coating of cationic water-soluble chitooligosaccharide onto poly (D,L lactide-co-glycolide) (PLGA) particle surface has been demonstrated the macrophage uptake reduction and the circulation half-life extension (Amoozgar et al., 2013; Sheng et al., 2009). The primary hydroxyl and amine groups of chitooligosaccharide allow for further chemical modification to control its physical properties. The hydrophobic conjugated chitooligosaccharide may form self-assembled nanoparticles that can encapsulate a large number of hydrophobic drugs such as PTX and a variety of hydrophobic moieties have been reported to develop amphiphilic chitooligosaccharide derivatives, including bile acids (e.g., cholic acid, deoxycholic acid and 5β-cholanic acid,) and fatty acids (e.g., stearic acid and oleic acid) (Garcia-Fuentes and Alonso, 2012; Hu et al., 2002; Hyung Park et al., 2006; Kim et al., 2001b; Park et al., 2010).

It is well-known that the positive charge can usually enhance the endocytosis of various nanoparticles by cells (Amoozgar et al., 2013; Harmon et al., 2011; He et al., 2010; Wang et al., 2005; Yim et al., 2013). In this work, the objective was to develop a new mixed polymeric micellar formulation comprised of PEG-PDLLA and COS for enhanced antitumor efficacy of PTX. COS was first modified by coupling with deoxycholic acid (DOCA). The DOCA modified chitooligosaccharide(COS-DOCA) was amphiphilic and desired to form the mixed micells with mPEG-PDLLA. The PTX-loaded mixed micelles (PTX-M) were prepared by nanoprecipitation method. The particle size, morphology, in vitro release profile and cytotoxicity were investigated. Compared with Taxol® and single mPEG-PDLLA micelle, pharmacokinetics, toxic effects, and the antitumor efficacy of PTX loaded mixed micelles were evaluated.

2. Materials and methods

2.1. Materials

Paclitaxel was purchased from Knowshine Pharmachemicals Inc. (Shanghai, China). Cremophor-based paclitaxel injection (Taxol) was ordered from Yangtze River Pharmaceutical (Group) Co., Ltd. (Jiangsu, China). Deoxycholic acid (DOCA), chitooligosaccharide (COS) (Mn = 5000), *N*-hydroxysuccinimide (NHS), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and 3-(4, 5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) were purchased from Sigma–Aldrich (Shanghai local agent, China). Monomethoxy poly(ethylene glycol)-block-poly(D,L-lactide) (PEG-PDLLA) (PEG Mw = 5000 Da, PDLLA Mw = 10,000 Da) was purchased from Advanced Polymer Materials Inc. (Montreal, Canada). Penicillin-streptomycin, RPMI1640, fetal bovine serum (FBS) and 0.25% (w/v) trypsine 0.03% (w/v) EDTA solution were purchased from Hycolon (USA). All other reagents and buffer solution components were of analytical grade.

HeLa cell line, A549 cell line, MGC-803 cell line and J774A.1 cell line were obtained from the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). Culture plates and dishes were purchased from Corning Inc. (NY, USA).

Male Sprague-Dawley (SD) rats (250 ± 20 g) and female BALB/c nude mice (20 ± 2 g), supplied by Shanghai SLAC laboratory animal Co., Ltd. (Shanghai, China) and kept under SPF conditions.

2.2. Synthesis of COS-DOCA

COS-DOCA was synthesized by a conjugation reaction between amino-groups of COS and carboxyl groups of DOCA using EDC as a coupling agent (Hyung Park et al., 2006; Kim et al., 2001b). In brief, COS-DOCA was obtained as follows: 160 mg COS (Mn = 5000), 80 mg DOCA 194.7 mg EDC and 116.3 mg NHS were added to 100 mL round-bottomed flask and dissolved with 40 mL methanol/H_2O (1:1, v/v). Under stirring, the reaction mixture was maintained at 30 °C in a water bath. After 30 h, the solution was transferred into a dialysis bag (MWCO 3500) and dialyzed against doubled distilled water for 24 h to remove the unreacted substances. The final product was frozen and dried in a vacuum. The products of synthesized were determine by Fourier Transform Infrared spectroscopy (FT-IR). These dried products were mixed with KBr and pressed to the plate for measurements. FT-IR spectra were recorded on an FT-IR spectrometer (Bio-Rad FTS-6000). The conjugation of DOCA moiety to COS chain was further confirmed by ^{1}H NMR and gel permeation chromatography (GPC) measurements.

2.3. Preparation of PTX-loaded mixed micelles

PTX-loaded mixed micelles were prepared by a nanoprecipitation method. 2 mg of PTX powder and 20 mg mPEG-PDLLA were dissolved into 5 mL acetone. 2 mg freeze-dried COS-DOCA powder was also weighed into 25 mL beaker and dissolved in 11 mL H_2O. Under magnetic stirring, 5 mL acetone solution was dropped into 5 mL COS-DOCA solution at the speed of 60 mL/h. The available mixture was evaporated by rotary vacuum evaporation to remove the organic solvents at 37 °C. After evaporation, the PTX-loaded mixed micelles were obtained.

2.4. Characterization of PTX-loaded mixed micelles

Micelles were diluted 3-fold with water. The particle sizes of PTX-loaded mixed micelles were measured by dynamic light scattering (DLS) using Zetasizer (Malvern, UK) at 25 °C. The detection range was from 2 to 5000 nm. Each sample was analyzed

in triplicate. The morphology of samples was observed with transmission electron microscope (TEM) (JEM-2010; JEOL, Japan). A drop of sample after dilution was placed onto a carbon-coated copper grid to form a thin liquid film. The films on the grid were negatively stained with 0.1% (w/v) phosphotungstic acids. After excess solution was removed, the sample was air-dried at room temperature.

Drug-loading (DL%) and encapsulation efficiency (EE%) were calculated by the following equations.