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CPT loaded nanoparticles based on beta-cyclodextrin-grafted poly(ethylene glycol)/poly (L-glutamic acid) diblock copolymer and their inclusion complexes with CPT

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ABSTRACT

This research is aimed to develop a nanoparticle delivery system based on β-cyclodextrin-grafted diblock copolymer and camptothecin (CPT) inclusion complexes with the purpose of enhancing the stability of CPT in aqueous media. Firstly, mPEG-PBLG diblock copolymer was synthesized by the ring-opening polymerization of γ-benzyl-L-glutamate-N-carboxyanhydride (BLG-NCA) initiated with amine terminated poly (ethylene glycol) monomethyl ether (mPEG-NH_2). After removal of benzyl groups, mono-6-amine-β-cyclodextrin (EDA-β-CD) units were coupled to the carboxyl groups of the copolymer as side groups to produce the host macromolecule mPEG-PLG(CD). The highly hydrophobic anticancer drug CPT was employed as the guest component which could be included into the host macromolecule to form supramolecular inclusion-complex mPEG-PLG(CPT@CD). Interestingly, the supramolecular complexes were able to form spherical nanoparticles with an average size of 98 nm in aqueous media confirmed by dynamic light scattering (DLS) and transmission electron microscopy (TEM), suggesting their passive targeting potential to tumor tissue. Due to the protection effect from a dual lock system (the environment of nanoparticles and the exterior surface of β-CD), the active lactone ring of CPT showed remarkably enhanced stability against hydrolysis under physiological condition. The release profile of the mPEG-PLG(CPT@CD) nanoparticles in PBS buffer was found to be gradual and sustaining. It was worthy to note that the release could be accelerated by addition of adamantane carboxylate (ADC) as competitive guest compound, demonstrating the chemically stimulated release behavior of the nanoparticles. Compared with free CPT, the mPEG-PLG(CPT@CD) nanoparticles displayed essentially decreased cytotoxicity against MCF-7 cell line in 24 h because of a sustained release profile of CPT from the nanoparticles, moreover, the carrier mPEG-PLG(CD) itself showed almost no cytotoxicity, indicating its great potential as tumor tissue targeted drug delivery system.

1. Introduction

Camptothecin (CPT), a pentacyclic alkaloid isolated from the Chinese tree *Camptotheca acuminate*, exhibits a broad-spectrum antitumor activity against lung, prostate, breast, colon, stomach, bladder, ovarian and melanoma cancers [1]. Nonetheless, just as many other anticancer drugs, the clinical efficacy of CPT are significantly restricted by its poor solubility in aqueous media and highly toxic side effects, such as myelosuppression, nausea and vomiting [2,3]. It has been confirmed that the severe toxicity of CPT mainly stems from its carboxylate form [4]. As is known, CPT has a pH-dependent equilibrium between its active lactone ring form and an inactive carboxylate form in aqueous solution [5]. At pH above 7, the closed lactone ring form, which is an essential functional group for the antitumor activity of CPT, would be easily hydrolyzed into ring-open carboxylate form, resulting in the heavy loss of biological activity and unexpected toxicity. So how to protect the lactone form of CPT from being hydrolyzed under physiological condition becomes a major concern by researchers [6]. Numerous studies have focused on designing drug delivery carriers such as liposomes, nanocrystalline suspensions and polymeric micelle systems to overcome those drawbacks associated with CPT and its derivatives [7–9]. Among all of these approaches, the polymeric nanoparticular systems are recognized as the most promising

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drug carriers for prolonged the circulation time in blood, reduced drug toxic side effects, prevented drug degradation, controlled drug release and achieved specific targeting [10–12].

Cyclodextrins (CDs) are a family of cyclic oligomers composed of glucopyranose units linked by α-1, 4 glycosidic bonds [13]. CDs are characteristic of a hydrophilic exterior surface and hydrophobic interior cavity, which are capable of interacting with a large variety of guest molecules to form noncovalent inclusion complexes [14]. Recently, this inclusion interaction has been successfully employed as the driving force to build supramolecular polymers as well as supramolecular assemblies in aqueous media [15,16]. The new strategy was first proposed by Jiang and co-workers, who have fabricated micelles and hollow spheres from several abundant β-CD contained host polymers and a range of guest components [17–19]. These nanoparticles showed great potential as the delivery carriers of drugs. So in this study, we developed a mPEG-PLG (CPT@CD) nanoparticular system to enhance the stability of CPT. The physicochemical properties, such as critical aggregation concentration (CAC), morphology, size distribution, *in vitro* drug accumulative release from the mPEG-PLG (CPT@CD) nanoparticles and the cytotoxicity against MCF-7 cell line were investigated in details.

2. Materials and methods

2.1. Materials

Gamma-benzyl-L-glutamate-N-carboxyanhydride (BLG-NCA) was purchased from ISOCHEM-SNPE (Paris, France) and used as received. Ethanol, acetone, acetonitrile, ethylenediamine (EDA), dichloromethane (DCM), dimethyl sulfoxide (DMSO), N, N-dimethylformamide (DMF), triethylamine (Et_3N), and dimethylamino pyridine (DMAP) were purchased from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China. DMF and DMSO was dried with 4 Å molecular sieves and redistilled before use. Beta-cyclodextrin (β-CD) was acquired from Aladdin, Shanghai, China. CPT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), poly (ethylene glycol) monomethyl ether (mPEG, Mw: 5000 Da) were obtained from Sigma–Aldrich, Shanghai, China. Alpha-methoxy-ω-amino-poly (ethylene glycol) (mPEG-NH_2) was synthesized according to the literature [20].

2.2. Cell line and culture

Human breast cancer cells line MCF-7 was supplied by Institute of Biochemistry & Cell Biology, Chinese Academy of Sciences. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, CA, USA) supplemented with 1% L-glutamine, 10% fetal bovine serum (FBS, HyClone, Logan, UT), streptomycin at 100 μg/mL, and penicillin at 100 U/mL. Cells were incubated at 37 °C in humidified 5% CO_2 atmosphere. Cells were splited by using 0.25% trypsin/EDTA solution when almost confluent.

2.3. Synthesis of mPEG-PLGA

Firstly, mPEG-PBLG was prepared according to literature [21]. Briefly, 0.40 g of mPEG-NH_2 (0.07 mmol) and 0.73 g (2.8 mmol) of BLG-NCA were dissolved in 15 mL of anhydrous CH_2Cl_2. The reaction mixture was stirred under N_2 at room temperature for 76 h, and then poured into a large amount of cold diethyl ether. The precipitate was collected by filtration, and dried in a vacuum to yield 1.28 g (85%) of product.

To remove the benzyl protecting group [22], mPEG-PBLG (1 g) was dissolved in 12 mL of trifluoroacetic acid (TFA) under nitrogen atmosphere at 0 °C, then 1.2 mL of trifluoromethanesulfonic acid (TFMSA) and 1.4 mL of thioanisole was added dropwise. The reaction mixture was gently stirred at 0 °C for 1 h and then warmed to room temperature for 30 min. The resulting product was precipitated twice with an excess of cold diethyl ether and the white precipitate was collected by filtration and dried in a vacuum to yield 0.6 g (85.7%) of product.

2.4. Synthesis of mono (6-(2-aminoethyl) amino-6-deoxy)-β-cyclodextrin (EDA-β-CD)

Mono-6-deoxy-6-(*p*-tolylsulfonyl)-β-cyclodextrin (6-OTs-β-CD) was synthesized as described by the previously reported method [23], β-CD (20.0 g, 16.3 mmol) was suspended in 150 mL of water, and NaOH (2.16 g, 54.7 mmol) in 7 mL of water was added dropwise over 6 min. The suspension became homogeneous and slightly yellow before the addition was complete. *p*-toluenesulfonyl chloride (3.36 g, 17.6 mmol) in 10 mL of acetonitrile was added dropwise over 8 min, causing immediate formation of a white precipitate. After 2 h of stirring at 25 °C, the precipitate was removed by filtration and the filtrate was refrigerated overnight at 4 °C. The resulting white precipitate was collected by filtration and dried in a vacuum to yield 2.39 g (11%) of pure white solid.

Five gram of mono-6-OTs-β-CD was reacted with excess amount of EDA (30 mL) at 75 °C for 4 h. After the reaction was completed, the mixture was allowed to cool to room temperature, and then, 30 mL of cold acetone were added. The precipitate was repeatedly dissolved in 30 mL of water-methanol mixture, and poured into 50 mL of acetone several times for the removal of unreacted EDA. The sample obtained was dried at 50 °C for 3 days in a vacuum oven, and 4.3 g of EDA-β-CD was obtained.

2.5. Synthesis of mPEG-PLG(CD)

MPEG-PLGA (0.6 g, 0.05 mmol) and NHS (242 mg, 2.1 mmol) were dissolved in 5 mL of anhydrous DMSO. DCC (432 mg, 2.1 mmol), and a catalytic amount of 4-dimethylaminopyridine were added to the reaction solution. The reaction mixture was stirred at room temperature for 3 days. Then the solution of EDA-β-CD (2.3 g, 2.1 mmol) in DMSO was added dropwise into the reaction system, followed by constant stirring for another 5 days. The reaction was diluted with 20 mL of water and the dicyclohexylurea was filtered off. The filtrate was further purified by dialysis (molecular weight cut-off size: 7000 Da) against deionized water at room temperature for 3 days to remove the EDA-β-CD and lyophilized to yield 1.15 g (69%) of product.

2.6. Preparation of the complexes mPEG-PLG(CPT@CD)between mPEG-PLG(CD) and CPT

The mPEG-PLG(CPT@CD) was prepared in a phosphate buffer solution (PBS)/ethanol system by co-lyophilization technique [24]. Ten miligram of mPEG-PLG(CD) and 0.5 mg of CPT were dissolved in the mixed solvent system, *i.e.*, 1 mL of PBS and 1 mL of ethanol. The system was left to equilibrate under constant stirring for 24 h at 50 °C under the darkness. After the organic solvent completely evaporated under vacuum, the suspension was filtrated. At last, the filtrate containing mPEG-PLG(CPT@CD) was lyophilized to obtain the dry yellow power. The amount of CPT incorporated into mPEG-PLG(CD) was determined according to the following procedure. The lyophilized mPEG-PLG(CPT@CD) powder was dissolved in DMSO to obtain a clear solution, and the drug concentration was quantified by its UV absorbance at 367 nm. The calibration curves were generated using the known concentrations of CPT. The following formulas were used to calculate the drug encapsulation efficiency (EEwt.-%) and drug loading (DLwt.-%) of