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α-Tocopherol succinate-modified chitosan as a micellar delivery system for paclitaxel: Preparation, characterization and in vitro/in vivo evaluations

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ABSTRACT

α-Tocopherol succinate hydrophobically modified chitosan (CS-TOS) containing 17 α-tocopherol groups per 100 anhydroglucose units was synthesized by coupling reaction. The formation of CS-TOS was confirmed by ^{1}H NMR and FT-IR analysis. In aqueous medium, the polymer could self-aggregate to form micelles, and the critical micelle concentration (CMC) was determined to be 5.8×10^{-3} mg/ml. Transmission electron microscopy (TEM) observation revealed that both bare and paclitaxel-loaded micelles were near spherical in shape. The mean particle size and zeta potential of drug-loaded micelles were about 78 nm and +25.7 mV, respectively. The results of DSC and XRD analysis indicated that paclitaxel was entrapped in the micelles in molecular or amorphous state. In vitro cytotoxicity and hemolysis study revealed the effectiveness and safety of this delivery system, which was further confirmed by the in vivo antitumor evaluations. It can be concluded that the CS-TOS was a potential micellar carrier for paclitaxel.

1. Introduction

Paclitaxel (PTX), as one of the most exciting chemotherapeutic drugs, which exerts its antitumor effect primarily by stabilizing the microtubules during mitosis, has been successfully used in the clinical treatment of several cancer types, especially breast and ovarian cancer (Singla et al., 2002). Due to its poor water solubility (approximately <2 μg/ml) (Liggins et al., 1997), PTX is currently solubilized in a 50:50 mixture of Cremophor EL and dehydrated ethanol as Taxol®. Unfortunately, Cremophor EL was shown to induce severe side effects like hypersensitivity, neurotoxicity, and nephrotoxicity (Gelderblom et al., 2001). Therefore, many attempts have been made to find less toxic and better tolerated carriers for PTX delivery.

Among these new delivery systems, polymeric micelles have attracted increasing interest (Alani et al., 2010; Qu et al., 2009; Sawant et al., 2008; Zhang et al., 2010) because of their special characteristics, such as good solubilization efficiency (Montazeri Aliabadi et al., 2005), high stability upon dilution (Torchilin, 2007), and reducing non-selective reticuloendothelial system (RES) scavenge (Kataoka et al., 2000). The nanoscale dimensions of polymeric micelles also permit the efficient accumulation in tumor tissues via the enhanced permeability and retention (EPR) effect (Maeda et al., 2000). Polymeric micelles have unique core–shell architecture, which is composed of hydrophobic segments as the inner core and hydrophilic segments as the outer shell in aqueous medium. Poorly water-soluble drugs can be solubilized within the core by hydrophobic interactions (Kwon and Okano, 1996).

To date, numerous amphiphilic block or graft copolymers have been synthesized for micellar drug delivery applications. Among them, chitosan has been extensively studied due to its excellent biocompatibility, biodegradability, nontoxicity, and low immunogenicity (Chen et al., 2006; Francis Suh and Matthew, 2000). However, natural chitosan has its intrinsic limitation for it can be dissolved only in acidic water, and after hydrophobically modification, ideal micelles were hard to be prepared. In view of that, water-soluble chitosan with low molecular weight and high degree of deacetylation was chosen as the hydrophilic part of the polymer in our study, whose amphiphilic derivatives have been demonstrated as potential carriers for micelles of hydrophobic drugs by many researchers (Chen et al., 2003; Hu et al., 2008; Huo et al., 2010; Liu et al., 2004; Ngawhirunpat et al., 2009). On the other hand, α-tocopherol is an excellent solvent for many poorly soluble drugs because of its good lipophilic nature (Nielsen et al., 2001). It may therefore provide sufficient capacity for hydrophobic drugs.

Abbreviations: CS, chitosan; α-TOS, α-tocopherol succinate; CS-TOS, α-tocopherol succinate-modified chitosan; PTX, paclitaxel; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; NHS, N-hydroxysuccinimide; TNBS, 2,4,6-trinitrobenzene sulfonic acid; DS, degree of the substitution; EE, drug encapsulation efficiency; DL, drug loading.

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In consideration of that, α-tocopherol succinate was introduced to the polymer.

In this study, we developed the polymeric micelle system of α-tocopherol succinate-modified chitosan (CS-TOS) for paclitaxel delivery. The amphiphilic chitosan derivative was synthesized by coupling reaction. In aqueous medium, the conjugate can self-assemble to form micelles. Based on this property, paclitaxel was incorporated into the micellar core for intravenous delivery. The preparation, characterization, properties of the micelles, and in vitro/in vivo evaluations were studied in detail.

2. Materials and methods

2.1. Materials

Water-soluble chitosan (CS) with molecular weight of 30 kDa and degree of deacetylation > 90% was purchased from Kittolife Co., Ltd., Seoul, Korea. α-Tocopherol succinate (α-TOS) was a kind gift from Xinchang Pharmaceutical Co., Ltd., Zhejiang, China. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were purchased from Shanghai Medpep Co., Ltd., Shanghai, China. 2,4,6-Trinitrobenzene sulfonic acid (TNBS), pyrene (purity > 99%), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were obtained from Sigma Chemical Co., St. Louis, USA. Paclitaxel (PTX, purity of 99.9%) was purchased from Tianfeng Bioengineering Technology Co., Ltd., Liaoning, China. Cremophor EL was kindly supplied by BASF Corp., Ludwigshafen, Germany. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and penicillin–streptomycin mixture were purchased from Gibco BRL, Carlsbad, USA. All other chemicals and solvents were of analytical or chromatographic grade and used without further purification. Distilled water or Milli-Q water was used in all experiments.

2.2. Animals and cell line

The New Zealand rabbit (male, weighing 2 kg) and specific pathogen-free female Kunming mice (5–6 weeks old, weighing 20–25 g) were purchased from Laboratory Animal Center of Shenyang Pharmaceutical University, Liaoning, China. MCF-7 cells (human breast cancer cells) were acquired from American Type Culture Collection, Manassas, USA.

Procedures involving animals complied with ethical guidelines and were approved by the Shenyang Pharmaceutical University Animal Ethics Committee.

2.3. Synthesis of α-tocopherol succinate-modified chitosan (CS-TOS)

The CS-TOS was synthesized by the coupling reaction of carboxyl group of α-tocopherol succinate with amino group of chitosan in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS), referring to the procedure described by Lee et al. (1998) with minor modification.

Briefly, the water-soluble chitosan was dissolved in distilled water and α-TOS was dissolved in dimethylformamide, both by sonication treatment in water bath at room temperature (Sonic Purger Model KH7200DB, Kunshan Ultrasonic Instruments Co., Ltd., Shanghai, China). To activate the carboxyl group of α-TOS, equal amount (1.5 equivalents of α-TOS) of EDC and NHS were added into the chitosan solution, which allowed formation of the amide linkage by reacting with the primary amino groups of chitosan. Afterwards, the α-TOS solution was added to the chitosan solution in dropwise manner. After completely dripped, the reaction mixture was kept in agitation at room temperature in dark condition. Twenty-four hours later, the mixture was poured into methanol/ammonia solution (7/3, v/v). The resulting precipitate was collected by centrifugation, and washed thoroughly with methanol to remove the unreacted α-TOS.

After above processes, the yellow gel precipitate was dissolved in 20 ml acidic distilled water, and then dialyzed against distilled water by using dialysis membranes (MWCO: 3.5 kDa, Viskase Companies Inc., USA) for 48 h in order to remove other water-soluble by-products. Finally, the dialyzed solution was lyophilized to get the CS-TOS powder (Freeze Dryer Model FD-1C-50, Boyikang Experimental Instrument Co., Ltd., Beijing, China).

2.4. Characterization of CS-TOS

2.4.1. Proton nuclear magnetic resonance (^{1}H NMR) analysis

To confirm the formation of CS-TOS, high-resolution ^{1}H NMR spectra were collected using a Bruker Avance spectrometer (AV-600, Bruker, Karlsruhe, Germany) operated at 600 MHz. Native and modified chitosan were dissolved in the mixture of deuterated water (D_2O) and tetradeuteroacetic acid (CD_3COOD) at a concentration of 1% (w/v).

2.4.2. Fourier-transform infrared (FT-IR) analysis

In order to determine the chemical interaction between CS and α-TOS, FT-IR spectra of CS, α-TOS, their physical mixture and CS-TOS were recorded in KBr discs on Fourier-transform infrared spectrometer (Bruker IFS-55, Bruker, Switzerland) in the range from 4000 to 400 cm^{-1}.

2.4.3. Measurement of the degree of substitution (DS)

The degree of the substitution is defined as the number of α-tocopherol groups per 100 anhydroglucose units (amino groups) of CS-TOS, and it was determined by TNBS method (Bernkop-Schnürch and Krajicek, 1998), which measures the amount of remaining primary amino residues on the polymer using 2,4,6-trinitrobenzene sulfonic acid (TNBS reagent).

2.4.4. Determination of critical micelle concentration (CMC)

To prove the potential of hydrophobic microdomain formation, the CMC of CS-TOS in aqueous medium was determined using a spectrofluorophotometer (F-2500 FL Spectrophotometer, Hitachi Ltd., Japan) with pyrene as the fluorescence probe (Kalyanasundaram and Thomas, 1977; Zhao et al., 1990).

2.5. Preparation of PTX-loaded CS-TOS micelles

The incorporation of PTX into polymeric micelles was carried out by a probe-type ultrasonic method. The concrete steps were as follows: firstly, 10 mg of CS-TOS was dissolved in 10 ml of distilled water, then desired volume of PTX-acetone solution at the concentration of 1 mg/ml was added quickly into the aqueous phase under probe-sonication, and the resultant mixture was further ultrasonicated at 400 W for 30 min (JY92-II, Ningbo Scientz Biotechnology Co., Ltd., China). To keep the sample solution from being heated, the sonication was carried out in an ice bath, with the pulse function on for 3 s and off for 2 s. In order to remove unloaded PTX from the mixture, the product was centrifuged at 4000 rpm for 10 min. Subsequently, the resultant supernatant was lyophilized at a condenser temperature of −52 °C and pressure of less than 20 Pa to obtain PTX-loaded CS-TOS micelles.

2.6. Characterization of PTX-loaded CS-TOS micelles

2.6.1. Differential scanning calorimetry (DSC) analysis

Four samples including PTX, blank micelles, the physical mixture of PTX and blank micelles, and PTX-loaded micelles were