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Amphiphilic nanoparticles based on poly(vinyl pyrrolidone) and stearoyl modified chitosan as drug vehicles for paclitaxel delivery

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ABSTRACT

Poly(vinyl pyrrolidone) (PVP) and stearoyl chloride (SC) double-grafted chitosan (PCS) nanoparticles (NPs) were prepared for paclitaxel (PTX) delivery. PTX could be effectively loaded into PCS NPs with encapsulation efficiency of 92.8%. PTX-loaded PCS NPs (PTX/PCS NPs), which possessed particle sizes of 144 nm and Zeta potentials of −7.5 mV, exhibited a sustained release behavior. In H-22 tumor bearing mice, significantly enhanced antitumor efficacies were achieved by PTX/PCS NPs with the tumor inhibition ratio (TIR) of 76.1% as compared with PTX injection. Furthermore, no signs of sub-acute toxicity were detected in healthy mice, indicating the safety of PCS NPs. Therefore, PCS NPs could be served as an effective and safe carrier for PTX delivery.

1. Introduction

Chemotherapeutics play a major role in the treatment of cancer, however, which have suffered from some drawbacks including lack of selectivity and high toxicity, thus compromising their clinical utility [1,2]. To resolve these problems, drug delivery systems (DDS) have been developed to effectively encapsulate and selectively deliver chemotherapeutic drugs to their specific sites of action for improved therapeutic outcome [3]. Recently, polymeric nanoparticles (NPs) have attracted considerable attentions for intravenous delivery of chemotherapeutic drugs [1,4]. In particular, chitosan as a naturally occurring polymer emerges as an attractive candidate due to its biocompatibility, biodegradability, and non-toxicity [5]. However, its applications are largely limited by its poor solubility and lack of amphiphilicity, which prohibits it from forming NPs via self-assembly in physiological condition. Therefore, hydrophobic and hydrophilic components have been chemically attached to the amino and/or hydroxyl groups of chitosan to improve its solubility and amphiphilicity, thereby facilitating the formation of polymeric NPs [5,6]. In addition, hydrophobic segments, including alkyl and fatty acyl groups (stearoyl, palmitoyl, linoleoyl etc.), could self-assemble as the inner core of NPs and serve as a container to facilitate the encapsulation and sustained release of hydrophobically chemotherapeutic drugs [7]; hydrophilic segments, such as poly(ethylene glycol) (PEG) and phosphorylcholine, endow NPs with desired solubility and stability to prolong their systemic circulation [8,9].

Poly(vinyl pyrrolidone) (PVP) has been widely recognized as a hydrophilic polymer for drug delivery owing to its biocompatibility and low toxicity. PVP modified polymeric NPs can provide long systemic circulation, thereby resulting in improved therapeutic effect [10]. However, the studies concerning PVP modified amphiphilic chitosan NPs for drug delivery have not been reported.

In the present study, for the first time, PVP and stearoyl chloride (SC) double-conjugated chitosan (PCS) were developed as amphiphilic polymeric vectors for paclitaxel (PTX) delivery. PCS NPs were prepared via self-assembly in physiological condition and their in vivo toxicity was assessed in healthy mice. PTX-loaded PCS NPs (PTX/PCS NPs) were prepared via sonication and their physicochemical properties and in vitro release were investigated. In vivo antitumor efficacies of PTX/PCS NPs were evaluated in H-22 tumor bearing mice.

2. Experimental section

2.1. Materials and animals

Chitosan (molecular weight of 100 kDa) with deacetylation degree of 85% was purchased from Golden-Shell Biochemical Co.,

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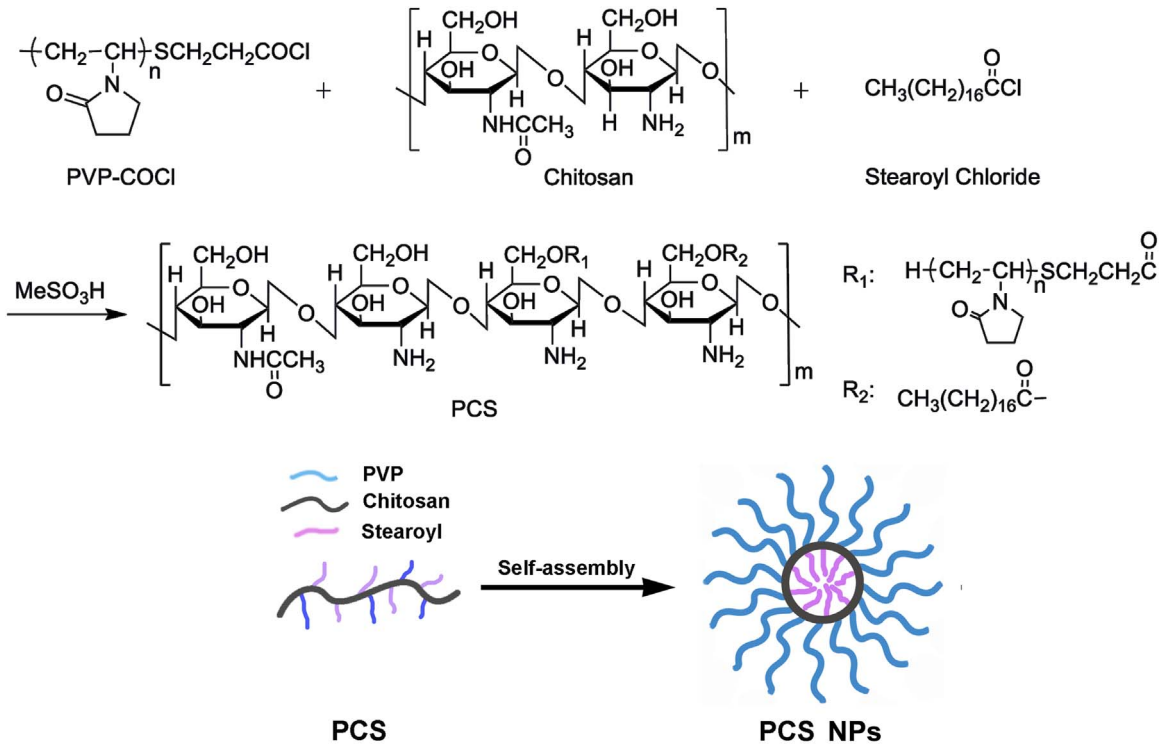

Fig. 1. Synthetic route of PCS and schematic illustration of the formation of PCS NPs.

Ltd. (Zhejiang, China). SC was obtained from Aladdin Reagent Co., Ltd. (Shanghai, China). PVP acyl chloride (PVP-COCl) was synthesized from N-vinyl pyrrolidone (The detailed information about this polymerization was presented in the section 1.1 of Supporting Information (SI)). Methanesulfonic acid ($MeSO_3H$) and Tween 80 were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). PTX was supplied by Hisun Pharmaceutical Co., Ltd. (Zhejiang, China). All other reagents were analytical grade.

Female Kunming mice (20 ± 2 g) were supplied by Shanghai Institute of Pharmaceutical Industry. All animal experiments were performed following the procedure approved by Institutional Animal Care and Use Committee, Fudan University.

2.2. Preparation, characterization, and in vivo toxicity of PCS

The synthetic route of PCS was illustrated in Fig. 1. Chitosan (0.2 g) and PVP-COCl (0.24 g) were dissolved in $MeSO_3H$ (6 mL). After stirring for 1 h, SC (0.1 g) was added and the reaction was allowed to process for 12 h under N_2 atmosphere. The reaction was quenched by crushed ice. Then the mixture was dialyzed for 6 h to remove most of acid, followed by neutralizing the remaining acid as well as ammonium salt with sodium hydroxide (1 M). For further purification, the solution was dialyzed again for 3 days and lyophilized. The detailed characterization of PCS were described in SI, including Fourier transformed infrared spectroscopy (FTIR) spectrum, ^{1}H nuclear magnetic resonance spectroscopy (^{1}H NMR), and elemental analysis. The PCS was dispersed in aqueous medium to form NPs via self-assembly (Fig. 1). The in vivo toxicity of PCS NPs was investigated as described in SI.

2.3. Preparation, characterization, and in vitro release of PTX/PCS NPs

To obtain PTX/PCS NPs, PTX (dissolved in ethanol, 10 mg/mL) was added to PCS NPs suspension, followed by sonication at 200 W for 20 min. The particle size, Zeta potential, and morphology of PTX/PCS NPs were evaluated as described in SI. The resultant PTX/PCS NPs was placed in a dialysis bag (MWCO, 3500 Da), immersed into 50 mL of phosphate buffered saline (PBS, pH 7.4) containing 0.1% (w/w) Tween 80, and incubated at 37 °C and 100 rpm/min. At designated time intervals, aliquots of 1 mL were withdrawn for PTX quantification according to SI and equal volume of fresh medium was replenished.

2.4. In vivo antitumor efficacy

H-22 ascites was inoculated subcutaneously at the right armpits of female Kunming mice. When the volume of tumors reached nearly 100 mm^3, tumor bearing mice were randomly divided into four groups ($n=6$) and intravenously injected with saline, blank PCS NPs, PTX injection, and PTX/PCS NPs, respectively. The administration was performed every three days for three times at the PTX dose of 10 mg/kg. The length (L, mm) and width (W, mm) of the tumor were measured every other day and tumor volumes (V, mm^3) were calculated as $L \times W^2/2$. When the tumor volume of saline group reached 2000 mm^3, all mice were sacrificed and the tumors were excised, weighed, and photographed. Tumor inhibition ratio (TIR) was calculated as $(1-W_t/W_s) \times 100\%$, where W_t and W_s stand for the average tumor weight of treatment and saline groups, respectively.

3. Results and discussion

3.1. Preparation, characterization, and in vivo toxicity of PCS

Compared with ^{1}H NMR spectrum of chitosan (Fig. 2A), new peaks at 0.9 and 1.3 ppm in PCS were attributed to the terminal methyl and methylene of stearoyl, indicating the linkage of SC with chitosan, and the characteristic peaks at 1.4–2.3 and 2.9–3.8 ppm proved the existence of PVP in PCS. Compared with FTIR spectrum of chitosan (Fig. 2B), strong peaks at 2921 and 1664 cm^{-1} in PCS were ascribed to the stretching vibration peaks of CH_2 and C=O, respectively, thereby proving the covalent linkage of PVP and SC with chitosan. In addition,