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Characteristics of paclitaxel-loaded chitosan oligosaccharide nanoparticles and their preparation by interfacial polyaddition in O/W miniemulsion system

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ABSTRACT

Paclitaxel-loaded chitosan oligosaccharide (CSO) nanoparticles were prepared by interfacial polyaddition between amino group of CSO and epoxy group of ethylene glycol diglycidyl ether (EGDE) in an O/W miniemulsion system. Using Span 85 and Tween 20 as the surfactants of oil phase and water phase, respectively, the stable O/W miniemulsion with about 200 nm droplet size could be obtained. When the molar ratio of EGDE to CSO was increased from 2 to 6, the average size of the obtained nanoparticles increased from 156.2 to 218.9 nm, the drug entrapment efficiency was increased from 83.68% to 92.30%, and the paclitaxel release rate was slowed. Fixing the molar ratio of EGDE to CSO at 4, the average size of prepared nanoparticles decreased from 282.1 to 140.4 nm when the total amount of CSO and EGDE was increased, while the drug entrapment efficiency increased, and the drug release rate in vitro decreased.

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1. Introduction

Chitosan is the natural alkaline polysaccharide derived from chitin by deacetylation and consists of 2-amino-2-deoxy-(1–4*b*)-D-glucopyranose residues (D-glucosamine units) and *N*-acetyl-D-glucosamine units (Kean & Thanou, 2005), which possess distinct physicochemical and biological properties and is widely accepted as carriers for gene delivery, oral protein delivery, and controlled release systems (Gazori et al., 2009). Because of the large molecular weight, insolubility in physiological pH condition and high viscosity of chitosan (Tommeraas et al., 2002), it is necessary to degrade chitosan to prepare chitosan oligosaccharide (CSO) with low molecular weight. Particularly, chitosan oligosaccharide presents more interesting characteristics for clinical applications, which include reduced toxicity, biocompatibility, biodegradability, and good solubility in physiological condition (Duceppe & Tabrizian, 2009).

The use of biodegradable materials for nanoparticle preparation can realize sustained drug release within the target site over a period of days or even weeks. Biodegradable nanoparticles formed by PLGA and PLA have been developed for sustained drug delivery and are especially effective for drugs with an intracellular target (Panyam & Labhasetwar, 2003). Chitosan nanoparticles have been applied in potential delivery systems for vaccines (Illum, Jabbal-Gill, Hinchcliffe, Fisher, & Davis, 2001; Yang et al., 2009a, 2009b), genes (Yoksan & Akashi, 2009), and anticancer agents (Li et al., 2009), which can be prepared by many methods, including solvent evaporation (Huang, Du, Yuan, & Hu, 2009), ion cross-linking (De campos, Sanchez, & Alonso, 2001; Huang, Ma, Khor, & Lim, 2002), emulsification covalent cross-linking (Lueßen et al., 1997), spray drying (Mi, Tan, Liang, & Sung, 2002), etc.

Paclitaxel (PTX) is one of the best anti-tumor drugs and exhibits a strong cytotoxic activity against a variety of solid tumors, such as breast cancer, ovarian cancer, lung cancer, and prostatic carcinoma. However, PTX has a poor solubility in water due to its hydrophobic properties, which limits its clinical applications (Zhang, Huo, Zhou, Yu, & Wu, 2009). Cremophor EL has to be used in the commercial Taxol® formulation (Cremophor EL:ethanol, 50:50, v:v), which is one of the first line formulations of paclitaxel. However, the use of Cremophor EL causes serious side effects including nephrotoxicity, neurotoxicity, and cardiotoxicity (Yang et al., 2009a, 2009b), which have limited the clinical application of Taxol. Therefore, it is important to develop a drug delivery system for paclitaxel without Cremophor EL. Many drug delivery systems for paclitaxel have been developed in the recent year, such as water soluble paclitaxel pro-drugs (Golik et al., 1996; Moosavi-Movahedi et al., 2003), liposome (Soepenberg et al., 2004; Zhang et al., 2005 and Yang et al., 2007), microsphere (Armstrong, Fleming, Markman, & Bailey, 2006; Azouz et al., 2008), emulsion (Kan, Chen, Lee, & Chu, 1999; Lundberg, Risovic, Ramaswamy, & Wasan, 2003), cyclodextrin inclusion compound (Alcaro et al., 2002; Liu et al., 2004 and

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Bouquet et al., 2009), polymeric micelle (Dabholkar, Sawant, Mongayt, Devarajan, & Torchilin, 2006; Huh et al., 2005; Lee et al., 2007) and polymeric nanoparticle.

In this paper, paclitaxel-loaded chitosan oligosaccharide nanoparticles were prepared by interfacial polyaddition in O/W miniemulsion system. The effects of the time of sonication dispersion, the proportion and concentration of EGDE and chitosan oligosaccharide during preparation on several characteristics of chitosan oligosaccharide nanoparticles such as particle size, drug entrapment efficiency and drug release behavior in vitro were evaluated.

2. Materials and methods

2.1. Materials

Chitosan (95% deacetylation degree, M_w = 450 kDa) was supplied by Yuhuan Marine biochemistry Co. Ltd. (Zhejiang, China). Chitosanase was purchased from Chemical Industries Co. Ltd. (Japan). Master sample of polysaccharide (Part No.: 2090-0100) was purchased from Polymer Laboratories Co. Ltd. (USA). The model drug, Paclitaxel was provided by Huzhou Zhanwang biochemical Co. Ltd., China. Ethylene glycol diglycidyl ether (EGDE) was purchased from Tokyo Kasei Kogyo Co. Ltd. (Japan). Tween 20, Span 85 and methylene dichloride were purchased from Shanghai Chemical Reagent Co. Ltd., China. Ethanol and other chemicals were analytical reagent grade.

2.2. Preparation of chitosan oligosaccharide

A 3% chitosan solution was prepared by dispersing 15 g chitosan in 500 mL of distilled water. After adding 6.25 mL of 36.5% (w/v) hydrochloric acid, the temperature of the mixture was raised up to 50 °C in a bath reactor, and 1 U/mL chitosanase was added. The reaction time of hydrolysis was controlled by molecular weight measurement of chitosan, monitoring through viscosity determination. The reaction mixture was then centrifugated for 10 min at 4000 rpm. The obtained supernatant was filtered with 0.45 μm filter, and then ultrafiltered by various molecular weight cut off (NMWCO) ultrafiltration membranes (Millipore Labscale TFF system, Millipore Co. USA). The low molecular weight of chitosan, chitosan oligosaccharide (CSO) was obtained by lyophilization.

The molecular weight of the final chitosan oligosaccharide (CSO) was determined by gel permeation chromatography (GPC) with TSK-gel column (G3000SW, 7.5 mm × 30 cm I.D.) at 25 °C (Hu et al., 2006). A weighted sample of lyophilized powder of CSO was dissolved in acetate buffer solution (pH 6.0) and the final concentration was adjusted to 1.0 mg/mL. Then, 10 μL of the sample was chromatographed using acetate buffer solution (pH 6.0) as the elution buffer and a flow rate of 0.8 mL/min. Master samples of polysaccharide with different molecular weight (M_w = 5.9, 11.8, 22.8, 47.3, 112, 212 K) were dissolved in acetate buffer solution (pH 6.0), and their final concentrations were set to 0.5 mg/mL. Calibration was performed by means of polysaccharide samples using the integral molecular weight distribution method.

2.3. Preparation of paclitaxel-loaded chitosan oligosaccharide nanoparticles

The oil in water (O/W) miniemulsion was prepared by probe-type ultrasonic treatment with methylene dichloride and CSO aqueous solution as the oil phase and aqueous phase, respectively. Paclitaxel-loaded chitosan oligosaccharide nanoparticles were prepared by polyaddition in O/W miniemulsion system at room temperature. The preparation recipes are shown in Table 1.

The aqueous phase was formulated by chitosan oligosaccharide (M_w = 8000 kDa) aqueous solution and Tween 20. The oil phase, consisted of methylene chloride, EGDE, paclitaxel, and Span 85 was added into aqueous phase by stirring (DC-40, Hangzhou Electrical Engineering Instruments, China) at 400 rpm for 5 min to form the pre-emulsion. The miniemulsion was then obtained by probe-type ultrasonic treatment (500 W, 10–50 cycles with 2 s active following 3 s duration, JY92-II, Scientz Biotechnology Co. Ltd., China) of the pre-emulsion in ice-bath and then stirred at room temperature over night.

2.4. Determination of particle size

One milliliter miniemulsion or nanoparticles dispersion was diluted to control the concentration of droplets or nanoparticles to 0.1 mg/mL. The droplet size of the resulted miniemulsion and nanoparticles were determined using a Zetasizer (3000 HS, Malvern Instruments, UK).

2.5. Determination of drug encapsulation efficiency

The content of paclitaxel was determined by high performance liquid chromatograph (HPLC). A Hypersil C18 column (150 mm × 3.9 mm) was used. The mobile phase consisted of acetonitrile and water (45:55, v/v) with a flow rate of 1 ml/min. The wavelength was set at 240 nm and the column temperature was 35 °C. The separated drug-loaded nanoparticles by centrifugation were re-dispersed into 30 ml of PBS (pH 7.4) with 2 M sodium salicylate and surged by vortexing (XW-80A, Instruments Factory of Shanghai Medical University, China) for 3 min to dissolve the unloaded drug. Then the dispersions were centrifuged at 20,000 rpm for 15 min (3K30, Sigma, Germany) and the supernatant was filtrated with 0.22 μm filter. The drug content in the obtained supernatant was measured by HPLC as described above. The drug entrapment efficiency (EE) was calculated from

$$EE = (W - W_0)/W \times 100\% \quad (1)$$

where W is the weight of the drug added in the system, W_0 is the analyzed weight of the drug in the supernatant after centrifugation.

2.6. In vitro release studies

The precipitate of drug-loaded nanoparticles were re-dispersed in 30 ml PBS (pH 7.4) containing 2 M sodium salicylate and surged by vortexing (XW-80A, Instruments Factory of Shanghai Medical University) for 3 min, and then shaken horizontally (SHEL-LAB1227-2E, SHELLAB, USA) at 37 °C and 60 strokes/min. One milliliter of the dispersion was withdrawn from the system at definite time intervals. The dispersions were centrifuged at 20,000 rpm for 15 min (3K30, Sigma, Germany) and the supernatant was filtered through a 0.22 μm filter. The paclitaxel content in the filtrate was determined by HPLC method as described above. The drug content at 0 time was considered as the content of unloaded drug.

3. Results and discussions

3.1. Preparation of stable O/W miniemulsion

The droplet diameter of the emulsion prepared by mechanical stirring usually ranges from several to tens of microns, while nano-sized miniemulsion with narrow size distribution can be obtained via applying probe-type ultrasonic treatment. In this paper, the miniemulsion was prepared by probe-type ultrasonic treatment with methylene chloride and chitosan oligosaccharide solution as the oil phase and aqueous phase, respectively. The

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