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Novel pH-sensitive chitosan-derived micelles loaded with paclitaxel

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ABSTRACT

A series of pH-sensitive graft copolymers, *N*-octyl-*N*-(2-carboxyl-cyclohexamethenyl) chitosan derivatives were synthesized and characterized by FTIR, ^{1}H NMR and elemental analysis, and their physical properties were measured with differential scanning calorimetry and X-ray diffraction spectrometry. The critical micelle concentrations (CMCs) of the modified chitosan determined by using pyrene as a hydrophobic probe in fluorescence spectroscopy were from 11 to 72 μg/ml. The graft polymers can form micelles solubilizing paclitaxel, with drug-loading rate ranging from 30.47% to 48.10% and entrapment efficiency from 42.22% to 59.24%. Cytotoxicities of carrier against tumor cells estimated that carriers were nearly non-cytotoxic. Additionally, the results of pH-sensitivity and drug release experiments showed that the micelles were highly sensitive to mild acidic conditions (pH 5.5) while reasonably stable at physiological conditions (pH 7.4). Therefore, chitosan-derived micelle may be a potential anti-tumor drug delivery system for chemotherapy of cancer.

1. Introduction

The selective control of drug concentration and distribution within the tumor microenvironment is one of the most important factors for achieving effective and safe cancer chemotherapy (Bae, Diezi, Zhao, & Kwon, 2007; Bae & Kataoka, 2009; Jain, 2001). Polymeric micelles from amphiphilic graft or block copolymers were recognized as one of the most promising anti-tumor drug carriers, characterized by excellent biocompatibility, high drug-loading content, and markedly improved bio-distribution (Nishiyama & Kataoka, 2006). They can increase the solubility of many poorly water-soluble anti-cancer drugs successfully (Kwon, 2003; Torchilin, 2004). In particular, the nano-scaled polymeric micelles exhibit tumor accumulation by enhanced permeability and retention (EPR) effect (Torchilin, 2001).

A number of natural or synthetic polymers have been used to form polymeric micelles. Among them, chitosan is the most attractive candidate due to its biochemical activity, biocompatibility, biodegradability and low toxicity, and it has been widely used in the pharmaceutical field (Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004). Grafting the hydrophobic and hydrophilic segments to the chitosan backbone would give rise to amphiphilic graft copolymers, which can form self-assembled micelles in water. Chitosan-derived micelles were studied in our group (Chen, Ding, Qu, & Zhang, 2008; Qu, Yao, Zhang, Wu, & Ping, 2009; Qu, Zhu, Zhang, & Ping, 2009; Yao, Zhang, Ping, & Yu, 2007; Zhang et al., 2008). Especially, *N*-octyl-*O*-sulfate chitosan (NOSC) micelle was reported to be a safe and effective carrier for delivering paclitaxel (Zhang et al., 2008). However, the problem of polymeric micelle without adequate drug release upon micelle accumulation in the tumor tissues is usually revealed by clinical trials.

In order to improve the therapeutic efficiency and reduce side-effect of anti-cancer drugs, many scientists began to develop 'smart' drug delivery systems. Acidic pH is known to be a prominent microenvironment (Rapoport, 2007) in solid tumors, interstitial fluid in tumors has a lower pH than that in normal tissues (6.75 vs. 7.23) (Lee et al., 2008; Rapoport, 2007). This phenomenon has been employed in the design of numerous pH-sensitive polymeric micelles for the delivery of anti-cancer drugs to tumors (Kale & Torchilin, 2007; Lee et al., 2008; Lee, Na, & Bae, 2003). In addition, the micelles end up in the acidic compartment of endosomes and lysosomes (pH 5.0–5.5) after endocytosis, so pH-sensitive micelles may overcome the intracellular barriers created by endosomal or lysosomal membranes. Thus the arrival of the drug to its target is attributed to the degradation of pH-sensitive micelles releasing the encapsulated drug. Polymeric micelles formed by poly(ethylene glycol)–poly(aspartate hydrazide adriamycin) have been reported as an effective drug delivery nanocarrier (Bae et al., 2007). Its response to intracellular acidic compartments, such as endosomes and lysosomes, resulted in the selective release of anti-cancer drug at low pH (<6). Hruby, Konak, and Ulbrich (2005) presented a novel pH-sensitive micelle drug delivery system based on hydrazone-bound doxorubicin. But pH-sensitive micelle

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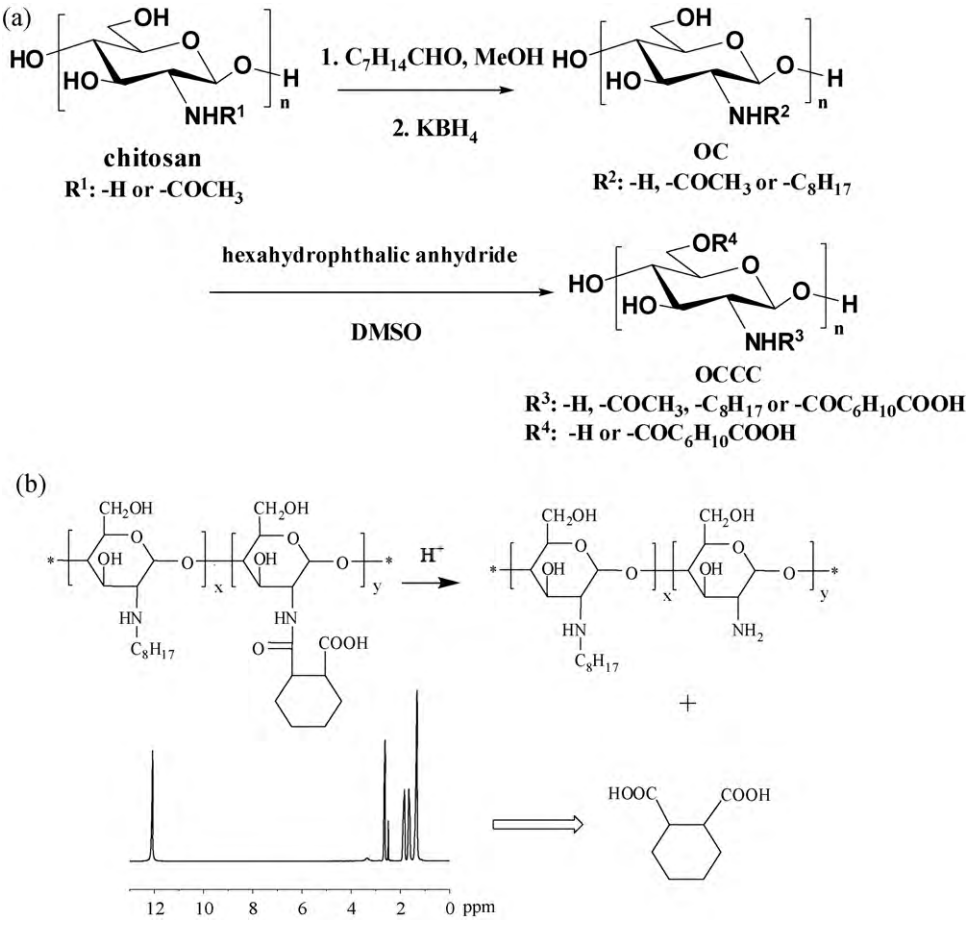

Scheme 1. (a) Synthesis route of OCCC and (b) hydrolyze route of OCCC-2.

delivery systems based on natural polymers have rarely been reported.

Amides with neighboring carboxylic acid groups were reported to exhibit pH-dependent hydrolysis. The amide of the secondary amine is almost instantly hydrolyzed at pH 5, slightly slower at pH 6, but only 50% even after 60 h at pH 7.4 (Xu et al., 2007). In our previous paper, *N*-octyl-*N*-(2-carboxylbenzoyl) chitosan (OCBC) as the material for drug carrier was synthesized (Li et al., 2009). It shows the pH sensitivity to mild acidic environment, but during the experiments there were many other things required to be improved, for example the water solubility of the polymers. Its relatively poor solubility possibly results from the hydrophobic benzyl group in the structure, so we come up with a way to make a modification of the polymer by replacing the benzyl group with other pH-sensitive groups, which are more hydrophilic. The hexahydrophthalic acid was hydrophilic due to the less hydrophobical cyclohexyl group compared with benzyl ring. In the current work, we describe a novel pH-sensitive drug delivery system using chitosan modified with amide linkage as a carrier for delivery of anti-cancer drug paclitaxel (PTX). Amphiphilic acylated chitosan was synthesized by the introduction of the octyl and carboxyl-cyclohexamethenyl moieties to part of amino groups of chitosan. The pH-sensitive micelle based on *N*-octyl-*N*-(2-carboxyl-cyclohexamethenyl) chitosan (OCCC) could remain stable at physiologic pH 7.4. In this research, the chemical structure of OCCC was confirmed by FTIR, ^{1}H NMR and elemental analysis. Furthermore, physical properties were measured with differential scanning calorimetry (DSC) and X-ray diffraction spectrometry (XRD) techniques. Transmission electron microscopy (TEM) techniques were exploited to evaluate the micelle-forming properties. The pH-sensitivity of the polymeric micelle was evaluated, and proved to be stable in neutral solution while highly sensitive to mild acidic environment. In addition, OCCC showed high capacity in solubilization of paclitaxel and pH-dependent drug release was investigated. Furthermore, the cellular cytotoxicity of OCCC and the paclitaxel-loaded micelle compared with Taxol®, the commercial paclitaxel for administration, were all evaluated in this paper. In conclusion, this study may lead to a novel pH-sensitive drug delivery system and improve the efficacy and safety for paclitaxel in chemotherapy.

2. Methods

2.1. Materials

Chitosan was provided by Nantong Shuanglin Biochemical Co. Ltd. (China), with a degree of deacetylation of 92% and viscosity average molecular weight of 70 kDa. Pyrene was purchased from Fluka Company (>99%). Paclitaxel was supplied by Taihua Natural Plant Pharmaceutical Co. Ltd. (China). HPLC/spectra-grade reagents were used as the mobile phase in HPLC analysis, and all commercially available solvents and reagents were used without further purification. Distilled and deionized water was used in all experiments.

2.2. Synthesis of amphiphilic chitosan derivative

N-Octyl-*N*-(2-carboxyl-cyclohexamethenyl) chitosan derivatives were prepared by introducing an octyl group to NH_2 on C_2 of the glucosamine unit in chitosan followed by a different degree of *N*-acylated as shown in Scheme 1a.