International Journal of Pharmaceutics 473 (2014) 501–509

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical nanotechnology

Enhancement of phototoxicity against human pancreatic cancer cells with photosensitizer-encapsulated amphiphilic sodium alginate derivative nanoparticles

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ARTICLE INFO

Article history:
Received 5 April 2014
Received in revised form 7 July 2014
Accepted 25 July 2014
Available online 30 July 2014

Keywords:
Photosensitizer
Sodium alginate
Phototoxicity
Reactive oxygen species
Apoptosis

ABSTRACT

Photosensitizer-encapsulated amphiphilic sodium alginate derivative (Photosan-CSAD) nanoparticles were prepared because of their ability to enhance phototoxicity in the photodynamic therapy of pancreatic cancer. These nanoparticles are spherical, 150–250 nm in size as determined by transmission electron microscopy, and have negative zeta potentials. Upon incubation with human pancreatic cancer cells, the Photosan-CSAD nanoparticles showed high fluorescence activity and reactive oxygen species generation, resulting in strong phototoxicity. However, no dark toxicity was observed. Apoptosis played a leading role in the cell death process induced by the Photosan phototoxicity. These results demonstrate that the Photosan-CSAD nanoparticles are a candidate for the photodynamic therapy of pancreatic cancer.

1. Introduction

Pancreatic cancer survival rates remain low, even with a complete resection of the tumor and/or pancreas, chemotherapy and radiotherapy (Bown et al., 2002; Samkoe et al., 2010). As a new therapy for the local destruction of pancreatic malignancy, photodynamic therapy (PDT) for the treatment of pancreatic cancer has recently been reported (Ayaru et al., 2004; Bown et al., 2002; Mitton and Ackroyd, 2008; Samkoe et al., 2010). During the PDT process, a photosensitizer is intravenously administered to the patient. Following the light activation of target tissues, reactive oxygen species (ROS) including singlet oxygen ($^1O_2$), superoxide (O_2^-) and peroxide anions (O_2^{2-}) are generated, and they oxidize subcellular organelles and other biomolecules leading to light-induced cell death (Nishiyama et al., 2009a). However, most photosensitizers have drawbacks in clinical applications. For example, low phototoxicity usually brings about low PDT efficacy because of the low quantum yield of ROS formation in tumor tissues. This is related to the photosensitizer's lack of a tumor tissue targeting property as well as the self-quenching of excited states in the photosensitizers (Lee et al., 2009; Park et al., 2011).

Amphiphilic polysaccharide derivatives are potential drug carriers because of their good cellular compatibility and biodegradability (Ayame et al., 2008; Liu et al., 2008). Additionally, amphiphilic polysaccharide derivatives may form nanoparticles, which increase tumor selectivity by the enhanced permeability and retention effect as well as avoiding rapid renal clearance and unwanted uptake by the reticuloendothelial system (Lee et al., 2009; Park et al., 2011). Encapsulated drugs can thus be delivered efficiently to tumor tissues and then can be released in a sustained manner (Lee et al., 2009; Park et al., 2011).

Sodium alginate is a linear anionic polysaccharide that consists of two kinds of hexuronic acid residues: 1,4-β-D-mannuronic acid and α-L-guluronic acid (Kang et al., 2002). As a natural polysaccharide, sodium alginate has found increasing biomedical applications because of advantages such as excellent cytocompatibility, biodegradability, non-toxicity, non-immunogenicity, chelating ability, and the possibility of chemical modification. In our previous work (Yang et al., 2007), we reported an amphiphilic sodium alginate derivative conjugated with cholersteryl residues (CSAD, Scheme 1A), which possesses good biocompatibility, potential interaction with cholesteryl receptors on cell surfaces, and a stronger ability to drive self-assembly into nanoparticles.

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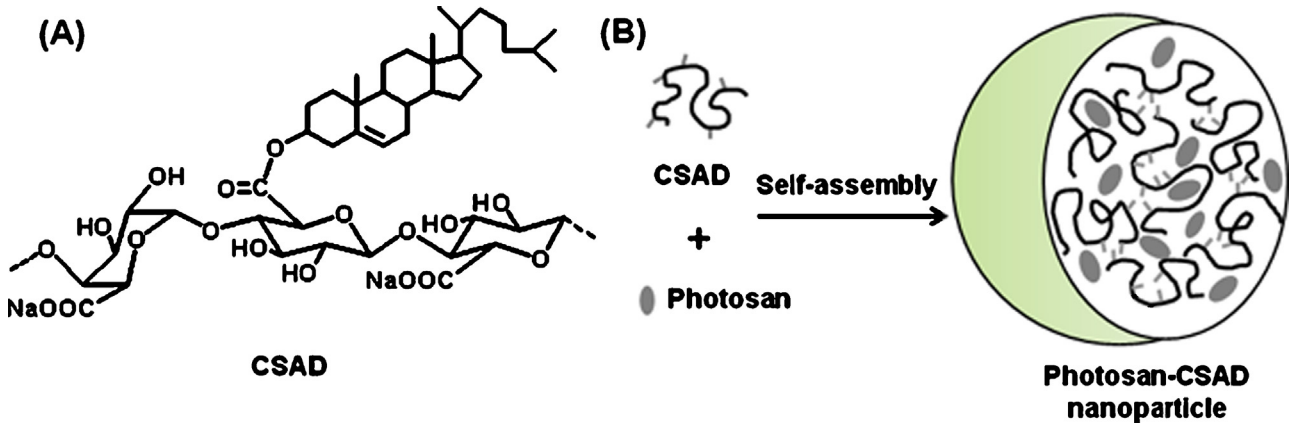
http://dx.doi.org/10.1016/j.ijpharm.2014.07.046
0378-5173/



Scheme 1. (A) Chemical structure of the CSAD derivative, and (B) preparation of the Photosan-CSAD nanoparticles by self-assembly.

Photosan (also referred to as Photofrin) is a porphyrin oligomer that contains sodium carboxylate groups, and it has been approved as an anionic water-soluble photosensitizer for clinical applications (Ding et al., 2011; Sadzuka et al., 2006). In this work, a CSAD derivative was developed to enhance the phototoxicity of Photosan against human pancreatic cancer (Panc-1) cells. As shown in Scheme 1B, Photosan-encapsulated CSAD (Photosan-CSAD) nanoparticles were prepared using a simple self-assembly method in aqueous solution. The fluorescence activity, ROS generation and phototoxicity of the nanoparticles as well as the cell death mechanism were assessed in Panc-1 cells.

2. Materials and methods

2.1. Materials

Sodium alginate, purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), was purified twice by dissolving in the distilled water, filtering, precipitating with ethanol and drying under vacuum at 40 °C. Its weight average molecular weight and number average molecular weight are 1.2×10^5 and 7.9×10^4 g/mol, respectively, and its polydispersity of 1.5 was determined by gel permeation chromatography. Cholesterol, *N,N′*-dicyclohexylcarbodiimide (DCC) and 4-(*N,N′*-dimethylamino)pyridine (DMAP) were purchased from Acros Organics (Janssens Pharmaceuticalaan, Belgium). Dimethyl sulfoxide (DMSO) was acquired from Aladdin Reagent Company (Shanghai, China), and was dried by soaking in molecular sieves and calcium hydride for a week before use. Photosan (Porfimer sodium), a freeze-dried injectable powder, was purchased from SeeLab Inc. (Wesselburen, Germany). RPMI 1640 culture medium and penicillin-streptomycin were purchased from Gibco Co. (Carlsbad, CA, USA), and fresh fetal calf serum was purchased from Hangzhou Jinuo Biomedical Co. (Hangzhou, China). A 2′,7′-Dichlorfluorescein-diacetate (DCFH-DA) kit and Hoechst 33342 were purchased from Beyotime Institute of Biotechnology (Shanghai, China).

2.2. Synthesis of the amphiphilic CSAD derivative

Sodium alginate was protonated in an aqueous HCl solution (2 mol/L). The resultant alginate acid precipitate was purified by washing with the distilled water and lyophilized. The CSAD derivative was then synthesized based on our previous work (Yang et al., 2007). Briefly, alginic acid (1.0 g, 5.68 mmol of uronic acid unit) was dissolved in 35 mL of water-free DMSO at room temperature overnight. Solutions of cholesterol (0.40 g, 1.03 mmol) in 2 mL chloroform and DCC (0.24 g, 1.16 mmol) and DMAP (0.14 g, 1.13 mmol) in 15 mL of DMSO were then added. The reaction was allowed to proceed at room temperature for 24 h, followed by purification with ethanol. The product was dissolved in the distilled water and neutralized by adding $NaHCO_3$ solution (4%, w/v). The solution was dialyzed against the distilled water for 3 days and lyophilized to obtain the CSAD derivative. FTIR: 3266 cm^{-1} (—OH vibration), 2920 cm^{-1} (—CH< and —CH_2— vibration), 1733 cm^{-1} (vibration of —C═O of carboxylic ester group,—COOR), 1600 and 1414 cm^{-1} (asymmetric symmetric and stretching vibrations of —C═O of sodium carboxylate group, —COONa), 1093 and 1033 cm^{-1} (vibration of —C—O—C— of glucosidic bond); ^{1}H NMR (D_2O, ppm): δ = 5.40 (glucose unit, H1), δ = 3.5–4.5 (glucose unit, H2–H6), δ = 1.0–2.5 (protons of the cholesteryl group). DS = 0.03 (DS: the degree of substitution, defined as the number of cholesteryl residues per hexuronic acid unit of sodium alginate as determined by the integration in the ^{1}H NMR spectrum).

2.3. Preparation of Photosan-CSAD nanoparticles

The CSAD derivative (30.0 mg) was dissolved in 15 mL of PBS (pH 6.2) with stirring at room temperature overnight, prior to the addition of 15 mL of phosphate buffer solution (PBS, pH 6.2) containing Photosan (150 μg/mL). The resulting mixture was stirred at room temperature in a dark flask for 24 h, followed by a period of dialysis in PBS (pH 6.2) in darkness for 1 day to remove un-encapsulated Photosan. A working curve for the Photosan concentration in PBS (pH 6.2) and a wavelength of 362 nm were used to determine the amount of Photosan in the solution outside of the dialysis bag (R^2 = 0.998), and this was analyzed by UV–vis spectrophotometry (UV-3150, Shimadzu, Japan). The amount of Photosan encapsulated in the nanoparticles was calculated from the reduction in the Photosan concentration. The Photosan encapsulating capacity was then calculated using Eq. (1).

$$\text{Encapsulating capacity}\ (\%) = \left[\frac{A-B}{C}\right] \times 100 \quad (1)$$

where *A* is the total weight of Photosan used, *B* is the weight of un-encapsulated Photosan, and *C* is the weight of the CSAD derivative. Consequently, the Photosan encapsulating capacity of the Photosan-CSAD nanoparticles was determined to be 5.2%. The UV–vis spectra of Photosan before and after encapsulation in the nanoparticles were obtained in PBS (pH 6.2) at a Photosan concentration of 2 μg/mL.

2.4. Characterization of Photosan-CSAD nanoparticles

2.4.1. Transmission electron microscopy (TEM)

The morphology of the Photosan-CSAD nanoparticles was investigated using TEM. The sample solution was dropped on to a