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MUC1 aptamer conjugated to chitosan nanoparticles, an efficient targeted carrier designed for anticancer SN38 delivery

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ABSTRACT

Molecularly targeted therapy is of great interest for diagnosis and treatment of cancerous cells due to its low toxicity for normal cells. In this study, chitosan was utilized as a promising carrier for delivery, and aptamer (Apt) was employed for active targeting of SN38 to colon cancer. SN38 cannot be used clinically due to its poor solubility and high toxicity. Developing nanoparticles (NPs) of drug–polymer conjugates can be a good candidate for overcoming such problems. *N*-Carboxyethyl chitosan ester (CS-EA) was synthesized as an intermediate for conjugation of SN38 to chitosan. MUC1 DNA aptamer with 5′-NH_2 functional group was conjugated to the self-assembled conjugate as a targeting agent. Prepared NPs had smooth and spherical morphology with 200 nm particle size. Conjugation of aptamer was confirmed by gel electrophoresis. In vitro cytotoxicity of NPs was assessed by HT-29 as MUC1 positive cell line through MTT assay. Aptamer conjugated NPs (Apt NPs) were more toxic than non-targeted NPs, however they were as toxic as free drug. Cellular uptake and targeting ability of prepared NPs were also confirmed via confocal microscopy. As a conclusion, prepared CS-SN38–Apt NPs can increase efficacy of drug SN38 through increasing solubility and specific delivery to the target tissue.

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1. Introduction

Interruption of DNA synthesis in cancerous cells is an attractive method of battling cancer diseases. Topoisomerase is an essential enzyme for controlling and facilitating DNA replication by breaking and unwinding the double stranded structure of DNA, which is necessary for cell replication. Accordingly, topoisomerase could be an appropriate target for inhibition of cancerous cells propagation (Champoux, 2001). Irinotecan (CPT-11) is a semisynthetic derivative of camptothecin, exhibiting proper water solubility and hindering cell nucleic acid synthesis by inhibiting topoisomerase I enzyme function (Bala et al., 2013; Moon et al., 2008). In spite of good solubility, only 5–10% of the administrated dose will be converted to the active form of the drug (Zhang et al., 2013; Zhao et al., 2000) by human carboxylesterase (hCE). Besides, this conversion shows unpredictable inter-patient varieties. In addition, the lacton ring of irinotecan can be easily converted to the open carboxylate form which is inactive (Gupta et al., 1997). In conclusion, these issues limit irinotecans application in cancer treatment. SN38, the active metabolite of irinotecan is 100–1000 times more potent than its prodrug, but the relatively high hydrophobicity of SN38 creates complications for its practical application (Ebrahimnejad et al., 2010). Moreover, instability of the active drug molecule at physiological pH is a major hindrance in attaining effective therapy (Bala et al., 2013).

Conventional chemotherapeutic agents affect cancerous cells as well as normal cells and therefore cause various side effects. Active targeting is a potential solution which has been extensively researched in order to decrease the undesirable and fatal side effects of anticancer drugs. In active targeting, carriers such as NPs are modified with targeting agents such as antibodies or aptamers. The targeting agents will escort the drug to the desired tissue, and the amount of administered dose will be reduced; consequently, normal cells are protected from cytotoxic effects of drugs. Accordingly, preparation of appropriate NPs to enhance SN38 pharmacokinetic and efficacy and implementing targeting agents can overcome such limitations. Various macromolecular prodrugs (e.g., EZN-2208, IMMU-130)

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(Bala et al., 2013) and nanomedicine formulations (i.e., nanoemulsions, polymeric micelles, lipid nanocapsules/nanospheres and liposomes) (Duan et al., 2010; Gu et al., 2012; Guo et al., 2012; Marier et al., 2011; Pal et al., 2005) of SN38 have been investigated to improve SN38 drug delivery to the cancer tissue, but each had advantages and weaknesses as well. Among various carriers, polymeric carriers are favorable because structural modification is relatively easy and conjugations with biocompatible polymeric carriers via covalent linkage could improve solubility and biocompatibility of the drugs. Prodrugs such as EZN-2208, which is a 40 kDa PEG conjugate (Zhao et al., 2008), PEGylated nanographene oxide (Liu et al., 2008) and HPMA_SN38 (Williams et al., 2012) are examples of conjugated drugs. Chitosan as a hydrophilic carbohydrate polymer is especially desired due to its biodegradability, biocompatibility and ability to increase drug solubility. Polymeric NPs benefit from passive targeting by enhanced permeability and retention (EPR) effect and leakage of vasculature around tumor tissue which facilitate permeation of NPs from blood vessels to the tumor tissue, besides, lack of lymphatic drainage keeps NPs at tumor site and causes accumulation of NPs at tumor tissue (Maeda et al., 2000). Implementation of active targeting in conjugation with other techniques leads to more efficient chemotherapy (Levy-Nissenbaum et al., 2008). Different kind of targeting agents have been investigated. Aptamers are oligonucleotides that fold by intramolecular interaction and acquire specific three dimensional conformation by which, they specifically bind to their antigen target with high affinity. Aptamers have attracted more attention in recent years as targeting agents, because of their many favorable characteristics such as high affinity and specificity to the target molecule, versatile selection process, ease of chemical synthesis, small physical size and lack of immunogenicity. The membrane associated glycol form of mucin glycoprotein is reported as an attractive target for anticancer drug delivery owing to its over expression in most adenocarcinomas such as colon, breast and ovarian cancers (Ferreira et al., 2009). In the current study, controlled release and targeting property were employed by using polymeric NPs which is targeted by MUC1 aptamer. As cancer cells have high expression rates of MUC1 receptor, we specifically designed chitosan NPs as a carrier for SN38 and bioconjugated MUC1 aptamer to the surface of NPs.

2. Material and method

2.1. Materials

SN38 was purchased from Knowshine Pharmachemicals Inc. (Shanghai, China). Low molar mass chitosan with 90% degree of deacetylation was supplied from Primex (Karmoy, Norway). Pyridine, ethyl acrylate, sodium nitrate, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) were obtained from Merck (Darmstadt, Germany). Di-*tert*-butyl dicarbonate (BOC) (purity approximately 98%) was purchased from Sigma–Aldrich, Inc. (St. Louis, MO, USA). MUC1 aptamer (Apt) (DNA based and 5′-NH_2 modification) was supplied from TAG A/S (Copenhagen, Denmark). All other chemical reagents were of analytical grade.

2.2. Methods

2.2.1. Depolymerization of chitosan

Low molar mass chitosan was prepared as reported previously (Akhlaghi et al., 2010; Atyabi et al., 2008). Briefly, 10 mL nitrite sodium with different concentrations (2.7, 7, 14 mg/mL) was added to 100 mL of 2% (w/v) chitosan solution in 6% (v/v) acetic acid. The reaction was continued for 1 h at room temperature while stirring. The solution pH was adjusted up to 9 by adding NaOH (5 N) drop-wise to precipitate depolymerized chitosan. The white-yellowish precipitated chitosan was filtered and washed with acetone and dissolved in acetic acid 0.1 N. Purification was carried out by subsequent dialysis against deionized water (2 × 1 L for 90 min and 1 × 1 L overnight). The product was freeze dried (LyoTrap plus LTE Scientific, UK) and stored for further studies.

2.2.2. Synthesis of N-carboxyethyl chitosan (CS-AC)

N-Carboxyethyl chitosan ethyl ester (CS-EA) was synthesized according to the method reported by Sashiwa et al. (2003a). Briefly, 250 mg of prepared chitosan was dissolved in 2% (v/v) acetic acid and then diluted with 50 mL ethanol. 0.5 mL ethyl acrylate was then added to the solution (10 equivalents/NH_2), and the reaction was stirred for 48 h at 50 °C temperature. The product was dialyzed against deionized water for 2 days and lyophilized to obtain CS-EA. To convert the ester of the prepared product to carboxyl group, 150 mg of lyophilized CS-EA was dissolved in 4.5 mL acetic acid (5% v/v) and kept under stirring for two days at room temperature. The reaction was ended by adding solution of NaOH (1 M) drop-wise to precipitate CS-AC. The precipitate was filtered and dried under vacuum.

2.2.3. Synthesis of conjugated di-tert-butyl dicarbonate with SN38 (BOC–SN38)

BOC–SN38 (conjugated di-*tert*-butyl dicarbonate with SN38) was prepared as reported by Zhao et al. (2008). Briefly, 2.45 g of SN38 was suspended in 250 mL of anhydrous dichloromethane. Furthermore, 1.764 g di-*tert*-butyl dicarbonate and 15.2 mL pyridine were poured into the suspension and stirred overnight at room temperature. The solution was filtered through celite, washed by HCl (0.5 N) (3 × 150 mL) and saturated with $NaHCO_3$ (1 × 150 mL). Subsequently, the organic phase was dried over $MgSO_4$ and then filtered and evaporated under vacuum.

2.2.4. Conjugation of N-carboxyethyl chitosan and SN38 (CS-SN38)

Conjugation of SN38 to chitosan was achieved by dissolving 100 mg of CS-AC in 5 mL PBS (pH 5.8). The reaction was continued by the addition of 200 mg EDC (5.5 molar equivalents to carboxyl group of chitosan) and was carried on for 4 h while stirring at room temperature. Afterward, 105 mg NHS (5 molar equivalents to active carboxyl group of chitosan) was added, and the reaction was stirred for 24 h at room temperature. 350 mg BOC–SN38 was dissolved in 35 mL DMSO, and the aforementioned aqueous part was added drop-wise into the solution. The reaction continued for 24 h. The reaction was ended by adjusting the pH to 8. The solution was dialyzed against methanol, deionized water (1 × 1 L 2:1 for 4 h and 1 × 1 L 1:1 for 6 h and 1 × 1 L 1:2 overnight) and deionized water (1 × 1 L for 4 h). Afterwards, the solution was lyophilized to obtain the yellowish powder that resembled chitosan. The chemical structure and reaction preparation is shown in Fig. 1.

2.2.5. Molar mass measurement

The molar mass of depolymerized chitosan was measured by Zetasizer ZS (Nano-ZS, Malvern, Worcestershire, UK) instrument using the process of static light scattering (SLS) as follows: three different concentrations of the polymer (with unknown molar mass) were prepared, and the intensity of the scattered light was measured in similar way to dynamic light scattering. Static light scattering measures the time-averaged intensity of scattered light instead of measuring the time dependent fluctuations in the scattering intensity of the molar mass and 2nd virial coefficient of the polymer can be determined. The SLS theory is based on

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