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Research Paper

Bile acid-conjugated chondroitin sulfate A-based nanoparticles for tumor-targeted anticancer drug delivery

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ABSTRACT

Chondroitin sulfate A-deoxycholic acid (CSA-DOCA)-based nanoparticles (NPs) were produced for tumor-targeted delivery of doxorubicin (DOX). The hydrophobic deoxycholic acid (DOCA) derivative was conjugated to the hydrophilic chondroitin sulfate A (CSA) backbone *via* amide bond formation, and the structure was confirmed by ^{1}H-nuclear magnetic resonance (NMR) analysis. Loading the DOX to the CSA-DOCA NPs resulted in NPs with an approximately 230 nm mean diameter, narrow size distribution, negative zeta potential, and relatively high drug encapsulation efficiency (up to 85%). The release of DOX from the NPs exhibited sustained and pH-dependent release profiles. The cellular uptake of DOX from the CSA-DOCA NPs in CD44 receptor-positive human breast adenocarcinoma MDA-MB-231 cells was reduced when co-treated with free CSA, indicating the interaction between CSA and the CD44 receptor. The lower IC_{50} value of DOX from the CSA-DOCA NPs compared to the DOX solution was also probably due to this interaction. Moreover, the ability of the developed NPs to target tumors could be inferred from the *in vivo* and *ex vivo* near-infrared fluorescence (NIRF) imaging results in the MDA-MB-231 tumor-xenografted mouse model. Both passive and active strategies appear to have contributed to the *in vivo* tumor targetability of the CSA-DOCA NPs. Therefore, these CSA-DOCA NPs could further be developed into a theranostic nanoplatform for CD44 receptor-positive cancers.

1. Introduction

Anticancer agents ranged from small chemicals to biomacromolecules, targeting a wide range of cancer types, have been developed [1–4]. The intravenous route has been favored for most of these agents due to the rapid onset time, negligible drug loss during administration, and higher accessibility to the tumor region. However, drug administration without carriers has induced unwanted toxicity to normal tissues and organs. Therefore, efforts have been made to formulate anticancer drugs that can be delivered to the tumor site selectively [5–7].

Studies have shown that abnormal tumor vasculatures, such as the high ratio of proliferating endothelial cells, lack of pericyte, and unusual formation of basement membrane, can lead to enhanced vascular permeability [8]. This is also related to the immatured lymphatic system in the tumor region, resulting in insufficient drainage. Enhanced permeability and retention (EPR), which is a passive tumor targeting strategy, has thus been proposed based on the fact that nano-sized carriers can enter the tumor region and easily accumulate at that site [9]. EPR is based on the biological factors of tumor tissues and the physicochemical properties of the nanocarriers such as size, charge, and shape [10]. However, the lack of specificity for cancer targeting calls for selective ligands that have been adopted for interactions with receptors expressed in cancer tissues, as an active tumor targeting strategy [10,11].

An example of this active tumor targeting strategy is CD44 receptor-mediated targeting, which has been widely investigated in preparing nano-sized carriers [6,12,13]. The CD44 receptor is a cell surface molecule involved in the proliferation, differentiation, migration, and survival of cells. Ligands that have been reported for the CD44 receptor include hyaluronic acid (HA), osteopontin, collagens, and matrix metalloproteinases. For example [6,14,15], hyaluronic acid-ceramide (HACE)-based nanocarriers have been produced, and their functions *via* HA and CD44 receptor interaction for cancer diagnosis and therapy have been evaluated.

Chondroitin sulfate is classified as a linear, sulfated, and negatively charged glycosaminoglycan (GAG) [16]. Due to its similarity in structure to HA, chondroitin sulfate can also be used as a ligand for the CD44 receptor [17]. It is abundant in tendons, ligaments,

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the aorta, and the cartilage of vertebra [16,18]. Chondroitin sulfate has disaccharide repeating units, composed of glucuronic acid and *N*-acetyl galactosamine (GalNAc) with a β(1 → 3) or β(1 → 4) linkage. One of its isoforms, chondroitin sulfate A (CSA) composed of an alternating copolymer β-glucuronic acid-(1-3)-*N*-acetyl-β-galactosamine-4-sulfate was used in this study. In addition to its binding affinity for the CD44 receptor, its hydrophilicity, biocompatibility, and biodegradability can provide preferable characteristics for the preparation of nanocarriers for intravenous administration [19,20]. Thus, chondroitin sulfate-based nanocarriers have been developed and evaluated for anticancer drug delivery [18,20,21].

Deoxycholic acid (DOCA) is a secondary bile acid and metabolic byproduct of intestinal bacteria and has been used as a hydrophobic moiety in the development of nanoparticles (NPs) [22–24]. In this study, CSA as the hydrophilic backbone grafted with a DOCA derivative as the hydrophobic segment was synthesized for the production of self-assembled NPs. Herein, we report on the encapsulation of a hydrophobic anticancer drug into the CSA-DOCA NPs, their physicochemical properties and the drug release, cellular uptake, and *in vivo* tumor targetability.

2. Methods and materials

2.1. Materials

Chondroitin sulfate A (CSA; approximately 37 kDa), deoxycholic acid (DOCA), ethylenediamine (EDA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), *N*-hydroxysuccinimide (NHS), pyrene, triethylamine (TEA), and deuterium oxide (D_2O) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Doxorubicin hydrochloride (DOX) was obtained from Boryung Pharmaceutical Co., Ltd. (Seoul, Korea). FCR-675 amine, a near-infrared fluorescent (NIRF) dye, was purchased from BioActs (Incheon, Korea). Dimethyl sulfoxide-d_6 (DMSO-d_6) was purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). Dulbecco's modified Eagle's medium (DMEM), RPMI 1640 cell culture medium, penicillin, streptomycin, and fetal bovine serum (FBS) were obtained from Gibco Life Technologies, Inc. (Carlsbad, CA, USA). All other reagents were of analytical grade.

2.2. Synthesis and characterization of CSA-DOCA

CSA grafted with DOCA (CSA-DOCA) was synthesized *via* amide bond formation (Fig. 1). First, DOCA was modified with EDA to form aminoethyldeoxycholamide (EtDOCA) for subsequent reaction with the carboxylic acid group of CSA. Briefly, DOCA (1.177 g; 3.0 mmol) was dissolved in methanol (MeOH; 5 ml) mixed with hydrochloric acid (36.5–38.0%; 0.18 ml). The mixture was stirred, refluxed for 6 h at 60°C, concentrated in an evaporator, and thoroughly dried under vacuum. The resultant white powder was washed with ice-cold water and lyophilized to obtain methyldeoxycholate (DOCA-OMe). DOCA-OMe was dissolved in EDA (50 times molar equivalent). The solution was stirred for 8 h at 120°C under reflux before cooling to room temperature. The mixture was precipitated with water and filtered. The filtrate, EtDOCA, was washed with excess water three times and freeze-dried.

CSA was then hydrophobically modified with EtDOCA to make amphiphilic CSA-DOCA. Briefly, CSA (100 mg) was dissolved in formamide (20 ml) at 80°C and cooled to room temperature, followed by the addition of EDC (36.8 mg) and NHS (61.3 mg). The mixture was stirred for 20 min at room temperature to activate the carboxylic acid groups of CSA. EtDOCA (34.8 mg) dissolved in DMF (20 ml) was slowly added to the CSA solution. The reaction mixture was stirred for 20 h at room temperature, dialyzed against a water/methanol mixture (from 25:75 to 75:25, v/v) for 1 day and water for 2 days, and freeze-dried. The resulting white powder, CSA-DOCA, was stored at 2–8°C for further experiments.

For the determination of the DOCA content in CSA-DOCA, ^{1}H-nuclear magnetic resonance (^{1}H-NMR; Varian FT-500 MHz;

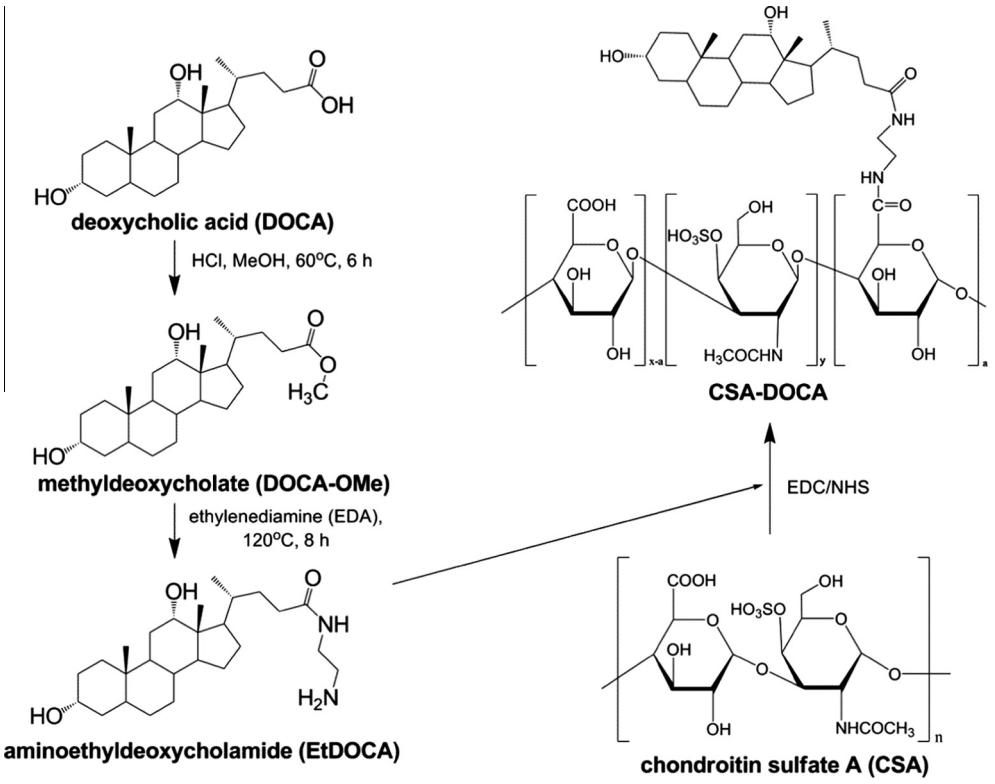

Fig. 1. Synthetic scheme of CSA-DOCA.