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Chemosensitizing indomethacin-conjugated chitosan oligosaccharide nanoparticles for tumor-targeted drug delivery

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ABSTRACT

A chitosan oligosaccharide (CSO)-indomethacin (IDM) conjugate (CI) was synthesized to fabricate chemosensitizing nanoparticles (NPs) for tumor-targeted drug delivery. IDM was conjugated to a CSO backbone *via* amide bond formation, of which successful synthesis was confirmed by proton-nuclear magnetic resonance analyses. Doxorubicin (DOX)-loaded CI (CI10/DOX; CI:DOX = 10:1 [w/w]) NPs with <75 nm of mean diameter, polydispersity index of ~0.2, and positive zeta potential were prepared. The release of DOX from the NPs was enhanced at acidic pH (pH 5.5 and 6.8) compared to physiological pH (pH 7.4). The release of IDM increased in the presence of A549 cell lysates. In A549 cells (human lung carcinoma cells), more efficient cellular uptake of CI10/DOX NPs than that of free DOX was observed by using confocal laser scanning microscopy and flow cytometry. The *in vitro* cytotoxicity of CI10/DOX NPs in A549 cells was higher than those of free DOX and CI NPs with free DOX groups. *In vivo* pharmacokinetic studies after intravenous administration in rats showed significantly lower clearance of DOX from NPs compared with the free DOX group. Tumor targetability of the developed CI NPs was also verified by a real-time optical imaging study. In summary, the chemosensitizing CI/DOX NP with enhanced anticancer activity, prolonged blood circulation, and passive tumor targeting can be a promising anticancer drug delivery system for tumor-targeted therapy.

Statement of Significance

Chemosensitizing nanoparticles (NPs) based on amphiphilic chitosan oligosaccharide-indomethacin (CSO-IDM; CI) conjugate were developed for tumor-targeted delivery of doxorubicin (DOX). IDM was introduced to the CSO backbone as a hydrophobic residue to synthesize an amphiphilic conjugate and a chemosenstizer of DOX for improving antitumor efficacies. IDM, conjugated to CSO, may inhibit the efflux of cellular uptaken DOX via multidrug resistance-associated protein (MRP) and subsequently augment the anti-proliferation potentials of DOX in A549 cells (MRP-expressed human lung cancer cells). Chemosensitizing properties of developed CI NPs were assessed in cell culture models and the tumor targetability of CI/DOX NPs was demonstrated in A549 tumor-xenografted mouse model by a real-time optical imaging. Developed CI NPs can be used as a multifunctional nanosystem for the therapy of MRP-expressed cancers.

1. Introduction

Multidrug resistance (MDR) is one of the most significant hurdles in anticancer drug delivery [1,2]. Its mechanisms have been found to be mainly related to P-glycoprotein (P-gp), also known as the multidrug resistance-associated protein (MRP). MRP is commonly overexpressed in cancer cell membrane, and its main role is

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to pump out the anticancer drugs with broad spectrum, thus maintaining low drug levels in the cytoplasm [3].

Interestingly, in MRP-expressed cells, several non-steroidal anti-inflammatory drugs (NSAIDs) including indomethacin (IDM) at non-toxic levels have been reported to exhibit enhancement of cytotoxic effects of many anticancer agents which are MRP substrates. This is well-known as the "chemosensitizing effect" [1]. A study demonstrated that the enhancement of doxorubicin (DOX) and vincristine cytotoxicity by IDM was due to the inhibition of MRP pumping and glutathione-S-transferase activities, and the consequent reversal of MRP-mediated efflux of anticancer drugs [4].

In this study, the possibility of introducing a chemosensitizing agent in chitosan oligosaccharide (CSO)-based nanoparticles (NPs) with an anticancer agent (*i.e.,* DOX) has been explored. Conjugating IDM as a chemosensitizing agent to CSO could result in an amphiphilic polymer for site-specific drug delivery of self-assembled NPs. In an aqueous *milieu*, amphiphilic polymers which have been widely investigated can form distinctive structures, composed of hydrophilic shell and hydrophobic core [5–8]. The hydrophilic shell of NPs composed of CSO, for example, can provide less recognition and elimination by the reticuloendothelial system (RES). Their nano-size could also give them an enhanced permeability and retention (EPR) effect, which can lead to the effective accumulation of drugs at tumor sites by passive targeting mechanism. Hydrophobic drugs (*i.e.,* DOX) can be loaded into the inner core of micelle-like nanocarriers, and their aqueous solubility could be improved [7–9]. These structural properties of self-assembled NPs may guarantee an effective tumor-targeted delivery of poorly water-soluble anticancer drugs [10,11].

Chitosan (CS), produced by the *N*-deacetylation of chitin, is a polycationic natural polymer composed of *N*-acetylglucosamine (GlcNAc) and glucosamine (GlcN) residues. CS has come under the spotlight as a biomaterial for its biocompatibility with mammalian cells [12,13]. In addition, its hydrophilic and mucoadhesive properties also can be advantages for the development of various drug delivery systems [14,15]. As the reactive amino groups on the GlcN units of CS can be modified chemically, various CS derivatives have been synthesized [16–19]. Conjugating IDM to the CSO backbone may prolong its blood circulation and subsequently enhance tumor targeting efficiency of DOX, compared to when DOX is administered with free IDM (IDM solution). Moreover, the uptake of IDM contained in the structure of NPs could be improved in cancer cells, followed by the augmented chemosensitizing effects that will be produced by inhibiting the efflux functions of MRP. The simultaneous arrival of DOX and IDM in the tumor region can be accomplished by DOX-loaded CSO-IDM (CI) NPs, which may guarantee improved antitumor efficacies after intravenous administration.

Therefore, herein, we report on the preparation and evaluation of the amphiphilic CI conjugate, where IDM as a chemosensitizing hydrophobic moiety was conjugated to the CSO backbone. This conjugate would form self-assembled NPs in the aqueous environment where DOX can be loaded into their hydrophobic cavity. After intravenous injection, NPs are expected to be highly localized near the tumor tissue by the EPR effect, after which IDM released from the NPs would enhance the cytotoxicity of DOX by chemosensitizing effect (Fig. 1). The physicochemical properties of drug-loaded NPs and the *in vitro* DOX and IDM release patterns were studied. Cytotoxicity and cellular uptake efficiency in human lung adenocarcinoma (A549) cells, *in vivo* pharmacokinetics of DOX in rats, and *in vivo* near-infrared fluorescence (NIRF) imaging in a mouse model were also investigated.

2. Materials and methods

2.1. Materials

CSO (average molecular weight: ~5 kDa, degree of deacetylation >90%) was obtained from Kitto Life Co., Ltd. (Seoul, Korea). IDM, triethylamine (TEA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), *N*-hydroxysuccinimide (NHS), pyrene, 4-(2-hydro

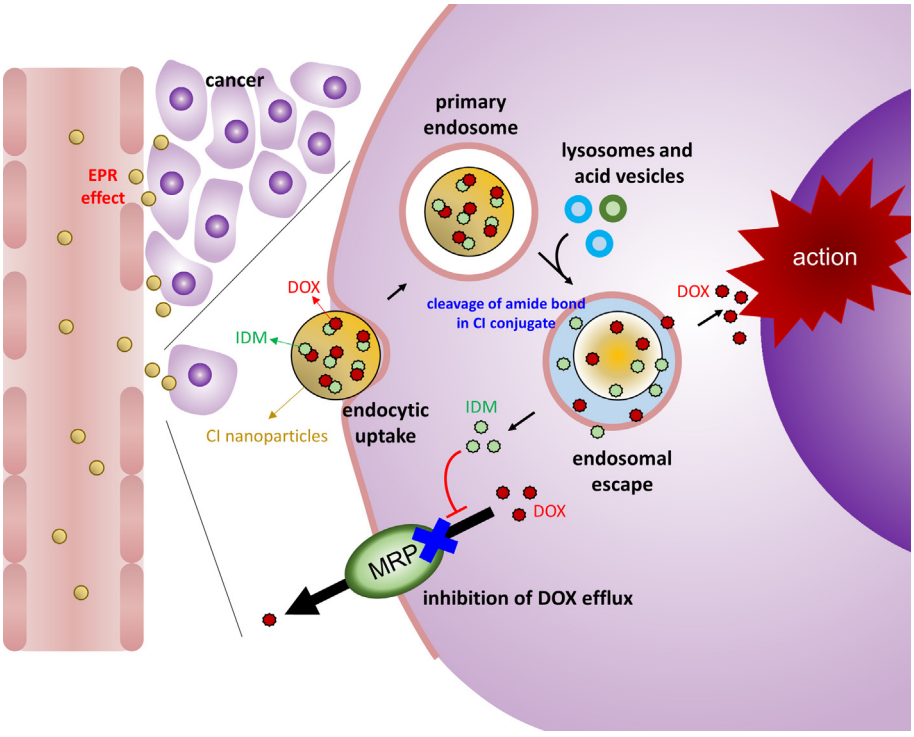

Fig. 1. Schematic illustration of the chemosensitizing mechanism of action of CI/DOX NPs for cancer therapy.