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Hypoxia-responsive polymeric nanoparticles for tumor-targeted drug delivery

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ABSTRACT

Hypoxia is a condition found in various intractable diseases. Here, we report self-assembled nanoparticles which can selectively release the hydrophobic agents under hypoxic conditions. For the preparation of hypoxia-responsive nanoparticles (HR-NPs), a hydrophobically modified 2-nitroimidazole derivative was conjugated to the backbone of the carboxymethyl dextran (CM-Dex). Doxorubicin (DOX), a model drug, was effectively encapsulated into the HR-NPs. The HR-NPs released DOX in a sustained manner under the normoxic condition (physiological condition), whereas the drug release rate remarkably increased under the hypoxic condition. From *in vitro* cytotoxicity tests, it was found the DOX-loaded HR-NPs showed higher toxicity to hypoxic cells than to normoxic cells. Microscopic observation showed that the HR-NPs could effectively deliver DOX into SCC7 cells under hypoxic conditions. *In vivo* biodistribution study demonstrated that HR-NPs were selectively accumulated at the hypoxic tumor tissues. As consequence, drug-loaded HR-NPs exhibited high anti-tumor activity *in vivo*. Overall, the HR-NPs might have a potential as nanocarriers for drug delivery to treat hypoxia-associated diseases.

1. Introduction

Hypoxia, a pathological condition in which tissue is deprived of a supply of adequate oxygen, is a hallmark of various intractable diseases such as cancer, cardiopathy, ischemia, rheumatoid arthritis, and vascular diseases [1,2]. For example, experimental and clinical studies have demonstrated that tissue partial pressures of oxygen, measured from ischemic stroke and cancer patients, are near zero mm Hg, which is substantially lower than in normal tissue (~30 mm Hg) [2,3]. Since hypoxia is involved in many aspects of the biology of such diseases, it significantly affects therapeutic responses. In particular, hypoxia is a negative factor for cancer therapy as it contributes to chemoresistance, radioresistance, angiogenesis, invasiveness, and metastasis [4]. Nevertheless, owing to its unique features, which are rarely seen in normal tissue, hypoxia is emerging as a primary target in the development of diagnostic agents and therapeutic drugs. Representative approaches for hypoxia-targeted cancer therapies are based on regulation of hypoxia inducible factor-1 [5] and on the use of bioreductive prodrugs that can be activated in the reductive environment of hypoxia [2,4]. For hypoxia imaging, many nitroaromatic or quinone derivatives with hypoxia-responsive moieties have been employed in the molecular design of diagnostic agents [6–9]. Of the derivatives investigated to date, 2-nitroimidazoles (NIs) have been most widely utilized in the development of imaging agents and bioreductive prodrugs because of their high sensitivity to hypoxia [10,11]. It has been demonstrated that, under hypoxic conditions, NIs are converted to hydrophilic 2-aminoimidazoles *via* a series of selective bioreductions, which are highly reactive to macromolecules in hypoxic tissues [11,12]. There,

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however, are no literature available for targeted drug delivery systems using hypoxia-responsive nanoparticles.

Self-assembled polymeric nanoparticles composed of amphiphilic polymers have emerged as promising nanocarriers for various anticancer drugs [13–16]. They exhibit unique characteristics as drug carriers, including enhancement of drug solubility, high thermodynamic stability, and preferential accumulation in tumor tissue *via* the enhanced permeation and retention (EPR) effect [14,17]. Conventional nanocarriers, however, often show limited anti-tumor efficacy because they release the drug in a sustained manner even at the target site of action [18]. Polymeric materials that respond to the pathophysiological conditions in tumors have been recently utilized to construct nanoparticles for drug delivery with enhance therapeutic efficacy [19,20]. Such stimuli-responsive nanocarriers are expected to reach the tumor site *via* the EPR effect and release the drug rapidly when they are exposed to tumor tissue [21]. To date, many stimuli for the development of smart nanocarriers have been explored, including ultraviolet radiation [22,23], glutathione [24–26], pH [27–29], and temperature [30–32]. Some of these stimuli-responsive drug carriers have advanced to clinical trials [33].

2. Materials and methods

2.1. Materials

Carboxymethyl-dextran sodium salt (CM-Dex, M_n = 10000–20000 Da), 1-ethyl-3(3-dimethylaminopropyl)carbodiimide (EDC), *N*-hydroxysuccinimide (NHS), 2-nitroimidazole (NI), doxorubicin·hydrochloride (DOX·HCl), 1.25 M HCl in methanol, and 6-(Boc-amino)hexyl bromide were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). The water used in the experiments was prepared by an AquaMax-Ultra water purification system (Younglin Co., Anyang, Korea). All other chemicals were of analytical grade and used without further purification.

2.2. Synthesis of hypoxia-responsive conjugate

In this study, we attempted to prepare hypoxia-responsive nanoparticles (HR-NPs) that allow for the facilitated release of hydrophobic drugs at hypoxic tumor tissue (Fig. 1). In order to prepare the amphiphilic conjugate which can form HR-NPs in an aqueous condition, the NI derivative was chemically conjugated to the backbone of water-soluble CM-Dex through amide formation (Fig. 2). First, NI was converted to 6-(2-nitroimidazole)hexylamine for reaction with the carboxylic acids of CM-Dex (Fig. S1). In brief, NI (0.6 g, 5.3 mmol) was dissolved in DMF, to which K_2CO_3 (1.1 g, 7.95 mmol) was added. 6-(Boc-amino)hexyl bromide (1.56 g, 5.57 mmol) in DMF was then added dropwise and stirred at room temperature (RT) overnight. The reaction mixture was filtered and washed with methanol, after which the residual solvent was evaporated. The solid obtained was suspended in water and extracted with ethyl acetate. The organic layer was separated, dried over sodium sulfate, and concentrated to obtain the product for step 1. The resulting product was dissolved in methanol and cooled to 0 °C, to which 10 ml of 1.25 M HCl in methanol was added and stirred at RT for 24 h. The solvent was removed from the reaction mixture using a rotary evaporator. The crude solid was recrystallized from ethanol to obtain amine-functionalized 2-nitroimidazole (NI derivative).

Next, the NI derivative was conjugated to CM-Dex in the presence of EDC and NHS (Fig. 2). In brief, CM-Dex (0.2 g, 0.9 mmol) was dissolved in a 1:1 mixture of formamide and dimethyl formamide, after which EDC (0.6–2.07 g, 3.6–10.88 mmol) and NHS (0.41–1.24 g, 3.6–10.88 mmol) were added and stirred for 15 min. The NI derivative (0.19–0.573 g, 0.9–2.7 mmol) in DMF was slowly added to the reaction mixture and stirred for 1 day. The resulting solution was dialyzed against an excess of water/methanol (1/3–1/1 v/v) for 1 day and against distilled water for 2 days before being lyophilized. The amount of NI derivative to CM-Dex was spectrophotometrically determined from the characteristic peak of the NI derivative at 325 nm using a UV–vis spectrophotometer (Optizen 3220UV, Mecasys Co., Ltd., Daejeon, Korea).

For cellular experiments and animal tests, Cy5.5-labeled hypoxia-responsive nanoparticles (Cy5.5-HR-NPs) were prepared, as previously described [17].

2.3. Characterization

The chemical structures of the NI derivatives and the conjugate were characterized using ^{1}H NMR (JNM-AL300, JEOL, Tokyo, Japan) operating at 300 MHz, for which the samples were dissolved in CD_3OD, D_2O, or DMSO-D_6. The sizes of the

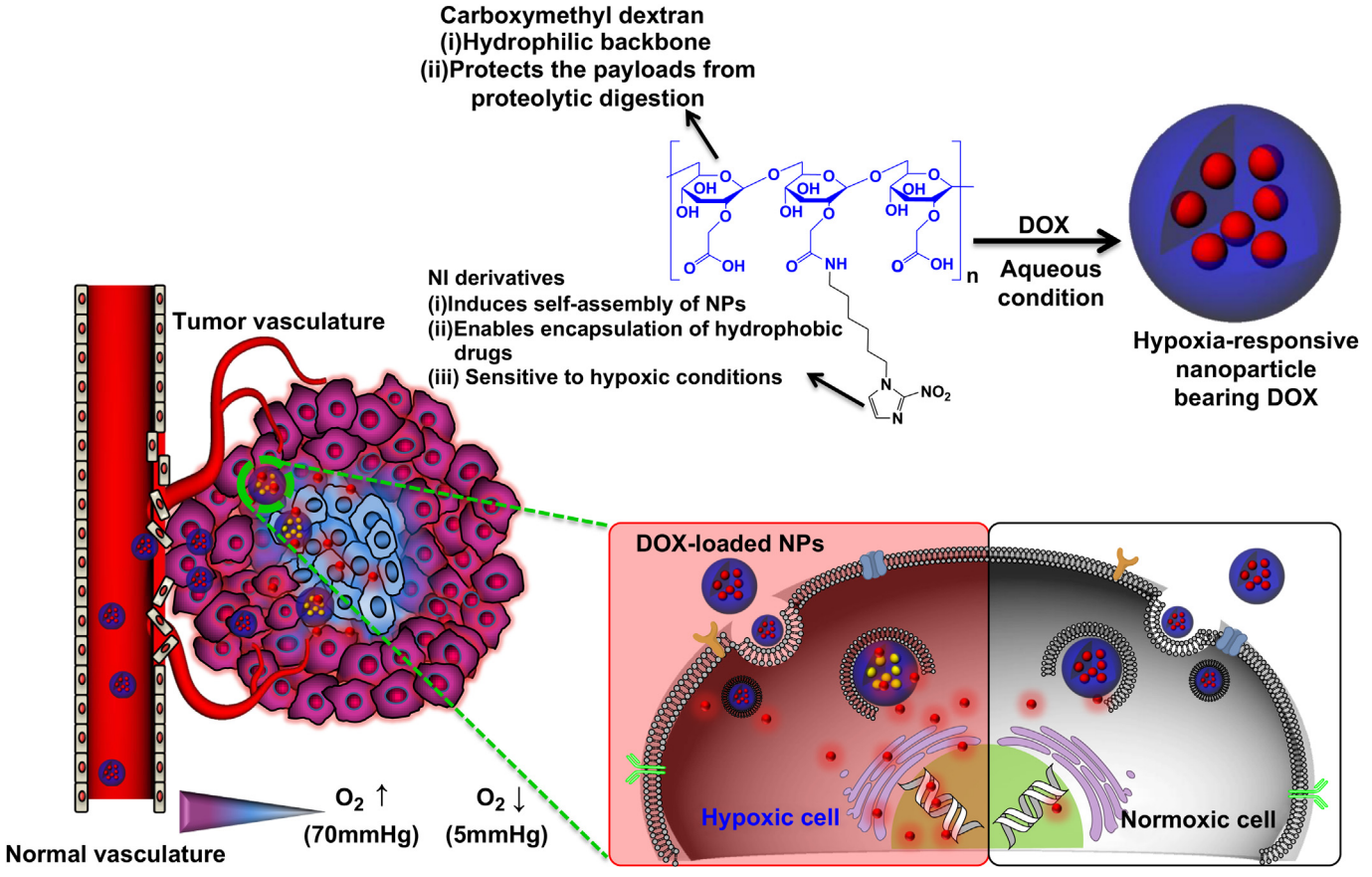

Fig. 1. Schematic illustration of the formation of drug-loaded HR-NPs and *in vivo* tumor-targeting pathways. The HR-NPs can reach the tumor site *via* the EPR effect, followed by intracellular drug release at hypoxic tissue.