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Boronic acid-tethered amphiphilic hyaluronic acid derivative-based nanoassemblies for tumor targeting and penetration

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ABSTRACT

(3-Aminomethylphenyl)boronic acid (AMPB)-installed hyaluronic acid–ceramide (HACE)-based nanoparticles (NPs), including manassantin B (MB), were fabricated for tumor-targeted delivery. The amine group of AMPB was conjugated to the carboxylic acid group of hyaluronic acid (HA) *via* amide bond formation, and synthesis was confirmed by spectroscopic methods. HACE-AMPB/MB NPs with a 239-nm mean diameter, narrow size distribution, negative zeta potential, and >90% drug encapsulation efficiency were fabricated. Exposed AMPB in the outer surface of HACE-AMPB NPs (in the aqueous environment) may react with sialic acid of cancer cells. The improved cellular accumulation efficiency, *in vitro* antitumor efficacy, and tumor penetration efficiency of HACE-AMPB/MB NPs, compared with HACE/MB NPs, in MDA-MB-231 cells (CD44 receptor-positive human breast adenocarcinoma cells) may be based on the CD44 receptor-mediated endocytosis and phenylboronic acid-sialic acid interaction. Enhanced *in vivo* tumor targetability, infiltration efficiency, and antitumor efficacies of HACE-AMPB NPs, compared with HACE NPs, were observed in a MDA-MB-231 tumor-xenografted mouse model. In addition to passive tumor targeting (based on an enhanced permeability and retention effect) and active tumor targeting (interaction between HA and CD44 receptor), the phenylboronic acid-sialic acid interaction can play important roles in augmented tumor targeting and penetration of HACE-AMPB NPs.

Statement of Significance

(3-Aminomethylphenyl)boronic acid (AMPB)-tethered hyaluronic acid-ceramide (HACE)-based nanoparticles (NPs), including manassantin B (MB), were fabricated and their tumor targeting and penetration efficiencies were assessed in MDA-MB-231 (CD44 receptor-positive human adenocarcinoma) tumor models. MB, which exhibited antitumor efficacies *via* the inhibition of angiogenesis and hypoxia inducible factor (HIF)-1, was entrapped in HACE-AMPB NPs in this study. Phenylboronic acid located in the outer surface of HACE-AMPB/MB NPs (in the aqueous *milieu*) may react with the sialic acid overexpressed in cancer cells and intramolecular B O bond can be formed. This phenylboronic acid-sialic acid interaction may provide additional tumor targeting and penetration potentials together with an enhanced permeability and retention (EPR) effect (passive tumor targeting) and HA-CD44 receptor interaction (active tumor targeting). Developed HACE-AMPB NP may be one of promising nanocarriers for the imaging and therapy of CD44 receptor-expressed cancers.

1. Introduction

Accurate drug delivery to tumor regions has been regarded as one of major tasks in cancer therapy. It can improve antitumor efficacies and reduce unwanted effects of anticancer agents.

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Ultimately, it can contribute to extensions in the survival rate of patients. Nano-sized carriers have been investigated as principal delivery approaches in the case of intravenous administration route [1–3]. Due to the leaky vasculatures and immature lymphatic system of tumor tissue, macromolecules can be easily extravasated and accumulated in tumor region [4]. This phenomenon, called the enhanced permeability and retention (EPR) effect, can enable nanomedicines to be distributed in tumor region (generally referred to as "passive tumor targeting") [5]. However, passive tumor targeting based on the pathophysiological characteristics of the tumor region also has pitfalls in tumor-targeted drug delivery. The lack of tumor specificity may hamper the precise delivery of anticancer drugs to a tumor region. To overcome the limit of the EPR effect, ligand-receptor interactions have been introduced as an active tumor targeting strategy [6]. Ligand-modified nanocarriers (*i.e.,* micelles, nanoparticles, and liposomes) have displayed marked improvement in antitumor efficacies by a combination of passive and active tumor targeting strategies [7–13].

However, the delivery of nanocarriers to the boundary of tumor tissue cannot ensure deep penetration and homogeneous distribution [14]. Due to high density of tumor cells and high interstitial fluid pressure (IFP), nanocarriers can cross only a few layers. Treatment with extravasation- and penetration-enhancing inflammatory mediators [*e.g.,* tumor necrosis factor (TNF)-α], inhibitors of fibrosis, and matrix-degradation enzymes can enhance penetration efficiency and subsequent antitumor efficacies [6]. Interestingly, several functional groups or enzymes are introduced to nanocarriers for efficient tumor penetration [15–19]. Among them, phenylboronic acid has been adopted as a tumor targeting ligand and penetration moiety [20–22]. During tumorigenic and metastatic processes, the glycosylation of proteins and lipids on the cell surface can be altered [23]. The increase of sialyltransferases may improve the expression of the terminal sialic acid residue of glycans on cancer cells [23,24]. Reversible cyclic boronate esters can be formed between the exocyclic polyol group of sialic acid and boronic acid group of phenylboronic acid [24]. Intramolecular B—O bond formation between phenylboronic acid and sialic acid (expressed in cancer) is known as one of main binding mechanisms [24,25]. In our previous report [20], (3-aminomethylphenyl)boronic acid (AMPB) was installed to chondroitin sulfate-A derivative-based nanoparticles (NPs), *via* amide bond formation, and they exhibited improved tumor targeting and penetration efficiency in CD44 receptor-expressed cancers.

In this investigation, AMPB-conjugated hyaluronic acid-ceramide (HACE) was synthesized and HACE-AMPB NPs were fabricated for tumor targeting and penetration. To elevate tumor targetability of NPs, several functional groups were attached to HACE conjugates, or biofunctional materials were mixed with HACE to prepare NPs [1,26–29]. The carboxylic acid group of HA has been mainly used for covalent bonding with amine groups or hydroxyl groups, resulting in amide or ester bond formation. As an anticancer agent, manassantin B (MB) was encapsulated into the HACE-AMPB NPs in this study. MB can be extracted from *Saururus chinensis*, and it has several potentials for cancer therapy such as inhibition of tumor-induced angiogenesis, inhibitory activities against DNA topoisomerase I and II, and inhibition of hypoxia-inducible factor-1 [30–32]. The anti-proliferation potentials of MB delivered with HACE-AMPB NPs were evaluated in MDA-MB-231 cells (CD44 receptor-positive human breast adenocarcinoma cells). Herein, the physicochemical properties of NPs (for passive tumor targeting), HA-CD44 receptor interaction (for active tumor targeting), and phenylboronic acid-sialic acid interaction (tumor targeting and penetration) were assessed in *in vitro* and *in vivo* models.

2. Materials and methods

2.1. Materials

The HA oligomer (4.7 kDa) and DS-Y30 (ceramide 3B; mainly *N*-oleoylphytosphingosine) were purchased from Bioland Co., Ltd. (Cheonan, Republic of Korea) and Doosan Biotech Co., Ltd. (Yongin, Republic of Korea), respectively. Adipic acid dihydrazide (ADH), chloromethylbenzoyl chloride, coumarin 6 (C6), deuterium oxide (D_2O), hexadeuterodimethyl sulfoxide (DMSO-d_6), *N*-hydroxysuccinimide (NHS), *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC), tetra-*n*-butylammonium hydroxide (TBA), 1-hydroxybenzotriazole (HOBt), and AMPB were purchased from Sigma–Aldrich (Saint Louis, MO, USA). Cy5.5-amine (FCR-675 amine) was purchased from BioActs (DKC Corp., Incheon, Korea). RPMI 1640 (developed by Roswell Park Memorial Institute), penicillin, streptomycin, and heat-inactivated fetal bovine serum (FBS) were obtained from Gibco Life Technologies, Inc. (Grand Island, NY, USA). All other reagents were of analytical grade and purchased from commercial sources.

2.2. Synthesis and characterization of HACE-AMPB

HACE was synthesized according to the reported method [26]. Briefly, HA (12.21 mmol) and TBA (9.77 mmol) were added to double-distilled water (DDW; 60 mL) and stirred for 30 min. Activated HA-TBA was acquired by freeze-drying. To synthesize the DS-Y30 linker, DS-Y30 ceramide (8.59 mmol) and triethylamine (9.45 mmol) in tetrahydrofuran (THF; 25 mL) and 4-chloromethylbenzoyl chloride (8.59 mmol) in THF (10 mL) were mixed. It was stirred for 6 h at 60 °C, and DS-Y30-containing linker was acquired by concentration and recrystallization. HA-TBA (8.10 mmol) and DS-Y30-containing linker (0.41 mmol) were dissolved in the mixture of THF and acetonitrile (4:1, *v/v*) and stirred for 5 h at 40 °C. HACE was obtained by eliminating impurities and organic solvents. HACE-AMPB was synthesized by an EDC/NHS-coupled reaction between the —COOH group of HA and the —NH_2 group of AMPB. HACE (84.8 mg) was dissolved in dimethyl sulfoxide (DMSO; 20 mL), and the pH was adjusted to 4 by adding 1N HCl. EDC (81.9 mg, 0.427 mmol) and NHS (49.2 mg, 0.427 mmol) were added to that solution, and it was stirred for 15 min. By adding 1N NaOH to that solution, the pH value was adjusted to 7. AMPB (40 mg, 0.213 mmol) was solubilized in DMSO (6 mL) and then slowly added to the HACE/EDC/NHS solution. This mixture was stirred for 24 h and dialyzed against distilled water (DW) for 2 days within a dialysis membrane [molecular weight cut-off (MWCO): 3.5 kDa]. The dialyzed resultant was lyophilized for further uses.

The synthesis of HACE-AMPB was verified by proton-nuclear magnetic resonance (^{1}H-NMR), Fourier-transform infrared (FT-IR), and fluorescence spectroscopic analysis. HACE-AMPB was dissolved in the mixture of D_2O and DMSO-d_6 (1:1, *v/v*) for ^{1}H-NMR (Varian FT-500 MHz; Varian, Inc., Palo Alto, CA, USA) analysis. The content of AMPB in HACE-AMPB was determined from the regression line of the integration ratio (7.3–7.4 ppm to 1.8 ppm) to weight ratio between AMPB and HACE. Peaks at 7.3–7.4 ppm and 1.8 ppm indicated AMPB and HA, respectively. The synthesis of HACE-AMPB was verified by FT-IR spectroscopy with an attenuated total reflectance (ATR) accessary (Frontier FT-IR, PerkinElmer Inc., Waltham, MA, USA). Transmittance (%) of HACE-AMPB was scanned in the 400–4000 cm^{-1} wavenumber range. The content of AMPB in HACE-AMPB was also measured by fluorescence intensity. HACE (1 mg/mL), AMPB (20–500 μM), or HACE-AMPB (1 mg/mL) was dissolved in the mixture of MeOH and DW (1:1, v/v). The excitation wavelength was fixed at 270 nm, and the emission spectra were obtained at wavelengths from 280–500 nm