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Comparison of adsorption and conjugation of Herceptin on poly(lactic-*co*-glycolic acid) nanoparticles – Effect on cell internalization in breast cancer cells

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ABSTRACT

Surfaces of nanoparticles with are commonly modified to enhance the targeting effect. In this study, we performed surface modifications of docetaxel (DTX)-loaded poly(lactic-*co*-glycolic acid) nanoparticles (PNPs) with Herceptin® (HCT) to improve the internalization and cytotoxicity in breast cancer cells. The PNPs were prepared with surfactant, poly(ethylene-*alt*-maleic anhydride) (PEMA), including a number of carboxyl groups for conjugation. Three types of PNPs were prepared via different methods such as adsorption, charged adsorption, and bio-conjugation. The PNPs were evaluated in terms of physical properties, stability, cellular uptake and cytotoxicity. The docetaxel-loaded PNPs with HCT were successfully surface-modified with mean particle sizes of 338.4 ± 59.8 nm (DTX-PNPs), 353.9 ± 67.5 nm (HCT-A-DTX-PNPs), 544.8 ± 301.7 nm (HCT-C-DTX-PNPs), and 499.1 ± 71.9 nm (HCT-B-DTX-PNPs). Cellular uptake of HCT-B-PNPs was 5.0-, 4.4-, and 4.6-fold higher than that of PNPs in BT-474, SK-BR-3, and MCF-7 cells, respectively, at 2 h. At 40 μg/mL, HCT-B-DTX-PNPs showed a higher cytotoxicity toward BT-474, SK-BR-3, and MCF-7 cells than the other formulations. In conclusion, HCT-B-DTX-PNPs were found to possess a higher affinity for breast cancer cells and induce a stronger cytotoxicity than that of other PNPs.

1. Introduction

Most anti-cancer drugs have limited pharmaceutical use due to their poor water solubility. To improve drug solubility, many formulations have been developed, such as solid dispersions [1, 2], nanoparticles and microparticles (NPs and MPs) [3, 4], solid lipid nanoparticles [5, 6], liposomes [7], micelles [8] and drug nanocrystals (NCs) [9, 10]. Since anti-cancer drugs have no targeting ability, they cannot readily reach cancer sites as well as can cause severe systemic toxicity to normal cells [11]. Recently, drug delivery systems (DDS) have widely adopted NP based systems because of their ability to target the cancer site via enhanced permeability and retention (EPR) effects. Likewise, because cancer vasculature has unique properties, the NPs of size ranges 100–500 nm can efficiently pass through the cancer site [12, 13]. Since passive targeting of drugs does not utilize a targeting moiety, it has low drug efficiency at cancer sites [14]. Therefore, in addition to passive targeting, active targeting is needed to improve drug efficiency in tumors [15].

Surface modification of NPs has improved the active targeting and controlled release of drugs. The following agents have been widely used to modify the surface of NPs. Poly(ethylene glycol) (PEG), which is hydrophilic and nontoxic polymer, has been used to modify NPs (PEGylated NPs) for prolonged circulation time (i.e., stealth) in the body [16]. Hyaluronic acid (HA), which targets CD44-overexpressing cancer cells, also is hydrophilic, biocompatible, and nontoxic [17]. Transferrin (Tf) has similar advantageous properties because cancer cells can overexpress the Tf-receptor (TfR); thus, Tf-NPs have been widely used for cancer therapy [18, 19]. Herceptin® (HCT) is a humanized IgG(1) kappa monoclonal antibody that specifically binds to human epidermal growth factor receptor 2 (HER2/neu); thus, HCT is widely used for targeting HER2-positive breast cancer cells [20]. HCT has been approved by the United States Food and Drug Administration

Abbreviations: HCT, Herceptin®; FE-TEM, field emission transmission electron microscopy; FT-IR, Fourier transform infrared; PBS, phosphate-buffered saline; DW, distilled water; PEMA, poly(ethylene-alt-maleic anhydride); PVA, poly(vinyl alcohol); PNPs, poly(lactic-co-glycolytic acid) nanoparticles; HCT-A-(DTX)-PNPs, Herceptin® adsorbed on (docetaxel loaded) PNPs; HCT-C-(DTX)-PNPs, Herceptin® adsorbed on PAH adsorbed on (docetaxel loaded) PNPs; HCT-B-(DTX)-PNPs, Herceptin® bio-conjugated on (docetaxel loaded) PNPs; PDI, polydispersity index

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(US FDA) for the treatment of breast and gastric cancers [21]. HCT was administered with paclitaxel (PTX) and docetaxel (DTX) in combination therapy, and the results showed an increased overall survival compared with that of HCT monotherapy [22]. Recently, HCT-conjugated or -adsorbed NPs have been widely studied, including dendrimers [23, 24], polymeric NPs [25, 26], and NCs [10, 27].

DTX is commonly selected as a model drug to be formulated in NPs. DTX, a member of the taxane class, which also includes PTX, has shown anti-cancer activity against various cancer types, such as breast, head and neck, gastric, and hormone-refractory prostate cancers; it has been approved by the US FDA [28]. A critical limitation of DTX is its poor water solubility. Thus, commercial products (e.g., Taxotere®) contain a mixture of dehydrated alcohol and Tween® 80 (polysorbate 80) that increases drug solubility. However, Tween® 80 can induce toxicological side effects, such as acute hypersensitivity reactions and peripheral neuropathy, accumulative fluid retention [29]. Poly(lactic-*co*-glycolic acid) (PLGA) is a biodegradable, biocompatible, and non-toxic polymer. It was approved by the USFDA and the European Medicines Agency [30]. Many researchers have developed drug-loaded NPs and MPs for disease treatment using PLGA [3, 31]. PLGA formulations were developed commercially as Lupron Depot® and Zoladex® (both injectable and implantable) [32]. Poly(allylamine hydrochloride) (PAH) has a positive charge and is widely used in nano and film systems, particularly in the drug and polymer electrolyte layer (LbL) nanoshell, which plays a crucial role in NP stability [33].

In this study, we have developed several surface-modified PNPs to compare their effect on cell internalization with that of HCT. Three categories of surface modification methods were selected: 1) an adsorption method, 2) a charged adsorption method, and 3) a bio-conjugation method. To compare the surface modification methods, four types of NPs were evaluated for their physical properties, namely particle morphology, particle size, surface charge, particle interaction with HCT, and stability. The in vitro drug release of NPs was evaluated independently in pH 7.4 phosphate-buffered saline (PBS) containing 0.5% (w/v) Tween® 80, and in cell culture media. Cell internalization was observed using confocal microscopy and qualification. Cytotoxicity was evaluated in breast cancers and keratinocyte cell line.

2. Materials and method

2.1. Materials

Docetaxel (DTX) was obtained from Korea United Pharm. Inc. (Seoul, Korea). Herceptin® (HCT) was purchased from Roche Ltd. (Basle, Switzerland). Poly(D, L-lactide-*co*-glycolide) (PLGA, Mw 38,000–54,000 as Resomer® RG 504 H), albumin–fluorescein isothiocyanate conjugate (albumin-FITC), coumarin 6 (Cou-6, 98%), poly(vinyl alcohol) (PVA, Mw 30,000–70,000), poly(ethylene-*alt*-maleic anhydride) (PEMA, Mw 100, 000–500,000), poly(allylamine hydrochloride) (PAH), *N*-hydroxysuccinimide (NHS), *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDC), Tween® 80, dimethylsulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and 4′,6-diamidino-2-phenylindole (DAPI) were purchased from Sigma-Aldrich (St. Louis, MO, USA). BT-474 and MCF-7 cells were purchased from the Korean Cell Line Bank (KCLB) (Seoul, Korea). SK-BR-3 and HaCaT cells were obtained from the College of Pharmacy, Chungnam National University. Fetal bovine serum (FBS), antibiotics, Dulbecco's modified Eagle medium (DMEM), and Roswell Park Memorial Institute (RPMI) 1640 medium were obtained from Hyclone (Logan, UT, USA). Methylene chloride was purchased from Samchun Chemicals (Pyeongtaek, Korea). All other chemicals were commercial products of analytical or reagent grade and were used without further purification.

2.2. Preparation of PNPs

PNPs were prepared by an emulsion-solvent evaporation method with some modification [34], and PEMA, a surfactant with many carboxyl groups, was used. Briefly, PLGA (300 mg) and DTX (20 mg) were dissolved in methylene chloride (10 mL). This PLGA/DTX solution (1 mL) was added dropwise to 1.0% (w/v) PEMA aqueous solution (30 mL) in an ice bath, and the mixture was sonicated (210 W for 1 min, with stirring at 300 rpm) using a probe-sonicator (KFS-300N, Ultrasonic Processor, Korea Process Technology, Seoul, Korea). The emulsion was stirred at 500 rpm for 3 h at room temperature to evaporate the organic solvents (Table S1). DTX-PNPs were collected by centrifugation at 16,000 ×*g*, for 20 min (MEGA 17R, Hanil Centrifuge, Hanil Scientific Industrial Co., Ltd., Incheon, Korea) and then washed with distilled water (DW) at three times. The collected DTX-PNPs were dispersed in 1 mL DW. In order to compare the binding efficiency of albumin-FITC or HCT, we prepared PNPs with PVA, a surfactant, as described above. For analysis of cellular uptake, PLGA (300 mg) and Cou-6 (1 mg) were dissolved in methylene chloride (10 mL) and then subjected to the method described above.

2.3. Albumin-FITC or HCT labeling of PNPs

Three types of HCT-labeled PNPs were prepared through an adsorption method (A), charged adsorption method (C), and bio-conjugation method (B). For binding of HCT on PNPs, PNPs (14, 7, 3.5, or 1.8 mg/mL) were mixed with HCT solution (1.0 mg/mL in DW) with stirring at 300 rpm for 1 h (final HCT concentration; 0.5 mg/mL) (Table S2). The optimized ratio was PNPs (14 mg) vs HCT (1.0 mg). In the adsorption method (A), a PNP dispersion (1 mL, approximately 14 mg PNPs) was mixed with albumin-FITC or HCT solution (1 mL, 1.0 mg/mL in DW) with stirring at 500 rpm for 1 h. HCT-A-PNPs were collected by centrifugation at 10,000 ×*g*, for 10 min (MICRO-12 microcentrifuge; Hanil Scientific Industrial Co., Ltd. Korea), and then were dispersed in DW. For the charged adsorption method (C), PNP dispersion (1 mL) was mixed with PAH (5 mg) with stirring at 500 rpm for 1 h. PAH-coated PNP dispersions (1 mL) were mixed with albumin-FITC or HCT solution (1 mL, 1.0 mg/mL in DW) with stirring at 500 rpm for 1 h. HCT-C-PNPs were collected via centrifugation at 10,000 ×*g*, for 10 min, and then dispersed in DW. For the bio-conjugation method (B), PNP dispersion (1 mL) was mixed with EDC (5 mg) and NHS (5 mg) with stirring at 500 rpm for 1 h. Activated carboxylate group on PNPs were collected via centrifugation at 10,000 ×*g*, for 10 min, and were then dispersed in DW (1 mL). Activated carboxylate group of PNP dispersions (1 mL) were mixed with albumin-FITC or HCT solution (1 mL, 1.0 mg/mL in DW) with stirring at 500 rpm for 1 h. HCT-B-PNPs were collected via centrifugation at 10,000 ×*g*, for 10 min, and then dispersed in DW.

2.4. Morphological observations

Micrographs of four types of PNPs were evaluated using field emission transmission electron microscopy (FE-TEM; model TECNAI G2 F30 S-Twin, FEI, OR, USA). Each PNP dispersion was placed dropwise onto TEM grids and dried for 1 day under vacuum. Samples were operated under an acceleration voltage of 300 kV. The sizes were measured manually using ImageJ software (National Institutes of Health, Bethesda, MD) and averaged to obtain the mean particle size.

2.5. Particle size and zeta-potentials

The particle sizes of the four types of PNPs were determined using a Zetasizer Nano-90 (Malvern Instruments Ltd., Malvern, UK), and the surface charges were determined by measuring the Zeta-potential (Zetasizer Nano-Z, Malvern Instruments Ltd.). The particle size and surface charge of each sample were measured in triplicate, and average values were calculated.