International Journal of Pharmaceutics 473 (2014) 426–433

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical nanotechnology

Development of poly(lactic-co-glycolic) acid nanoparticles-embedded hyaluronic acid–ceramide-based nanostructure for tumor-targeted drug delivery

Ju-Hwan Park [a], Jae-Young Lee [a], Ubonvan Termsarasab [a], In-Soo Yoon [b], Seung-Hak Ko [c], Jae-Seong Shim [c,d], Hyun-Jong Cho [e,*], Dae-Duk Kim [a,**]

[a] College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul 151-742, Republic of Korea
[b] College of Pharmacy and Natural Medicine Research Institute, Mokpo National University, Jeonnam 534-729, Republic of Korea
[c] Biogenics Inc., Daejeon 305-510, Republic of Korea
[d] Skin & Tech Inc., Seongnam 461-713, Republic of Korea
[e] College of Pharmacy, Kangwon National University, Chuncheon 200-701, Republic of Korea

ARTICLE INFO

Article history:
Received 15 March 2014
Received in revised form 21 May 2014
Accepted 25 July 2014
Available online 28 July 2014

Keywords:
Cancer diagnosis
Docetaxel
Embedding
Hyaluronic acid–ceramide
PLGA nanoparticle
Tumor targeting

ABSTRACT

A hyaluronic acid–ceramide (HACE) nanostructure embedded with docetaxel (DCT)-loaded poly(D,L-lactide-co-glycolide) (PLGA) nanoparticles (NPs) was fabricated for tumor-targeted drug delivery. NPs with a narrow size distribution and negative zeta potential were prepared by embedding DCT-loaded PLGA NPs into a HACE nanostructure (DCT/PLGA/HACE). DCT-loaded PLGA and DCT/PLGA/HACE NPs were characterized by solid-state techniques, including Fourier-transform infrared (FT-IR) spectroscopy, differential scanning calorimetry (DSC), and powder X-ray diffraction (PXRD). A sustained drug release pattern from the NPs developed was observed and negligible cytotoxicity was seen in NIH3T3 cells (normal fibroblast, CD44 receptor negative) and MDA-MB-231 cells (breast cancer cells, CD44 receptor positive). PLGA/HACE NPs containing coumarin 6, used as a fluorescent dye, exhibited improved cellular uptake efficiency, based on the HA-CD44 receptor interaction, compared to plain PLGA NPs. Cyanine 5.5 (Cy5.5)-labeled PLGA/HACE NPs were injected intravenously into a MDA-MB-231 tumor xenograft mouse model and demonstrated enhanced tumor targetability, compared with Cy5.5-PLGA NPs, according to a near-infrared fluorescence (NIRF) imaging study. Considering these experimental results, the DCT/PLGA/HACE NPs developed may be useful as a tumor-targeted drug delivery system.

1. Introduction

Nano-sized vehicles have attracted much interest for anticancer drug delivery and cancer diagnosis (Cho and Kwon, 2011; Koo et al., 2012; Termsarasab et al., 2013a,b; Yoon et al., 2013a,b; Zhang et al., 2013). Due to the innate cytotoxicity of anticancer drugs to normal tissues and organs, tumor targeting has long been regarded as desirable for anticancer drug delivery. Numerous approaches to tumor targeting have been developed and some have been used successfully in clinical cancer therapy (Cheng et al., 2012; Ma et al., 2011).

Tumor targeting strategies are generally classified into passive and active targeting (Danhier et al., 2010; Lammers et al., 2012; Maeda et al., 2013). Passive tumor targeting is based on the "enhanced permeability and retention" (EPR) effect, which may be caused by large clefts in the endothelium of blood vessels and an impaired lymphatic drainage system in tumors. Thus, macromolecules can readily accumulate in the tumor region and drug-loaded nanovehicles may exert improved anti-cancer efficacy. Major drawbacks of the passive targeting strategy are insufficient tumor targetability and the continuing toxic potential to normal cells. To address this limitation, specific ligands (small molecules, peptides, proteins) that have a high binding affinity for receptors overexpressed on cancer cells have been introduced to nanovehicles to improve tumor targeting efficiency (Danhier et al., 2012; Kamaly et al., 2012).

For anticancer drug delivery and cancer diagnosis, diverse biocompatible materials have been used to fabricate nanovehicles (Bunschoten et al., 2012; Lee et al., 2011; Yoon et al., 2013a,b).

* Corresponding author. Tel.: +82 33 250 6916; fax: +82 33 259 5631.
** Corresponding author. Tel.: +82 2 880 7870; fax: +82 2 873 9177.
E-mail addresses: hjcho@kangwon.ac.kr (H.-J. Cho), ddkim@snu.ac.kr (D.-D. Kim).

http://dx.doi.org/10.1016/j.ijpharm.2014.07.038
0378-5173/

Among them, poly(D,L-lactide-co-glycolide) (PLGA) has been widely investigated because of its biodegradability and biocompatibility (Acharya and Sahoo, 2011; Park et al., 2013; Yoo and Mitragotri, 2010). Although PLGA nanoparticles (NPs) exhibit favorable performances in anticancer drug delivery, the insufficient tumor targetability remains an issue to be resolved. Thus, various tumor-targeting moieties have been introduced to PLGA NPs to enhance targeting efficiency (Graf et al., 2012; Zhou et al., 2012).

Hyaluronic acid (HA) has been widely used as a tumor targeting moiety, aimed primarily at the CD44 receptor overexpressed in several types of cancer (Ganesh et al., 2013; Hiscox et al., 2012; Misra et al., 2011). In our previous studies (Cho et al., 2011, 2012a,b; Jin et al., 2012), hyaluronic acid–ceramide (HACE), as an amphiphilic HA oligomer derivative, was synthesized and used for the preparation of self-assembled NPs. HACE NPs provided sustained drug release and tumor targetability in drug delivery and cancer diagnosis based on the interaction between HA and the CD44 receptor. Improved anti-cancer activity was also observed after intravenous injection of HACE-based NPs.

In this study, the individual advantages of biodegradable PLGA NPs and tumor-targetable HACE nanostructures were combined to prepare new nano-sized drug delivery systems. Instead of chemical conjugation or adsorption of HA onto the surface of PLGA NPs, embedding of PLGA NPs into self-assembled HACE nanostructure was attempted (Fig. 1). Although the concept of embedded nanostructures has been used with inorganic material-based NPs, its application to the development of organic polymer-based NPs has not been investigated previously. Here, we report the fabrication and characterization of nano-sized PLGA NPs-embedded HACE nanostructure. Drug release, cellular distribution, and in vivo tumor targetability were also investigated.

2. Materials and methods

2.1. Materials

Docetaxel (DCT) was purchased from Taihua Co. (Xi'an, China). Hyaluronic acid oligomer (4.7 kDa) and DS-Y30 (ceramide 3B; mainly *N*-oleoylphytosphingosine) were purchased from Bioland Co., Ltd. (Cheonan, Korea) and Doosan Biotech Co., Ltd. (Yongin, Korea), respectively. Coumarin 6, chloromethylbenzoyl chloride, and tetra-n-butylammonium hydroxide (TBA) were obtained from Sigma–Aldrich Co. (St. Louis, MO, USA). Poly(D,L-lactide-co-glycolide) (PLGA; 25–35 kDa) and amine-functionalized PLGA (PLGA-NH_2, 28 kDa) were purchased from Polyscitech (West Lafayette, IN, USA). The N-hydroxysuccinimidyl ester form of cyanine 5.5 (Cy5.5-NHS) was from Amersham Biosciences (Piscataway, NJ, USA). Cell culture media (RPMI 1640, Waymouth), 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.

2.2. Preparation and characterization of DCT-loaded nanoparticles

HACE conjugate was prepared according to a reported method (Cho et al., 2011). Briefly, HA (12.21 mmol) and TBA (9.77 mmol) were solubilized in 60 ml double-distilled water (DDW) and subsequently stirred for 30 min. Activated HA-TBA was obtained by freeze-drying. DS-Y30 ceramide (8.59 mmol) and triethylamine (9.45 mmol) in 25 ml tetrahydrofuran (THF), and 4-chloromethylbenzoyl chloride (8.59 mmol) in 10 ml THF were blended to make the DS-Y30 linker. DS-Y30 linker was obtained by concentration and recrystallization processes after stirring for 6 h at 60 °C. HA-TBA (8.10 mmol) and the DS-Y30 linker (0.41 mmol) were solubilized in THF/acetonitrile mixture (4:1, v/v) and stirred for 5 h at 40 °C. HACE was finally obtained after further processing to eliminate impurities and organic solvents.

To fabricate DCT/PLGA NPs-embedded HACE nanostructures (DCT/PLGA/HACE NPs), the core part (DCT/PLGA NPs) was prepared in advance. PLGA NPs were prepared based on a modified solvent evaporation method (Keum et al., 2011; Mo et al., 2012). DCT (6 mg) and PLGA (60 mg) were dissolved in dichloromethane (1.5 ml) and poured into 30 ml of DW including 2% poly(vinyl alcohol). After sonicating for 20 min with a probe-type sonicator (Vibra-Cell VC 750 ultrasonic processor, Sonics & Materials, CT, USA), the mixture was stirred for 1 h to eliminate the organic solvent. Samples were centrifuged (13,200 rpm, 10 min) and NP pellets were suspended with DW (30 ml) by vortexing. To develop the PLGA NPs-embedded HACE nanostructure, a HACE film, coated in tube, was prepared. HACE (3 mg/ml) was dissolved in methanol and heated at 65 °C for 1 h to remove the organic solvent. The DCT/PLGA NPs suspension prepared was added to the HACE film-coated tube and vortexed for 5 min. By centrifuging (13,200 rpm, 10 min), uncoated HACE was removed and the DCT/PLGA/HACE NPs suspension was collected.

The features of the drug-loaded NPs developed (particle size, polydispersity index, and zeta potential) were studied by electrophoretic light scattering method (ELS-Z; Otsuka Electronics, Tokyo, Japan). To measure drug encapsulation efficiency (EE), DCT-loaded NPs were diluted with dimethyl sulfoxide (DMSO) and analyzed quantitatively using high-performance liquid chromatography (HPLC). DCT was analyzed using a Waters HPLC system (Waters Co., Milford, MA) equipped with a reverse phase C-18 column (Gemini, 250 × 4.6 mm, 5 μm; Phenomenex, Torrance, CA, USA), a pump (Waters 515), an automatic injector (Waters 717plus), and a UV/vis detector (Waters 2487). The mobile

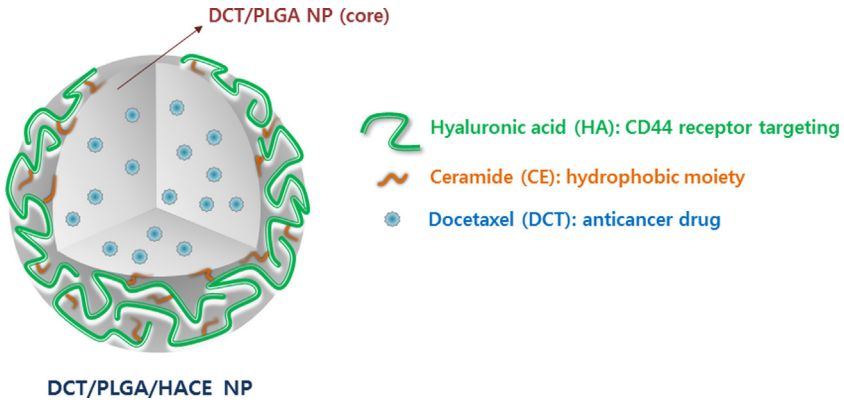

Fig. 1. Preparation of DCT/PLGA/HACE NPs. Schematic illustration of DCT/PLGA/HACE NP is presented.