Biomaterials 35 (2014) 550–566

Contents lists available at ScienceDirect

Biomaterials

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

Biodegradable self-assembled nanoparticles of poly (D,L-lactide-co-glycolide)/hyaluronic acid block copolymers for target delivery of docetaxel to breast cancer

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ARTICLE INFO

Article history:
Received 16 July 2013
Accepted 24 September 2013
Available online 15 October 2013

Keywords:
Self-assembled nanoparticles
Docetaxel
Poly (D,L-lactide-co-glycolide)
Hyaluronic acid
CD44
Breast cancer

ABSTRACT

To develop biodegradable docetaxel-loaded self-assembled nanoparticles of poly (D,L-lactide-co-glycolide)/hyaluronic acid block copolymers were successfully synthesized. These copolymers could form nanoparticles with small size (<200 nm), an acceptable CMC (~7.9 mg/L), typical core/shell structure and superior stability in one week. DTX-loaded $PLGA_{502H}$-b-$HA_{5.6k}$ nanoparticles (DTX/SANPs) showed a biphasic release pattern within 120 h, and exhibited enhanced cytotoxicity toward CD44-overexpressing MDA-MB-231 cells. Cellular uptake study indicated that $PLGA_{502H}$-b-$HA_{5.6k}$ nanoparticles (SANPs) were taken up in MDA-MB-231 cells by CD44-mediated endocytosis. Pharmacokinetics study revealed DTX/SANPs could prolong the circulation of DTX in the blood. In vivo studies demonstrated that SANPs exhibited enhanced tumor targeting and antitumor activity with lower systemic toxicity. In conclusion, DTX/SANPs have great potential for targeted chemotherapy for CD44-overexpressing breast cancer.

1. Introduction

Self-assembled nanoparticles composed of amphiphilic block copolymers have received intensive attentions in the field of drug delivery systems [1,2]. Such nanoparticles always have a core–shell structure consisting of a hydrophobic core and a hydrophilic shell [1,3,4]. Typically, the hydrophobic block forms the hydrophobic core of the nanoparticles, while the hydrophilic block forms the outer or hydrophilic shell. The hydrophobic core serves as a compartment for loading hydrophobic drugs, and the hydrophilic shell made of a brush-like corona can stabilize nanoparticles in aqueous solution. Furthermore, hydrophilic shell is expected to help prolong circulation time of self-assembled nanoparticles, due to the steric stabilization which provides protection from opsonization in blood stream. In addition, self-assembled nanoparticles often possess a small size of <200 nm which facilitates the extravasation of nanoparticles at leaky sites of tumors owing to the enhanced permeability and retention (EPR) effect [5].

Although nanoparticles formed from block copolymers have passive targeting to tumors, their therapeutic effect may be limited by insufficient cellular uptake by tumor cells, due to lack of active targeting of unmodified nanoparticles [6,7]. To enhance the active targeting, nanoparticles are generally modified with targeting moieties such as peptide ligands [8–10], nucleotide aptamers [11,12], and antibodies [13–16]. However, the application of these targeting moieties severely suffers from the following disadvantages [17,18]: potential immunogenicity, impaired binding affinity after conjugation and high cost. Hyaluronic acid (HA), a naturally occurring polysaccharide composed of *N*-acetyl-D-glucosamine and D-glucuronic acid, could specifically bind to its receptors CD44 and RHAMM (receptor for HA-mediating motility) which are well-validated targets in a variety of tumors and be internalized via receptor-mediated endocytosis [19,20]. Thus, HA is regarded as a targeting moiety for tumor targeting. It is noteworthy that,

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0142-9612/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biomaterials.2013.09.089

compared with other types of targeting moieties, HA possesses unique advantages such as non-toxicity, non-immunotoxicity [21], good biocompatibility, biodegradability [22] and modification flexibility [23].

Hyaluronic acid (HA) has been extensively used as a targeting moiety in HA-drug conjugates [24,25], HA nanogels [26] and HA self-assembled nanoparticles [5,20,27,28] for cancer targeting delivery of chemotherapy drugs. In addition to its targeting ability to tumors, HA is a hydrophilic molecule and possesses the ability to form the hydrophilic shell in self-assembled nanoparticles after conjugation with hydrophobic polymers such as poly (D,L-lactide-co-glycolide) (PLGA) [29–33]. Generally, the self-assembled nanoparticles composed of HA/PLGA copolymers are categorized into two types: one type is nanoparticles composed of PLGA-grafted HA copolymers, in which hydrophobic PLGA chain is grafted onto the backbone of hydrophilic HA [30–33], and the other one is composed of PLGA/HA block copolymers [29]. Both nanoparticles composed of PLGA/HA copolymers can generally form a core–shell structure, and showed effective cellular targeting and significant cytotoxic effects toward CD44-overexpressing tumor cells [29,30,33]. Notably, compared with the nanoparticles composed of PLGA-grafted HA copolymers, one of the main advantages of the nanoparticles composed of PLGA/HA block copolymers is that hydrophilic HA can be freely extended and directed toward aqueous solution while the hydrophobic PLGA incorporate hydrophobic drugs [29]. This unique advantage may contribute to longer circulation and more active targeting toward CD44-overexpressing tumor cells for the nanoparticles composed of PLGA/HA block copolymer. Until now, there have been very few studies about the nanoparticles composed of PLGA/HA block copolymers. Such copolymer was first reported to form typical core–shell nanoparticles in aqueous condition by Dae Hwan Kang's group, in which the nanoparticles based on PLGA/HA block copolymer had been proven to possess active targeting ability to CD44-overexpressing HCT-116 human colon carcinoma cells [29]. Furthermore, hydrophobic drug (doxorubicin) was successfully loaded into such nanoparticles with high drug loading content, which achieved enhanced cytotoxicity in HCT-116 cells. In addition to its biodegradability and compatibility, this copolymer is a superior material for targeted delivery to CD44-overexpressing cancer. However, the preparation procedure was not optimized, and the effects of molecular weight ratio of HA to PLGA on the characteristics of the nanoparticles such as size and critical micelle concentration (CMC) were not investigated. Furthermore, in vivo tumor targeting, pharmacokinetics and antitumor efficacy of drug loaded nanoparticles based on PLGA block HA should be further investigated. Up to now, there is still no study about docetaxel-loaded PLGA/HA block copolymers based nanoparticles for breast cancer targeting.

In this study, to obtain an optimized type of self-assembled nanoparticles composed of PLGA/HA block copolymers, we synthesized a series of PLGA/HA block copolymers of different molecular weight using an end to end coupling strategy and developed a series of nanoparticles composed of these block copolymers. The size, CMC and zeta potential of these nanoparticles were investigated. Finally, the optimal nanoparticles, $PLGA_{502H}$-b-$HA_{5.6k}$ nanoparticles (SANPs), with the smallest size and a suitable CMC were developed for further investigation. Characteristics of blank or drug loaded nanoparticles were evaluated and the mechanism of endocytosis against CD44-overexpressed cells was also investigated. Coumarin-6 and DiR were loaded into $PLGA_{502H}$-b-$HA_{5.6k}$ nanoparticles to track the behavior of the nanoparticles to elucidate the targeting pathway. Pharmacokinetics and in vivo antitumor activity of docetaxel (DTX) loaded $PLGA_{502H}$-b-$HA_{5.6k}$ nanoparticles (DTX/SANPs) were investigated in Sprague–Dawley (SD) rats and tumor-bearing BALB/c nude mice, respectively.

2. Materials and methods

2.1. Materials

Hyaluronic acid with a different molecular weight (M_w) of 5.6, 7.3 or 8.9 kDa was purchased from the Shandong Freda Biopharmaceutical Co., Ltd. (Shandong, China). PLGA (50:50) polymers, Resomer® RG 502 H (MW 13, 600) and RG 503 H (MW 35, 700), were purchased from Boehringer Ingelheim (Ingelheim, Germany). Docetaxel was purchased from ShangHai Biochempartner Co., Ltd. (Shanghai, China). 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), 1,4-Diaminobutane, Triton X-100, 4,6-Diamidino-2-phenylindole (DAPI), sodium cyanoborohydride and coumarin-6 were purchased from Sigma–Aldrich Co. LLC. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl), *N*-Hydroxysuccinimide (NHS) and *N*,*N*-Diisopropylethylamine were obtained from Aladdin Reagent Co., Ltd. (Shanghai, China). 1,1-Dioctadecyl-3,3,3′,3′-tetramethyl indotricarbocyanine iodide (DiR) was obtained from Biotium (CA). Cell Cycle and Apoptosis Assay Kit and Annexin V-FIT Kit were purchased from Beyotime Institute of Biotechnology (Shanghai, China). Cell culture medium (RPMI 1640 and High Glucose DMEM), trypsin–EDTA, penicillin, streptomycin and fetal bovine serum (FBS) were provided from GIBCO (USA). Two breast cancer cell lines (MCF-7 and MDA-MB-231) were purchased from the Cell Culture Center of the Shanghai Institutes for Biological Sciences of the Chinese Academy of Sciences (Shanghai, China). All the other reagents used were of analytical grade and used without further purification.

Sprague–Dawley (SD) rats (male, 200 ± 20 g) and BALB/c nude mice (female, five weeks, ~16 g) were purchased from Shanghai Slac Laboratory Animal Co, Ltd (China, Shanghai) and maintained in laminar flow room under constant temperature and humidity. All animal experiments were performed in accordance with protocols evaluated and approved by the ethics committee of Second Military Medical University.

2.2. Synthesis of PLGA/HA block copolymers (PLGA-b-HA)

PLGA/HA block copolymers (PLGA-b-HA) were synthesized by an end to end coupling strategy as described before [29]. Here, we take one of the PLGA-b-HA copolymers, $PLGA_{502H}$-b-$HA_{5.6k}$, as an example to illustrate the procedures of the synthesis of the block copolymers.

(a) Synthesis of amino-functionalized HA

The synthetic strategy was based on a terminal reductive amination reaction [34] between hyaluronic acid (HA) and 1,4-Diaminobutane with sodium cyanoborohydride ($NaCNBH_3$) as a reducing agent (Fig. 1A). In detail, 1 g of HA (5.6 kDa, 0.18 mmol) was dissolved in an acetate buffer (30 ml, pH = 5.6, 2% w/w). Then, 1.0 ml of 1,4-Diaminobutane (11.4 mmol) was added into the HA solution under magnetic stirring. Twenty-four hours after stirring at 50 °C, HA reacted with 1,4-Diaminobutane and resulting imine was obtained in the mixture. Subsequently, 0.2 g of sodium cyanoborohydride (3.2 mmol) was added to the mixture each day for three days under stirring. The mixture was purified by dialysis with a dialysis bag (Spectra/Por®, MWCO 3500) against deionized water for 72 h to remove excess 1,4-Diaminobutane and sodium cyanoborohydride. The final product was collected and lyophilized.

(b) Synthesis of *N*-Hydroxysuccinimide PLGA (PLGA-NHS)

The *N*-Hydroxysuccinimide PLGA (PLGA-NHS) was synthesized as described before [35]. Briefly, 1 g of PLGA-COOH (RG 502H, 0.083 mmol) was added into methylene chloride (10 ml) in which 48.0 mg of *N*-Hydroxysuccinimide (NHS, 0.42 mmol) and 80.0 mg of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC·HCl, 0.42 mmol) were dissolved. Then, the mixture was stirring for another 24 h at room temperature (Fig. 1B). Following that, the final product PLGA-NHS was precipitated with ethyl ether (5 ml), and washed for three times in an ice-cold mixture of ethyl ether and methanol (50:50, v/v) to remove residual NHS and EDC·HCl. Finally, purified PLGA-NHS was dried under vacuum.

(c) Synthesis of PLGA-b-HA

First, 0.5 g of PLGA-NHS (0.042 mmol) was dissolved in dimethyl sulfoxide (DMSO, 20 ml), and 0.35 g of amino-functionalized hyaluronic acid (0.063 mmol) and 20 μl of *N*,*N*-Diisopropylethylamine (0.16 mmol) were added subsequently. The mixture was stirred at 50 °C for 48 h (Fig. 1C). Then, the mixture was purified by dialysis with a dialysis bag (Spectra/Por®, MWCO 12000–14000) against deionized water for 72 h to remove excess amino-functionalized hyaluronic acid. The final product PLGA-b-HA was lyophilized and used to prepare SANPs without further treatment.