Biomaterials 116 (2017) 82–94

Contents lists available at ScienceDirect

Biomaterials

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

Targeted hydroxyethyl starch prodrug for inhibiting the growth and metastasis of prostate cancer

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ARTICLE INFO

Article history:
Received 23 September 2016
Received in revised form
16 November 2016
Accepted 21 November 2016
Available online 23 November 2016

Keywords:
Hydroxyethyl starch prodrug
Schiff base bond
Targeted drug delivery
Chemotherapy
Prostate cancer therapy

ABSTRACT

Prostate cancer is one of the most prevalent malignancies among men. Although chemotherapy has been an effective therapeutic approach for treating metastatic prostate cancer, serious undesired side effects have hampered its wide application clinically. In this work, a pH-responsive LHRH-conjugated hydroxyethyl starch-doxorubicin (HES-DOX/LHRH) prodrug was facilely synthesized by conjugating oxidized HES (HES-CHO) with DOX and LHRH through an acid-sensitive Schiff base bond. The resulting prodrug spontaneously self-assembled into nanoscopic micelle with a radius of about 55 nm in an aqueous environment. HES-DOX/LHRH significantly improved the *in vivo* tissue distribution of the drug. Compared to its non-targeted counterpart, targeted HES-DOX/LHRH demonstrated a greater *in vitro* anti-proliferative capability toward mouse RM-1 prostate cells. More importantly, targeted HES-DOX/LHRH exhibited higher levels of anti-tumor and anti-metastasis activities against an RM-1-xenografted mouse model, with lower systemic toxicity compared to free DOX·HCl and non-targeted HES-DOX. Hence, these results revealed that targeted HES-DOX/LHRH possesses great potential application in clinical chemotherapy of metastatic prostate cancer.

1. Introduction

In the countries of Europe and the Americas, such as the United States, prostate cancer is one of the most common type of cancer, accounting for nearly 21% of newly diagnosed cancers and 8% of cancer-related deaths among men in 2016 [1]. As prostate cancer progresses, it can metastasize to the rectum, bladder, bone, lymph node, lung, and liver [2]. The standard first-line therapy for treating metastatic prostate cancer is androgen deprivation therapy (ADT), performed through surgical orchiectomy or by a luteinizing hormone-releasing hormone (LHRH, also known as gonadotropin-releasing hormone, GnRH) agonist or antagonist [3,4]. Continuous stimuli of pituitary LHRH receptors by the LHRH analogs lead to the down-regulation of these receptors and the inhibition of luteinizing hormone (LH) release, followed by a decrease in the level of androgen synthesis [5]. However, most patients with metastatic prostate cancer can only benefit from ADT for less than 24 months [4,6]. Moreover, metastatic castration-resistant prostate cancer (mCRPC) produces painful osteoblastic bone metastases that severely impair the patient's quality of life [2], and patients with mCRPC are expected to survive less than 19 months [7]. Although chemotherapeutic treatment of mCRPC has been shown to improve the patient's quality of life and lengthen the period of survival to a certain extent [8], the undesired serious side effects have hampered its further application [9]. For instance, doxorubicin (DOX), a type of anthracycline antibiotic drug, has been widely used in clinical chemotherapeutic treatment of a diverse range of malignant tumors, such as the lung, prostate, bladder, bone, brain, cervix, and breast cancers [9]. DOX inhibits DNA replication and transcription by intercalating into the DNA duplex, which significantly suppresses the reproduction of tumor cells [10]. Compared to docetaxel

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http://dx.doi.org/10.1016/j.biomaterials.2016.11.030
0142-9612/

(DTX), which is currently the standard medication for CRPC, DOX is preferred for eradicating the metastatic lesions caused by mCRPC [11]. However, short half-life and circulation time [12], in addition to serious side effects (*e.g.*, alopecia, hematopoietic depression, gastrointestinal disorders, and cardiotoxicity) [13], have limited the further application of DOX to the clinic.

In recent years, polymer-drug conjugates have been intensively researched to improve the efficacy and reduce the toxicity of traditional small-molecule drugs (SMDs) [12,14]. Polymer-drug conjugates possess several significant advantages over SMDs: (1) significantly improved aqueous solubility of hydrophobic drugs through conjugation to a hydrophilic polymer, (2) potential for drugs to be delivered by controlled release under certain conditions (*e.g.,* a change in pH or temperature), (3) extended circulation time through escape from the prompt uptake by the reticuloendothelial system (RES), and (4) selective intratumoral accumulation *via* the enhanced permeability and retention (EPR) effect [15,16]. Efficacy and safety are always the foremost concerns in the development of polymer-drug conjugates, as they are directly associated with the drug release behaviors of the prodrugs [15]. Generally, adequate stability in normal physiological tissues and quick drug release in the lesion site are desired [17].

Drug release at the targeted site is activated by sharp intelligent responses to specific microenvironmental stimuli, with pH changes being the most suitable and commonly used one among these stimuli [18]. In contrast to normal tissues (pH 7.2–7.4), the tumor tissue has a lower pH (pH ~ 6.8) by virtue of the Warburg effect, which could be attributed to the constant accumulation of lactic acid generated by incomplete catabolism of glucose in cancer cells [19]. Furthermore, early endosomes (pH 5.9–6.2) and late endosomes/lysosomes (pH 5.0–5.5) in cancer cells have lower pHs than that of the extracellular microenvironment [20]. Hence, the pH-responsive polymer-drug conjugates have great potential as a drug delivery system that can promptly release the loaded drug in an acidic microenvironment.

Passive tumor-targeting through the EPR effect has proven effective for treating hypervascular solid tumors, but the effect on hypovascular or avascular solid tumors (*e.g.*, prostate and pancreatic cancers) has been unsatisfactory. Moreover, passive targeting seldom applies to migratory tumors like leukemia. Given these circumstances, active targeting was developed by conjugating peptides onto nanoparticles that can couple with the receptors overexpressed on cancer cells [21]. Overexpressed LHRH membrane receptors have been found in many cancer cells, including prostate (about 90%; such as DU145, PC3, and R-3327-H), breast (around 50%; such as MCF-7 and MDA-MB-231), and ovarian and endometrial (approximately 80%) cancer cells [6,22,23]. Both metastatic lymph nodes and lesions of prostate cancer have been found to express LHRH receptors, whereas receptor expression is scarce in normal tissues [5,8]. More importantly, the LHRH-receptor-targeting nanoparticles demonstrated no obvious impact on the physiological effects of the pituitary-gonadal axis [24]. Hence, these findings reveal that the LHRH receptors are ideal targets for targeted chemotherapy of prostate cancer, especially mCRPC.

Although a large number of hydrophilic polymers have been exploited as matrices of polymer-drug conjugates, very few are appropriate for clinical application [16]. HES is a semi-synthetic biodegradable polysaccharide, which has been widely used as a plasma substitute and volume expander for the clinical treatment of cerebral ischemia, hypovolemic shock, or artery occlusive disease over the years, and it is degraded by serum α-amylase *in vivo* [25,26]. HES has been proven to possess similar or superior performances compared to poly(ethylene glycol) (PEG). Its efficacy can be attributed to several valuable characteristics, such as (1) high water solubility due to hydroxyethylation, (2) low hypersensitivity as a result of similar molecular structure of HES to human glycogen, (3) controllable biodegradation behavior through regulating the molar mass of HES and degree of hydroxyethylation, and (4) enhanced microcirculation of the organism through elevated blood viscosity [26,27]. These excellent properties make HES a promising biomedical material for producing nanocarriers.

HES with weight-average molecular weight (M_w) of 130 kDa and a molar substitution of 40% (HES (130 Da/0.4)) used in this work has attracted more attention due to its excellent local and systemic tolerability, and high renal excretion rate [26]. The HES–DOX conjugates with an acid-sensitive Schiff base bond were prepared through an efficient Schiff base reaction between the aldehyde group of HES-CHO and the amino groups of DOX and/or LHRH (Scheme 1A). Afterward, nanoscopic micelles were fabricated through spontaneous self-assembly of the resulting prodrugs (Scheme 1B). The prodrug micelles possessed steady drug-binding ability, sustained drug release behavior, and excellent stability [15]. The *in vitro* DOX release profiles, *in vitro* tumor cell internalization and cytotoxicity, tissue distribution, and *in vivo* anti-tumor and anti-metastasis efficacy and safety of HES–DOX conjugates are systematically elaborated. To our best knowledge, this is the first reported application of LHRH-targeted polymer-drug conjugate to the chemotherapeutic treatment of metastatic prostate cancer.

2. Materials and methods

2.1. Materials

All the materials used in this study have been listed in Supplementary data.

2.2. Syntheses of HES-DOX and HES-DOX/LHRH

HES-CHO was synthesized according to the previously reported oxidation reaction [28]. HES-DOX was synthesized through a Schiff base reaction [12]. Typically, 1.0 g of HES-CHO and 0.116 g of DOX·HCl were mixed in a buffered solution at pH 5.5 and stirred at 55 °C for 72 h. The unreacted DOX·HCl was removed through dialysis (molecular weight cut-off (MWCO) = 3500 Da) against deionized water for 72 h. After filtration, the red solution was collected and lyophilized to obtain the final product, yielding 79.7%. HES-DOX/LHRH was synthesized through the similar proposal. Typically, 1.0 g of HES-CHO and 0.04 g of LHRH were mixed in a buffered solution at pH 5.5 and stirred at 55 °C for 24 h. Then, 0.116 g of DOX·HCl was added, and the mixture was stirred for another 72 h. The unreacted DOX·HCl was removed through dialysis (MWCO = 3500 Da) against deionized water for 72 h. After filtration, the red solution was collected and lyophilized to obtain the final product, yielding 81.5%.

2.3. In vitro DOX release and cell experiment

The DOX release behaviors, targetability, cell uptake, and cytotoxicity of HES–DOX conjugates were determined according to the previously reported techniques [12,29]. In addition, the detailed experimental procedures were described in Supplementary data.

2.4. Animal procedures

Six-week-old male C57BL/6 mice weighing from 18 to 20 g and Sprague-Dawley rats weighing from 225.0 to 235.0 g were purchased from the Laboratory Animal Center of Jilin University. Utmost care was given to minimize the suffering of the animals, and all animal procedures were performed under the guidelines