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Intracellular delivery and antitumor effects of a redox-responsive polymeric paclitaxel conjugate based on hyaluronic acid

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ABSTRACT

Polymer–drug conjugates have demonstrated application potentials in optimizing chemotherapeutics. In this study a new bioconjugate, HA-ss-PTX, was designed and synthesized with cooperative dual characteristics of active tumor targeting and selective intracellular drug release. Paclitaxel (PTX) was covalently attached to hyaluronic acid (HA) with various sizes (MW 9.5, 35, 770 kDa); a cross-linker containing disulfide bond was also used to shield drug leakage in blood circulation and to achieve rapid drug release in tumor cells in response to glutathione. Incorporation of HA to the conjugate enhanced the capabilities of drug loading, intracellular endocytosis and tumor targeting of micelles in comparison to mPEG. HA molecular weight showed significant effect on properties and antitumor efficacy of the synthesized conjugates. Intracellular uptake of HA-ss-PTX toward MCF-7 cells was mediated by CD44-caveolae-mediated endocytosis. Compared to Taxol and mPEG-ss-PTX, HA9.5-ss-PTX demonstrated improved tumor growth inhibition in vivo with a TIR of 83.27 ± 5.20%. It was concluded that HA9.5-ss-PTX achieved rapid intracellular release of PTX and enhanced its therapeutic efficacy, thus providing a platform for specific drug targeting and controlled intracellular release in chemotherapeutics.

Statement of Significance

Polymer–drug conjugates, promising nanomedicines, still face some technical challenges including a lack of specific targeting and rapid intracellular drug release at the target site. In this manuscript we designed and constructed a novel bioconjugate HA-ss-PTX, which possessed coordinated dual characteristics of active tumor targeting and selective intracellular drug release. Redox-responsive disulfide bond was introduced to the conjugate to shield drug leakage in blood circulation and to achieve rapid drug release at tumor site in response to reductant like glutathione. Paclitaxel was selected as a model drug to be covalently attached to hyaluronic acid (HA) with various sizes to elucidate the structure–activity relationship and to address whether HA could substitute PEG as a carrier for polymeric conjugates. Based on a series of in vitro and in vivo experiments, HA-ss-PTX performed well in drug loading, cellular internalization, tumor targeting by entering tumor cells via CD44-caveolae-mediated endocytosis and rapidly release drug at target in the presence of GSH. One of the key issues in clinical oncology is to enhance drug delivery efficacy while minimizing side effects. The study indicated that this new polymeric conjugate system would be useful in delivering anticancer agents to improve therapeutic efficacy and to minimize adverse effects, thus providing a platform for specific drug targeting and controlled intracellular release in chemotherapeutics.

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1. Introduction

Nanomedicine plays an important role in improving biological efficacy of the conventional small-molecule chemotherapeutics [1–3]. Polymer–drug conjugate, also known as polymeric prodrug, has demonstrated significant potentials in nanomedicine for their delivery of active agents to the targets, by controlling drug release with minimal burst effect during transition from the blood circulation to the extracellular tumor matrix [4]. Several PEGylated enzymes and cytokines have been successfully developed and approved for clinical applications; polyglutamic acid-palitaxel has also shown particular potential in Phase III clinical trial in women with non-small-cell lung cancer [5,6]. Nevertheless developing such a drug delivery system still faces technical challenges including a lack of specific drug targeting and rapid intracellular drug release at the target site [7].

Introduction of various biological ligands or antibodies into novel drug delivery system has been proven beneficial for selective delivery of anticancer compounds to tumor cells [8]. Hyaluronic acid (HA) has recently received extensive attention for its high affinity to CD44 [9,10]; it also possesses numerous desirable physicochemical and biological properties such as biocompatibility, biodegradability and non-immunogenicity [11] for drug delivery applications. HA-based conjugates are capable of simultaneous passive targeting of solid tumors via enhanced permeation and retention (EPR) effect and active targeting of CD44-bearing cancer cells without additional targeting ligands [12]. HA-paclitaxel conjugates have shown improvement of drug efficacy in standard chemotherapeutics in brain metastases of breast cancer (HA MW 3–5 kDa) [13], in orthotopic OSC-19-luciferase and HN5 xenograft models (HA MW 35 kDa) [14], and in mice bearing ovarian cancer (HA MW 200 kDa) [15]. Despite the promising results in tumor targeting, it was unclear how variability of HA molecular weight had influenced the tumor-targeting ability; HA of low molecular weight (LMW HA) behaved quite differently than HA of high molecular weight (HMW HA) in CD44 binding affinities (MW 5–8 < 10–12 < 175–350 kDa). In addition, nanocarrier of HMW HA displayed faster clearance than that of LMW HA [16]. Therefore, it would be important to elucidate the structure–activity relationship between HA molecular weight and HA-modified conjugates.

To achieve rapid intracellular release of anticancer drug, nanocarriers should become adaptive to tumor microenvironment (e.g., pH, temperature, redox) timely and appropriately to exemplify their unique advantages in applications [17–19]. It is noteworthy that concentration of reducing glutathione (GSH) in cytosol is 100–1000 times higher than that of other body fluids, and that tumor tissues are highly hypoxic with at least 4-fold higher than the normal tissues [20,21]. Delivery vehicles with disulfide functionality thus are cleaved in the presence of reducing agents including L-cysteine and glutathione (GSH) [22,23] efficiently. As a result, several glutathione-responsive polymeric conjugates including CPT-SS-PEG-SS-CPT [4] and H-shaped PEGylated methotrexate conjugates [24] have been developed for selective drug release.

In this study, the concept of redox potential for anticancer drug paclitaxel (PTX) was designed and confirmed, the structure–activity relationship between HA molecular weight and HA-modified bioconjugates was investigated. mPEG-ss-PTX was also synthesized as a control to address whether or not hydrophilic polymer HA could substitute PEG as a carrier for polymeric bioconjugates. Scheme 1 depicts the hypothesis and mechanism of specific drug targeting and delivery, i.e., HA-ss-PTX is to be specifically transferred to the tumor site by the EPR effect and absorbed by tumor cells via CD44-mediated endocytosis. Upon entering the cells, the disulfide bond would be disrupted, releasing PTX rapidly from the micelles. A series of in vitro and in vivo experiments were subsequently carried out to evaluate the potential of this novel delivery system in tumor therapy.

2. Materials and methods

2.1. Materials and reagents

Sodium hyaluronate (MW 9.5 kDa, 35 kDa and 770 kDa) was purchased from Freda Biochem Co., Ltd. (Jinan, Shandong, China). Monomethoxy poly(ethylene glycol) (mPEG, MW 2 kDa), pyrene and thiazolylblue tetrazolium bromide (MTT) were obtained from Sigma–Aldrich Co. (St. Louis, MO, USA). 1-Ethyl-3 (3-dimethylaminopropyl) carbodiimide (EDC), *N*-hydroxysuccinimide (NHS), 4-dimethylaminopyridine (DMAP), 3,3′-dithiodipropionic acid (DPA), dithiothreitol (DTT) and glutathione (GSH) were purchased from Aladdin Reagent Database Inc. (Shanghai, China). Paclitaxel (PTX) was purchased from Shanghai Zhongxi Sunve Pharmaceutical Co., Ltd. (Shanghai, China). Coumarin-6 (C6) and 4′,6-diamidino-2-phenylindole (DAPI) were purchased from Shanghai H-Y Biological Technology Co., Ltd. (Shanghai, China) and Beyotime Institute of Biotechnology Co., Ltd. (Shanghai, China), respectively. RPMI1640 media, fetal bovine serum (FBS) and tyrisin were purchased from GIBCO Co., Ltd. (Grand Island, NY, USA). Near-infrared dye DiR was provided by Beijing Fanbo Science and Technology Co., Ltd. (Beijing, China). Purified deionized water was obtained from a Milli-Q® Plus System (Billerica, MA, USA). All other chemicals were of chromatographic or analytical grades and used without further purification.

2.2. Synthesis and preparation

2.2.1. Synthesis of adipic dihydrazido-functionalized HA (HA-ADH)

Raw sodium hyaluronate was purified and desalted prior to use. Briefly, 1.0 g sodium hyaluronate was dissolved in 20 mL deionized water, and the pH adjusted to 3.5. The liquid was incubated overnight, dialyzed (MWCO 3.5 kDa), and then lyophilized. HA-ADH was prepared by acylation process between ADH and HA [25,26]. In a representative example, 2.5 mmol ADH was added to 0.5 mmol HA in deionized water and the pH adjusted to 5.0. Subsequently, 0.25 mmol EDC was dissolved in a small amount of water and added to the solution under agitation. The pH of the mixture was readjusted to 5.0. After 4 h at room temperature, the reaction was discontinued by raising pH to 7.0. The reactant was purified by successive dialysis (MWCO 3.5 kDa) against 0.1 M NaCl, 25% (v/v) ethanol solution and deionized water. Then the solution was lyophilized and stored at 4 °C for further use. A series of HA-ADH with various carboxyl substitutes was sequentially prepared by adjusting the ratios of HA, ADH and EDC.

2.2.2. Synthesis of DPA-functionalized PTX (PTX-ss-DPA)

DPA was used as a donor to introduce disulfide bond to PTX in preparing PTX-ss-DPA. Fig. S1 illustrates the synthesis pathway. In brief, 1.5 mmol DMAP and EDC were added to equivalent DPA in 20 mL CH_2Cl_2 under nitrogen and agitation at 0 °C. The reaction continued for 0.5 h to activate the carboxyl group of DPA. 0.5 mmol PTX in CH_2Cl_2 was added drop-wise to the mixture, and the reaction continued for 24 h at room temperature. Esterification process was monitored by thin layer chromatography. The product was washed with 0.01 M HCl twice and deionized water three times, dried and concentrated under vacuum. Final residue was dissolved in CH_2Cl_2 and purified by silica gel column chromatography with CH_2Cl_2:CH_3OH = 40:1 (Rf = 0.31) to yield PTX-ss-DPA.

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