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Versatile preparation of intracellular-acidity-sensitive oxime-linked polysaccharide-doxorubicin conjugate for malignancy therapeutic

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ABSTRACT

Recently, chemotherapy has been one of the most important therapeutic approaches for malignant tumors. The tumor tissular or intracellular microenvironment-sensitive polymer-doxorubicin (DOX) conjugates demonstrate great potential for improved antitumor efficacy and reduced side effects. In this work, the acid-sensitive dextran-DOX conjugate (noted as Dex-*O*-DOX) was synthesized through the versatile efficient oximation reaction between the terminal aldehyde group of polysaccharide and the amino group in DOX in the buffer solution of sodium acetate/acetic acid. The insensitive one, *i.e.*, Dex-*b*-DOX, was prepared similarly as Dex-*O*-DOX with a supplemented reduction reaction. The DOX release from Dex-*O*-DOX was pH-dependent and accelerated by the decreased pH. The efficient intracellular DOX release from Dex-*O*-DOX toward the human hepatoma HepG2 cells was further confirmed. Furthermore, Dex-*O*-DOX exhibited a closer antiproliferative activity to free DOX·HCl as the extension of time. More importantly, compared with Dex-*b*-DOX, Dex-*O*-DOX exhibited higher antitumor activity and lower toxicity, which were further confirmed by the systemic histological and immunohistochemical analyses. Hence, the facilely prepared smart polysaccharide-DOX conjugates, *i.e.*, Dex-*O*-DOX, exhibited great potential in the clinical chemotherapy of malignancy.

1. Introduction

Malignancy has been one of the most serious diseases with the highest incidence and mortality, which brings an enormous burden on society all over the world [1]. In the field of basic research, the design and implementation of malignancy chemotherapy have become increasingly complex, while the ideal chemotherapy drugs with excellent efficacy and low toxicity have not been exploited [2,3]. Doxorubicin (DOX) is an anthracycline-based antibiotic drug, which has been widely used for tumor treatment in clinic [4]. DOX can inhibit the biosyntheses of nucleic acids through embedding DNA, which plays an important role in clinical tumor suppression [5]. In practice, DOX has strong efficacy for most cancers, such as breast cancer, lung cancer, ovarian cancer, and soft tissue sarcoma. However, DOX belongs to small molecule drugs, which have many unfavorable properties: short circulation time and half-life, and significant systemic toxicity [6].

The polymer-drug conjugates mean making the general clinical drugs, including gene, antibiotic, interferon, hormone, immunosuppressant, and so on, connect to polymers for improved therapeutic effect and reduced side effects [7,8]. Back in 1986, SMANCS, a polystyrene-maleic acid-conjugated neocarzinostatin as a kind of conjugate, was approved in Japan for the treatment of hepatocellular carcinoma [9]. Recent years, the polymer-drug conjugates based on environment-friendly polymeric matrices have been a hotspot. It is because of higher tumor killing effect and lower cytotoxicity due to the selective accumulation in tumor tissue, effective tumor cellular uptake, and sustained drug release. The prodrugs can reach to a good treatment efficacy by controlling the drug release from conjugates. Therefore, the polymer-drug conjugates are paid more attention in developing new drug formulations [10–12].

The solid tumors exhibit characteristic microenvironment attributed to the special origin, nutrient supply, growth form, metabolic pathway, *etc.* [13]. In detail, the tumor tissue has lower pH (pH ~6.8) than normal tissues (pH 7.2–7.4). At the same time, the early endosome (pH 5.9–6.2), and late endosome and even lysosome (pH 5.0–5.5) in tumor cells have the lowest pH [14]. In addition, there is a visible redox potential existing between the

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extracellular and intracellular microenvironments, attributing to the different reduced and oxidized glutathione concentrations [15,16]. Furthermore, for nanosized drug delivery systems, the enhanced permeability and retention (EPR) effect is widely employed to achieve passive targeting [17,18]. Based on the above research background, the polymer-drug conjugates with stimuli-responsiveness (*e.g.*, pH or redox) can play to their strengths excellently by the unique chemical structures and spontaneous self-assembly into nanoparticles [19,20], so as to achieve a desired efficacy and security.

Polysaccharides include dextran (Dex), lentinan, alginate, hyaluronic acid, and so on. Polysaccharides encompass many brilliant physical, chemical, and physiological properties, such as excellent biocompatibility, biodegradability, and polyfunctionality for facile chemical decoration [21]. Based on the above properties, polysaccharides are accomplished as excellent biomaterials for drug and gene delivery, and tissue engineering [22–24]. Polysaccharide-drug conjugates play an irreplaceable role in researching and developing novel antitumor drugs by different conjugate techniques [25,26]. Among all polysaccharides, Dex is a bacterial polysaccharide, which can improve the level of interleukin [5], and fully stimulate the body's immune system [27,28]. As an excellent medium, Dex has been widely used in a variety of antitumor drug formulations, such as conjugates [29,30], micelles [31,32], vesicles [33,34], and nanogels [35,36]. As expected, almost all the formulations exhibit superior performances in antitumor drug delivery.

The terminal group in most polysaccharides exhibits the characteristic of mutual transformation between hydroxyl and aldehyde groups [37]. As shown in Scheme S1, Supporting information, the typical transformation of Dex was described. In this work, a versatile oxime click reaction between the terminal aldehyde group of Dex and the amino group in DOX was employed to synthesize the intracellular acidity-sensitive Dex-*O*-DOX and insensitive Dex-*b*-DOX. Subsequently, the self-assembly behaviors, DOX release profiles, cellular internalization and cytotoxicities *in vitro*, and maximum tolerated doses (MTDs), tissue distribution, and antitumor efficacies and security *in vivo* of Dex-DOX conjugates were systematically revealed.

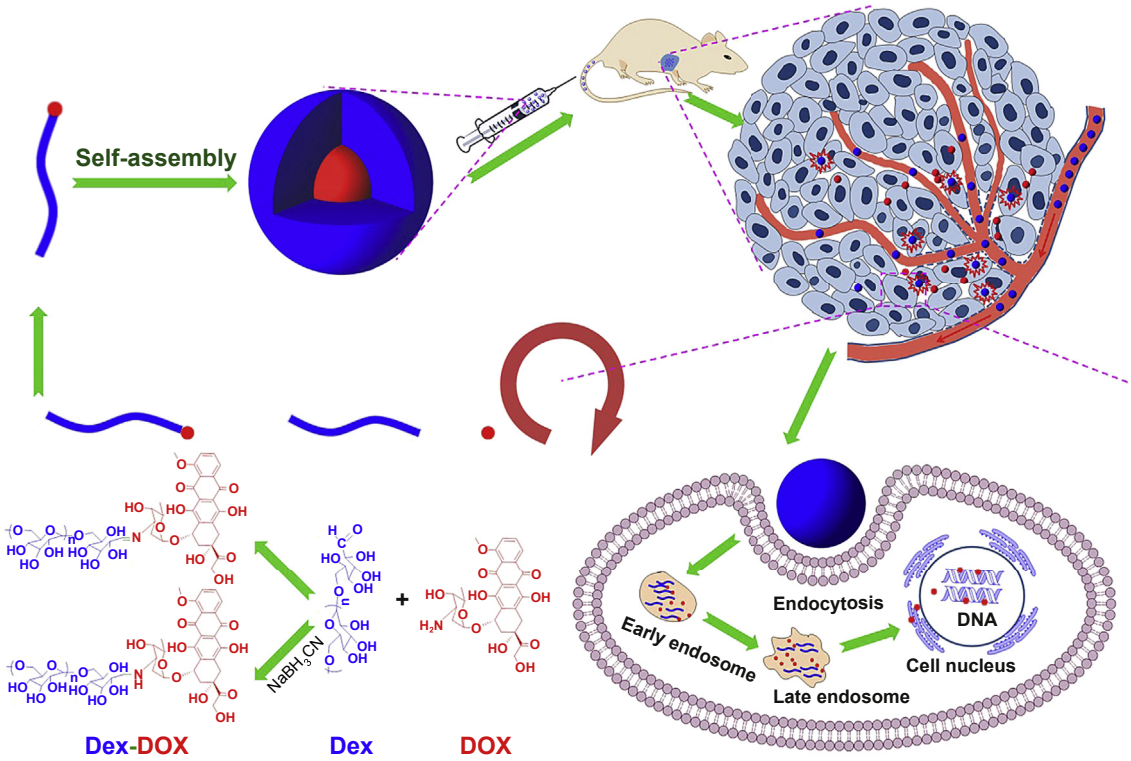
2. Materials and methods

2.1. Materials

Dex (relative molecular mass (M_r) = 5000 Da) was purchased from J&K scientific Ltd. (Beijing, P. R. China). Doxorubicin hydrochloride (DOX·HCl) was purchased from Zhejiang Hisun Pharmaceutical Co., Ltd. (Zhejiang, P. R. China). Acetic acid and sodium acetate were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, P. R. China) and used as obtained. Cell culture products, including Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS), were bought from Gibco (Grand Island, NY, USA). Penicillin and streptomycin were obtained from Huabei Pharmaceutical Co., Ltd. (Hebei, P. R. China). Sodium cyanoborohydride ($NaBH_3CN$), 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI), and Alexa Fluor 488 phalloidin (Alexa 488) were purchased from Sigma–Aldrich (Shanghai, P. R. China). Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) kit was purchased from Roche Company (Mannheim, Germany). Caspase-3 and survivin antibodies were purchased from Abcam Company (Cambridge, UK). Streptacidin/peroxidase 9710 and diaminobenzidine chromogenic kits were purchased from Fuzhou Maixin Biotechnology Development Co., Ltd. (Fuzhou, P. R. China). The purified deionized water was prepared by the Milli-Q plus system (Millipore Co., Billerica, MA, USA).

2.2. Syntheses of Dex-DOX conjugates

As shown in Scheme 1, the oxime-linked intracellular-acidity-sensitive Dex-*O*-DOX was prepared through the oximation reaction between the terminal reactive aldehyde group of Dex and the amino group in DOX [37–39]. In detail, Dex (50.0 mg, 0.01 mmol) was dissolved in acetate/acetic acid buffer solution at pH 5.0, and followed by the addition of DOX·HCl (29.0 mg, 0.05 mmol, 5 times equivalent to Dex). The reaction was performed at 45 °C for 72 h in darkness. The excess drug was removed by dialysis (molecular weight cut-off (MWCO) = 3500 Da) against purified deionized water replaced every 2 h for 24 h, and followed by lyophilization in the dark. In addition, Dex-*O*-DOX was washed three times with methanol and then



Scheme 1. Schematic illustration for syntheses and self-assembly of Dex-DOX conjugates, and *in vivo* circulation, selective accumulation in tumor tissue, and final pH-triggered intracellular DOX release after intravenous injection.