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Efficient nuclear drug translocation and improved drug efficacy mediated by acidity-responsive boronate-linked dextran/cholesterol nanoassembly

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ABSTRACT

The present study reported a lysosome-acidity-targeting bio-responsive nanovehicle self-assembled from dextran (Dex) and phenylboronic acid modified cholesterol (Chol-PBA), aiming at the nucleus-tropic drug delivery. The prominent advantage of this assembled nanoconstruction arose from its susceptibility to acidity-labile dissociation concurrently accompanied with the fast liberation of encapsulated drugs, leading to efficient nuclear drug translocation and consequently favorable drug efficacy. By elaborately exploiting NH_4Cl pretreatment to interfere with the cellular endosomal acidification progression, this study clearly evidenced at a cellular level the strong lysosomal-acidity dependency of nuclear drug uptake efficiency, which was shown to be the main factor influencing the drug efficacy. The boronate-linked nanoassembly displayed nearly no cytotoxicity and can remain structural stability under the simulated physiological conditions including 10% serum and the normal blood sugar concentration. The cellular exposure to cholesterol was found to bate the cellular uptake of nanoassembly in a dose-dependent manner, suggesting a cholesterol-associated mechanism of the intracellular internalization. The *in vivo* antitumor assessment in xenograft mouse models revealed the significant superiority of DOX-loaded Dex/Chol-PBA nanoassembly over the controls including free DOX and the DOX-loaded non-sensitive Dex-Chol, as reflected by the more effective tumor-growth inhibition and the better systematic safety. In terms of the convenient preparation, sensitive response to lysosomal acidity and efficient nuclear drug translocation, Dex/Chol-PBA nanoassembly derived from natural materials shows promising potentials as the nanovehicle for nucleus-tropic drug delivery especially for antitumor agents. More attractively, this study offers a deeper insight into the mechanism concerning the contribution of acidity-responsive delivery to the enhanced chemotherapy performance.

1. Introduction

Pathology-specific bio-responsive nanoparticles (NPs) represent the development trend of drug delivery vehicles [1], which are featured with the dynamic stability depending on the special microenvironments in the diseased sites [2]. pH-Sensitive polymeric NPs targeting at the late endosome/lysosome organelles (pH 4.0–5.5) are currently actively investigated for the improved tumor chemotherapy [3–6]. The pH-sensitive NPs remain intact at physiologically normal conditions in favor of the prolonged circulation duration and preferential tumor accumulation through the enhanced permeability and retention (EPR) effect [7]. Upon the endocytosis by tumor cells, those NPs can experience the programmed changes in response to the dramatically reduced pH of lysosome and in turn release the loaded drugs more readily [8]. Consequently, the high drug concentration gradient between cytoplasm and nucleus is thought to facilitate the nuclear entry of free drugs *via* the diffusion pathway. This sounds very appealing for tumor chemotherapy since many first-line antitumor drugs such as doxorubicin (DOX) and camptothecin have to induce cell death after the nuclear localization [9]. Nevertheless, it is noted that the direct supporting evidences of this mechanism have been actually

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lacking in a cellular level though lots of pH-sensitive NPs have been reported for this purpose. Moreover, the enhancement of drug efficacies in cells is insignificant and far from satisfactory in some cases [10,11]. Those problems largely hamper the advance of the researches and applications of lysosome-acidity responsive drug delivery.

Currently, pH-sensitive polymeric NPs are mostly prepared through chemical introduction of acidity-labile bonds into the polymer structure [12,13]. Compared with it, pH-dependent self-assembly approach shows apparent superiority in terms of the facile control over the composition and property of NPs, and possibly the more sensitiveness to pH stimuli. Nevertheless, the related studies are very rarely reported. Covalent boronic acid-diol coupling can spontaneously form at neutral condition but is susceptible to acidity-induced cleavage [14–19]. The unique pH-reversible characteristic makes boronate linking attractive as the driving force for the integrative assembly yet with the dynamic stability depending on the surrounding pHs.

Taking all those mentioned above into consideration, the present study reports a lysosome-targeted acidity-responsive nanoconstruction self-assembled from dextran (Dex) and phenylboronic acid modified cholesterol (Chol-PBA). The rationale behind choosing Dex and cholesterol is that the biomaterials derived from natural materials should be preferable as far as the systematic safety is concerned. Furthermore, the formed nanoassembly may share the merits from the parents including the serum-tolerability of Dex and the transmembrane ability associated with cholesterol [20,21]. Triggered by the lysosomal acidity, Chol-PBA/Dex nanoassembly is expected to undergo structural destruction and thus affect the release behavior of entrapped drugs (Scheme 1). Therefore, its potency as the lysosome-targeted acidity-responsive delivery NP has been herein *in vitro* and *in vivo* evaluated in terms of the nuclear drug translocation and antitumor efficacy. More attractively, this work intends to explore the dependence of the nuclear drug uptake efficiency and drug efficacy on the intracellularly lysosomal acidity by elaborately interfering with the endosomal acidification progression. We hope this study can offer a deeper insight about the contribution of acidity-responsive delivery to the antitumor chemotherapy.

2. Materials and methods

2.1. Materials

Dextran (Dex, M_n = 3000 Da), 3-aminophenylboronic acid (PBA, 98%), cholesteryl chloroformate (Chol, 98%) , cholesterol (98%), Alizarin Red S (ARS), glucose, Stearic acid (SA), N,N′-dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP) were obtained from Aldrich Chemical Co. Ltd. Doxorubicin hydrochloride (DOX·HCl) was purchased from Zhejiang Hisun Pharmaceutical Co., Ltd. (China). All other chemicals were purchased from Shanghai Chemical Reagent Ltd. and used without any treatments.

Dulbecco's modified Eagle's Medium (DMEM), fetal bovine serum (FBS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), Hoechst 33342, LysoTracker Red DND-99, and PBS were purchased from Invitrogen (Carlsbad, CA, USA).

2.2. Measurements

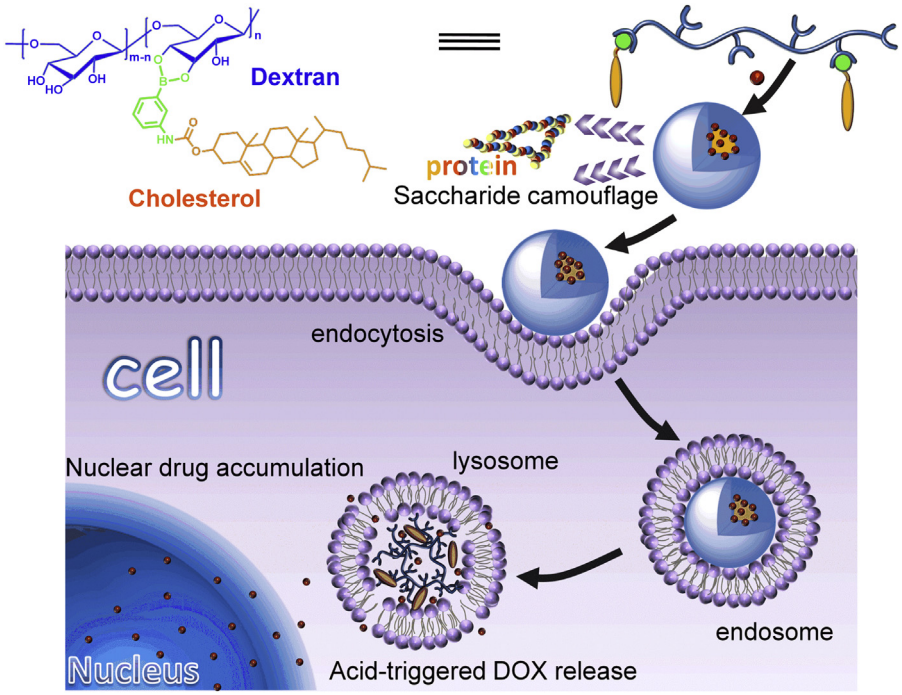
^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Varian Unity 300 MHz spectrometer using D_2O or d-DMSO as the solvent. Transmission electron microscopy (TEM) was carried out using a JEOL JEM-100CXII instrument operating at an acceleration voltage of 80 KV. Briefly, a drop of nanomicelle suspension was placed on a copper grid with formvar film and stained by a 0.2% (w/v) solution of phosphotungstic acid before photography.

2.3. Synthesis and characterization of cholest-5-en-3-ol(3β)-(3-boronophenyl) carbamate (Chol-PBA)

According to an amended method [22], cholesterol chloroformate (1.8 g, 4.0 mM) and 3-aminophenylboronic acid (1.1 g, 8.0 mM) were dissolved in 100 mL of dry distilled diethyl ether under the nitrogen protection at 30 °C. N-methylimidazole (0.82 g, 10 mM) was then added under stirring. The reaction process was monitored by TLC. After around 30 h, the resulting cloudy solution was diluted with diethyl ether to 150 mL followed by washing with deionized water (4 × 50 mL). The ether layer was dried over Na_2SO_4 and the solvent was then removed by rotary evaporation to give a white powder. The product was purified by column chromatography (petroleum ether:ethyl acetate = 2:1).

2.4. Nanoassembly preparation

Dex/Chol-PBA nanomicelles were prepared by the solvent evaporation method. Briefly, a predetermined amount of Chol-PBA was dissolved in 0.1 mL of THF. This solution was added dropwise into 15 mL of Dex solution (3 mg mL^{-1}), the solution was allowed standing at 30 °C for 24 h under stirring. The feeding ratios of Dex to



Scheme 1. Illustration of reversible pH-dependent Dex/Chol-PBA nanoassembly for lysosome-acidity-targeting bio-responsive drug delivery.