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Hyaluronic acid ion-pairing nanoparticles for targeted tumor therapy

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ABSTRACT

Hyaluronic acid (HA)-based doxorubicin (DOX) nanoparticles (HA-NPs) were fabricated *via* ion-pairing between positively charged DOX and negatively charged HA, which displayed near-spherical shapes with an average size distribution of 180.2 nm (PDI = 0.184). Next, HA-NPs were encapsulated in liposomal carriers to afford HA-based DOX liposomes (HA-LPs), which also showed near-spherical morphology with an average size of 130.5 nm (PDI = 0.201). HA-NPs and HA-LPs displayed desirable sustained-release profiles compared to free DOX, and moreover, HA-LPs were proven to prevent premature release of DOX from HA-NPs. Cell based studies demonstrated HA-NPs and HA-LPs were selectively taken up by $CD44^+$ tumor cells, and DOX was released intracellularly to target the cell nuclei. Both HA-NPs and HA-LPs showed comparable levels of penetration efficiency in tumor spheroids. *In vivo* studies revealed that HA-NPs and HA-LPs significantly prolonged the blood circulation time of DOX, decreased accumulation in the normal tissues and enriched drugs into the tumors. Furthermore, HA-NPs and HA-LPs greatly enhanced therapeutic efficacy of DOX in tumor-bearing mice and minimized systemic toxicity against vital organs. In sum, HA-NPs and HA-LPs represent promising nanocarriers for $CD44^+$ tumor-targeted delivery.

1. Introduction

Hyaluronic acid (HA) is an anionic polysaccharide consisting of repeating disaccharide units of N-acetyl-D-glucosamine (1-β-4) and D-glucuronic acid (1-β-3) (Fig. 1a) [1], which is a critical component of the extracellular matrix [2,3]. As a naturally derived macromolecule, HA has been extensively used in biomedical applications due to excellent biocompatibility, biodegradability, low-toxicity and low-immunogenicity. HA has been used as a moisture protectant in cosmetics and medical fields over the past few decades. Moreover, HA and modified HA have been extensively used for ophthalmic surgery [4], arthritis treatment [5], tissue engineering [6] and drug delivery [7,8].

Recently, cancer cells were found to overexpress CD44 receptor, which is a principal cell-surface receptor for HA [9–12]. HA was proven to specifically bind to surface CD44 receptors on cancer cells, and thus, HA has been explored as a targeting ligand in tumor-targeted drug delivery [13–17]. As a tumor-targeting vector, the main application of HA was in the following aspects. The structure of HA can be modified to generate a variety of HA derivatives, which retain biodegradability and compatibility with improved stability [18–21]. HA has rich carboxyl and hydroxyl groups which can be used as sites of chemical modification. Eun Ju Oh et al. designed and synthesized a series of HA derivatives successfully for the real-time imaging of HA derivatives using quantum dots (QDot), which confirmed the presence of CD44 receptors in human organs (liver, kidney and spleen) [22]. HA was also used in the surface modification of nanoparticles or liposomes by chemical coupling to achieve tumor targeting or improve biocompatibility [16,23–26]. Eliaz et al. modified the surface of cationic liposomes with HA by chemical coupling, which greatly improved the tumor target ability of liposomes [24–26]. Regarding modified HA derivatives, they become novel materials after chemical modification, and thus the biocompatibility, biodegradability and cytotoxicity of the materials remain to be further elucidated.

In the current study, we aimed to fabricate a HA-based nanoparticle that not only carries the advantages of HA but also maintains its structural integrity. Classical polymeric nanoparticles displayed the core-shell structure that usually consists of a hydrophilic shell and a hydrophobic core. Due to the presence of negatively charged carboxyl groups, HAs were hypothesized to interact with positively charged hydrophobic drugs by forming ion pair complexes thus resulting in the formation of self-assembled nanocomplexes with positively charged hydrophobic drugs in the core and negatively charged hydrophilic HAs in the shell. Owing to its high potency and broad spectrum of anti-tumor activity, doxorubicin (DOX) is a leading therapeutic in various cancer chemotherapy [27]. As a result, we selected DOX as the model drug (Fig. 1b), and fabricated HA-based DOX nanoparticles (HA-NPs) *via* electrostatic interactions (Fig. 1c). In our preliminary study, HA-NPs displayed poor stability during storage at room temperature and significant burst effect in the plasma. Next, HA-NPs were further encapsulated in phospholipid bilayers to afford HA-based DOX liposomes (HA-LPs) (Fig. 1d), which

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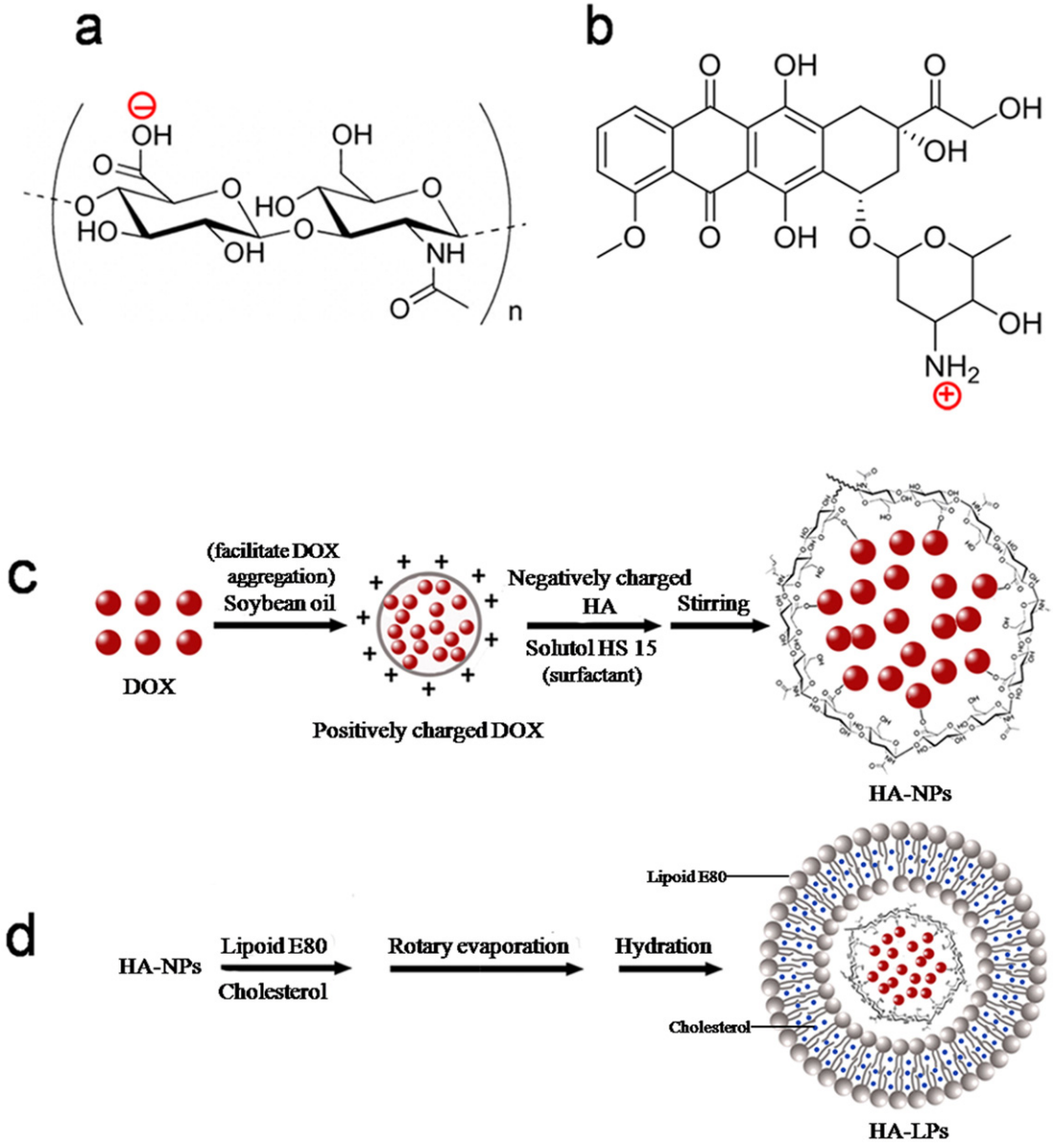

Fig. 1. Chemical structure of (a) HA and (b) DOX. Schemes of (c) HA-NPs and (d) HA-LPs fabrication.

were designed to reduce premature release of DOX from HA-NPs. The method for HA-NP fabrication was proven simple and convenient, which successfully maintained the structural integrity of HA without any chemical modification.

2. Materials and methods

2.1. Materials

Sodium hyaluronate was obtained from Freda Biopharm Co., Ltd. (Shandong, China). DOX hydrochloride was obtained from Huafeng Technology Co., Ltd. (Beijing, China). Cholesterol was obtained from Kelong Company (Chengdu, China). Lipoid E80 was obtained from Lipoid Co., Ltd. (Ludwigshafen, Germany). Solutol HS 15 was obtained from BASF Aktiengesellschaft (Ludwigshafen, Germany). Soybean oil for injection was purchased from Tieling Medical Oil Co., Ltd. Other reagents and chemicals were analytical grade and purchased commercially.

Wistar rats and Kunming mice were obtained from experiment animal center of Sichuan University (Chengdu, China). All animal studies presented were performed per the institutional guidelines and approved by the Ethics Committee of Sichuan University.

2.2. Preparation

DOX hydrochloride was neutralized by the following procedure. Briefly, 10 mg of DOX hydrochloride was dissolved in 2 ml of deionized water. Then the pH value was adjusted to about 10 to remove the hydrochloride salt. The solution was extracted with dichloromethane. Then dichloromethane was removed by rotary evaporator to yield DOX.

2.2.1. Preparation of HA-NPs

As shown in Fig. 1c, 3.6 mg of HA and 50 mg of Solutol HS 15 were dissolved in 10 ml of deionized water. 100 μl of soybean oil for injection was added to HA mixture and the reaction was stirred for 10 min at room temperature. Then, 2 ml of ethanol solution containing 5 mg of DOX was added dropwise to the above solution. This reaction was stirred for 30 min at room temperature. Next, the mixture was maintained for 30 min at 45 °C to evaporate the ethanol by rotary evaporator. The obtained suspension was sonicated for 3 min at 265 W (5 s pulses