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Hyaluronic acid coated PLGA nanoparticulate docetaxel effectively targets and suppresses orthotopic human lung cancer

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ABSTRACT

PLGA nanotherapeutics though representing a most promising platform for targeted cancer therapy are confronted with low stability and insufficient tumor cell uptake. Here, we report that hyaluronic acid (HA) coated PLGA nanoparticulate docetaxel (DTX-HPLGA) is particularly robust and can effectively target and suppress orthotopic human lung cancer. DTX-HPLGA was easily prepared with a small size of 154 nm and negative surface charge of −22.7 mV by nanoprecipitation and covalent coating with HA. DTX-HPLGA displayed a low IC_{50} of 0.91 μg/mL in CD44+ A549 cells and a prolonged elimination half-life of 4.13 h in nude mice. Interestingly, DTX-HPLGA demonstrated 4.4-fold higher accumulation in the cancerous lung than free DTX, reaching a remarkable level of 13.7 %ID/g at 8 h post-injection, in orthotopic human A549 lung cancer-bearing mice. Accordingly, DTX-HPLGA exhibited significantly better inhibition of tumor growth than free DTX, leading to healthy mice growth and markedly improved survival time. DTX-HPLGA with easy fabrication, excellent stability and tumor accumulation, effective tumor suppression, and low side effects is of particular interest for targeted chemotherapy of lung cancers.

1. Introduction

With increasing incidence and mortality, lung cancer especially non-small cell lung cancer (NSCLC) has emerged as a leading malignancy worldwide [1]. Docetaxel (DTX), a cell cycle-specific antitumor drug that can inhibit the depolymerization of microtubules, has been approved as first- and second-line treatment for NSCLC [2,3]. The clinical formulation of DTX in polysorbate 80 (Taxotere®), however, causes serious side effects including hypersensitivity reactions, nephrotoxicity, and cardiotoxicity [4,5]. To reduce systemic side effects and further improve therapeutic efficacy, various nanocarriers including polymeric micelles, nanoparticles, and liposomes have been explored for DTX delivery [6–8]. Interestingly, polymeric DTX nanoformulations based on e.g. poly(ethylene glycol)-b-poly(D,L-lactide) (PEG-PDLLA), PEG-b-poly(*N*-(2-hydroxypropyl)-methacrylamide-lactate), or cyclodextrin-PEG are under clinical investigation for treating various advanced solid tumors [9–11].

PLGA nanoparticles with excellent biocompatibility and biodegradability are one of the most attractive pharmaceutical delivery nanoplatforms [12–15]. Protein and peptide nanoformulations based on PLGA are currently used for the treatment of various diseases including cancer [16–18]. BIND-014, an active targeting micellar DTX formulation based on PEG-PLGA copolymer, has been under clinical translation for treating advanced solid tumors and NSCLC [19,20]. It should be noted, however, that current PLGA-based nanotherapeutics typically suffers from a low plasma stability, premature drug release, poor tumor accumulation and retention, and inefficient tumor cell uptake [21–23]. PLGA-based nanotherapeutics are usually fabricated using poly(vinyl alcohol), poloxamer, poly(vinyl pyrrolidone) and TPGS as surfactants [24–26], which gives an "inert" surface hindering target cell internalization. Moreover, these surfactants are prone to washing off during purification or in circulation, resulting in destabilization and aggregation of nanotherapeutics [27,28]. Interestingly, Zhang et al. reported that coating of PLGA nanotherapeutics with the plasma membrane of human platelets exhibited reduced cellular uptake by macrophage-like cells and enhanced therapeutic efficacy in a rat model of coronary restenosis and a mouse model of systemic bacterial infection [29]. Chen et al. demonstrated that PLGA nanoparticles following the lipid coating and AMD3100 decoration could efficiently deliver sorafenib and overcome acquired drug resistance in the orthotopic hepatocellular carcinoma model [30]. García-Salcedo et al. reported that PEG and antibody fragment covalently coated PLGA nanoparticulate pentamidine could cure all infected mice at a 10-fold lower dose than the minimal full curative dose of free drug using a murine model of African trypanosomiasis [31]. We recently reported a facile approach to prepare hyaluronic acid (HA) coated PLGA nanoparticles (HPLGA) using vitamin E-oligo(methyl diglycol L-glutamate) (VEOEG) as a surfactant [32]. Paclitaxel-loaded HPLGA exhibited effective inhibition of subcutaneously implanted MCF-7 breast tumor xenografts.

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In this paper, we report that HA coated PLGA nanoparticulate docetaxel (DTX-HPLGA) effectively targets and suppresses orthotopic human lung cancer (Scheme 1). It is known that lung cancers like A549 are overexpressing CD44 receptors [33–35]. Several studies have shown that HA based self-assembled nanoparticles can effectively deliver therapeutic nucleic acid, siRNA, and microRNA to both sensitive and resistant A549 lung cancer cells, achieving targeted treatment of solid and metastatic tumors *in vivo* [36,37]. HA coating of lipid/starch-based nanomedicines via electrostatic attraction has shown better antitumor efficacy toward lung cancer cells *in vitro* and *in vivo* [38,39]. DTX-HPLGA, unlike previous reported PLGA nanotherapeutics, is easy to fabricate and possesses multi-functions such as enhanced colloidal stability, superb tumor accumulation, and high specificity toward orthotopic human lung cancer in nude mice.

2. Materials and methods

2.1. Preparation of DTX-HPLGA

DTX-HPLGA was prepared by a modified nanoprecipitation method using VEOEG as a surfactant [40]. Briefly, 30 mg of PLGA and 2.6 mg of DTX were dissolved in acetone (3 mL). 0.9 mL of the resulting solution was dropwise added into VEOEG aqueous solution (0.45 mg/mL, 9 mL) under stirring at room temperature. After evaporating acetone, DTX-PLGA NPs were collected by centrifugation at 12,000 rpm for 10 min and then washed once with deionized water to remove free VEOEG surfactant and free drug. Taking advantages of positively charged amine groups in VEOEG located on the nanoparticles surface, negatively charged HA was facilely coated on the DTX-PLGA NPs via electrostatic interaction followed by covalent conjugation via EDC/NHS coupling method, similar to our previous report for HA coated PTX-PLGA NPs (PTX-HPLGA) [32]. The stability of DTX-PLGA NPs against extensive dilution and in 10% FBS was monitored by DLS measurement ($n = 3$). To monitor the intracellular behaviors and *in vivo* tumor-targetability, near infra-red fluorescence probe Cy5 was grafted on HA [32,41], and then coated on HPLGA to obtain Cy5-labeled HPLGA (Cy5-HPLGA).

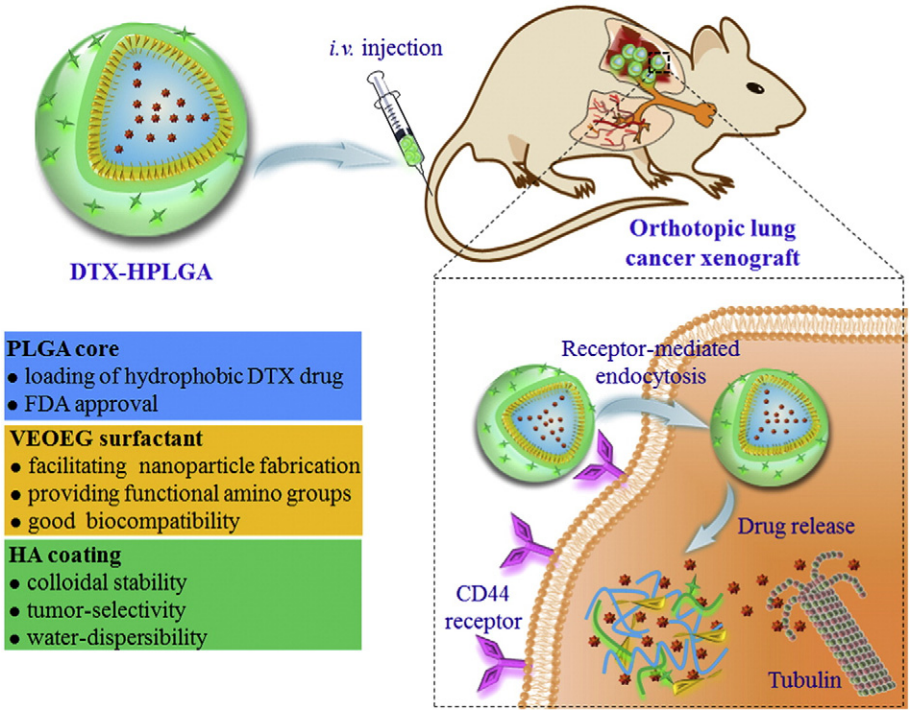
The DTX loading level in HPLGA was determined by dissolving DTX-HPLGA in acetonitrile/water (50:50, v/v), filtering through a filter (0.45 μm), and measuring using HPLC. The standard curve was acquired with DTX in acetonitrile/water (50:50, v/v) solutions ranging from 0.05 to 100 μg/mL. Drug loading content (DLC) and drug loading efficiency (DLE) were determined as a previous report [32].

2.2. Pharmacokinetics studies

Pharmacokinetics studies of DTX-HPLGA and free DTX were performed in nude mice (dosage: 5 mg DTX equiv./kg, $n = 3$). The mice were handled under protocols approved by Soochow University Laboratory Animal Center and the Animal Care and Use Committee of Soochow University. At predetermined time points post *i.v.* injection (0.05, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h for DTX-HPLGA while 2, 5, 10, 30, 60, 120 and 240 min for free DTX), 20 μL of blood was drawn, and briefly sonificated in 1.0 mL of acetonitrile/methanol (v/v = 1:1). After incubating overnight, the blood samples were centrifuged at 13,000 rpm for 20 min to obtain the supernatant. DTX was extracted by evaporating the solvent, and then re-dissolve in 250 μL of acetonitrile for HPLC measurement. The blood circulation followed a typical two compartment model, in which the distribution half-life ($t_{1/2\alpha}$) and elimination half-life ($t_{1/2\beta}$) were calculated according to the following formula: $y = A_1 \times \exp(-x/t_1) + A_2 \times \exp(-x/t_2) + y_0$, wherein $t_{1/2\alpha} = t_1 \times \ln 2$ and $t_{1/2\beta} = t_2 \times \ln 2$. The area under the curve (AUC) was estimated using the formula: $AUC = A_1 \times t_1 + A_2 \times t_2$.

2.3. Ex vivo imaging and biodistribution

Ex vivo imaging of HPLGA was evaluated in orthotopic human A549-Luc lung xenografts that were established by injecting 5×10^6 A549-Luc cells in 50 μL of matrigel/phosphate buffer (PB) mixture (1:4, v:v) into the left lung parenchyma of mice. The tumor size and sites were visualized by the measurement of bioluminescence through IVIS Lumina II imaging system (Caliper Life Sciences, U.S.A.) following the injection of D-luciferin potassium salt solution (15 mg/mL, 100 μL) in PBS. When the tumor luminescence intensity reached about



Scheme 1. Illustration of hyaluronic acid (HA) coated PLGA nanoparticulate docetaxel (DTX-HPLGA) that effectively targets and suppresses orthotopic human lung cancer.