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Self-assembled nanoparticles from hyaluronic acid–paclitaxel prodrugs for direct cytosolic delivery and enhanced antitumor activity

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ABSTRACT

A prodrug-based nanosystem obtained by formulating prodrug and nanotechnology into a system is one of the most promising strategies to enhance drug delivery for disease treatment. Herein, we report a new nanosystem based on HA–PTX conjugates (HA–PTX Ns), which penetrated across cell membranes into cytosol, thus enhancing paclitaxel (PTX) delivery. HA–PTX Ns were successfully obtained based on HA–PTX, and their average particle size was approximately 200 nm. Importantly, unlike other prodrug-based nanosystems, HA–PTX Ns obtained cellular entry without entrapment within the lysosomal–endosomal system by using pathways including clathrin-mediated endocytosis, microtubule-associated internalization, macropinocytosis and cholesterol-dependence. Due to significant accumulation in tumors, HA–PTX Ns had more than a 4-fold decrease in tumor volume on day 14 in contrast with PTX alone. In conclusion, HA–PTX Ns could enter cells, bypass the lysosomal–endosomal system and improve PTX delivery.

1. Introduction

Nanosystems for drug delivery (NsDD) possess great potential to improve the therapeutic effect of active compounds due to advantages such as enhanced solubility and drug stability, small nanoparticle size, the ability to target drugs to the disease site, changes in pharmacokinetics and tissue biodistribution and reduced side effects (Ashley et al., 2011; Cheng et al., 2012; Davis et al., 2008). However, conventional nanocarriers suffer from drawbacks such as poor stability in physiological conditions, a high tendency to recrystallize during storage, low drug-loading efficiency, premature burst release and poor target site accumulation, therefore resulting in limitations of clinical applications (Duhem et al., 2014; Mura et al., 2015; Zhang et al., 2013). Thus, novel NsDD that can overcome these drawbacks are highly desirable.

Prodrug-based NsDD that integrate prodrug strategies and nanotechnology into a system are some of the most promising ways to enhance the delivery of active compounds owing to their advantages including enhanced drug availability in target sites, high drug-loading capacity, noncrystallization upon encapsulation and controlled and prolonged drug release (Fang and Al-Suwayeh,

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2012; Luo et al., 2014). Generally, the currently available prodrug-based NsDD can be classified into three categories: (i) prodrug-nanoparticles prepared by formulating the prodrug into the nanocarriers via noncovalent interactions, (ii) small molecular weight prodrug-nanoparticles obtained by self-assembly of active compounds and (iii) NsDD based on amphiphilic polymer–drug conjugates, which are prepared by self-assembling the conjugates into nanoscaled particles. Due to the exceptionally high drug loading and excellent stability under physiological environments, NsDD based on polymer–drug conjugates are more efficient for drug delivery (Cai et al., 2015; Shen et al., 2010).

Paclitaxel (PTX), a highly hydrophobic drug with water-solubility less than 0.24 mg/L, is one of the most commonly used active compounds in clinics for cancer therapy. PTX suppresses microtubule dynamic instability, which is required for cellular division, and induces apoptosis (Li et al., 2015b; Schiff et al., 1979). However, like many other anticancer drugs, its clinical use is greatly limited by its inherent drawbacks including poor solubility, adverse side effects and poor tumor penetration (He et al., 2015b). In order to solubilize PTX, Cremophor EL and dehydrated ethanol have to be added into the product formulation, Taxol. However, the presence of Cremophor EL in Taxol would in turn generate severe side effects, such as allergy, hypersensitivity, and anaphylactic reactions, thus affecting 20–40% of patients and compromising the therapeutic index for PTX. Therefore, the development of a Cremophor EL-free PTX formulation is urgent.

Hyaluronic acid (HA), a naturally occurring biocompatible polyanionic polysaccharide, is an anionic biopolymer made up of alternating disaccharide units of D-glucuronic acid and *N*-acetyl-D-glucosamine with a β (1→4) interglycosidic linkage (Yang et al., 2015). Due to its well-tolerated and biocompatible nature, HA has been widely studied for biomedical applications including tissue engineering, drug delivery and molecular imaging (Li et al., 2015a; Martens et al., 2015). Importantly, HA is also utilized as a targeting ligand in drug carriers because it can effectively bind to the CD44 receptor, which is overexpressed in cancer cells (Liu et al., 2011; Zhong et al., 2015), thus achieving active targeting for anticancer drug delivery.

Herein, we reported a new NsDD based on HA–PTX conjugates. Indeed, other groups reported that HA–PTX is active for tumor therapy (Luo et al., 2000); however, few reports indicated that HA–PTX conjugates could form nanoparticles. Here, we demonstrate that HA–PTX conjugates can self-assemble into nanoparticles in aqueous conditions. Importantly, we demonstrated that HA–PTX Ns bypassed the lysosomal–endosomal system, were well taken up by cancer cells and were located in cytosol and nucleus. To our best knowledge, NsDDS that can obtain cellular entry without entrapment within the lysosomal–endosomal system have not been reported until now. Such a discovery would be beneficial to enlarge the application of based-polymer–drug conjugate nano-systems, severing as carriers for targeting delivery of protein or gene drugs into the cytosol. Such NsDDS also enhanced antitumor activities compared with free PTX, thus achieving active tumor therapeutic targeting without additional modification of other ligands. Using HA–PTX self-assembled nanopartiles is a promising strategy for enhancing PTX delivery for tumor treatment.

2. Methods and materials

2.1. Materials

PTX (99% purity) was obtained from Yew Biotechnology Co., Ltd. (Jiangsu, China). Taxol (marked product of PTX) was from Bristol-Myers Squibb (China) Investment Co., Ltd. (Shanghai, China). IR 783 probe (90% purity), fluorescein isothiocyanate (FITC, 98% purity), hyaluronidase and MTT (98% purity) were purchased from Sigma–Aldrich Co., Ltd. (St. Louis, MO, USA). Hyaluronic acid, *n*-hydroxy two imide, diphenyl phosphoryl chloride, adipic acid dihydrazide and succinic anhydride were purchased from Aladdin Industrial Inc. (Shanghai, China). The H22 cell line was purchased from Nanjin Key GEN Biotech Co., Ltd. (Nanjing, China). Anhydrous pyridine, carbodiimide hydrochloride (EDC) and triethylamine were from Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China). Fetal bovine serum, HBS, RPMI-1640, Dulbecco's modified Eagle medium and trypsin were obtained from Thermo Fisher Scientific Inc. (Waltham, MA, USA). DAPI and LysoTracker Red were obtained from Beyotime Institute of Biotechnology (Haimen, China). All of the other chemicals were of analytical reagent grade and were obtained from Sinopharm Chemical Reagent (Shanghai, China).

Male ICR mice (18–22 g) were purchased from the College of Veterinary Medicine at Yangzhou University (License No: SCXK (Su) 2012-0004, Yangzhou, China). The animals used in the experiments received care in compliance with the Principles of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals. The experiments followed the protocol approved by the China Pharmaceutical University Institutional Animal Care and Use Committee.

2.2. Preparation of and characterization of HA–PTX Ns

2.2.1. Synthesis of PTX–NHS

The synthesis of *n*-hydroxy two imide two phenyl phosphate (SDPP) was performed as described below. In brief, 230 mg *n*-hydroxy two imide, 34 μL diphenyl phosphoryl chloride and 280 μL triethylamine were added into dichloromethane in order, the mixture was stirred for 12 h under room temperature, and the reaction mixture was placed into a mortar and ground with ethyl ether. Subsequently, the deposition was dissolved with acetic ether and washed with water and saturated sodium chloride solution. Finally, the organic phase was mixed with anhydrous sodium and dried under reduced pressure.

The synthesis of PTX–semi succinyl salt (PTX–semi) was performed as described below. Briefly, 540 mg PTX and 76 mg succinic anhydride were dissolved in dichloromethane. Upon addition of 513 μL anhydrous pyridine, the reaction mixture was stirred for three days at room temperature. After concentration under vacuum conditions, the sample was purified with column chromatography using acetic ether and *n*-hexane (1:1, v/v) as eluents.

The synthesis of PTX–NHS was next. After dissolving 300 mg PTX–semi and 164 mg SDPP in acetonitrile, the reaction mixture was mixed with 176 μL triethylamine and stirred at room temperature for 24 h. Upon condensation under vacuum conditions, the mixture was separated with a mixture of acetic ether and *n*-hexane (1:1, v/v) as eluents (Greenwald et al., 1996).

2.2.2. Synthesis of HA–adipic acid dihydrazide (HA–ADH)

Low molecular weight (MW) HA was prepared as follows: 2 g high MW HA (1.5 MDa) was first dissolved in PBS (pH 6.5, 4 mg/mL), and then hyaluronidase (10 UI/mg) was added into the enzymatic reaction solution, which was kept at 37 °C for 1 h and stopped at 90 °C. After that, the sample was purified by dialyzing with a dialysis bag (MWCO = 3500 Da) and filtering through a 0.22-μm cellulose acetate membrane. Finally, the purified sample was freeze-dried for use.

HA–ADH synthesis is listed below. ADH (52 mg) was placed into 15 mL low MW HA solution (3 mg/mL), and the reaction mixture was adjusted to pH 4.75 with 0.1 M HCl. It was then blended with 12 mg EDC and reacted for 24 h at pH 4.75. The reaction was stopped by pH regulation to 7.0 with 0.1 M sodium hydroxide. The sample was dialyzed successively against 0.1 M sodium hydroxide, 25% ethanol/water and water (MW cut off 3.5 kDa). The purified