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Endothelial growth factor receptor-targeted and reactive oxygen species-responsive lung cancer therapy by docetaxel and resveratrol encapsulated lipid-polymer hybrid nanoparticles

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ABSTRACT

Special targeted therapy like endothelial growth factor receptor (EGFR) targeted therapy is available for the treatment of advanced non-small cell lung cancer (NSCLC). Biodegradable core-shell lipid-polymer hybrid nanoparticles (LPNs) can combine the beneficial properties of lipid and polymeric NPs for controlled drug delivery. In the present study, epidermal growth factor (EGF) conjugated LPNs were fabricated to co-deliver docetaxel (DTX) and resveratrol (RSV). In vitro and in vivo studies demonstrated that EGF DTX/RSV LPNs have significant synergistic effects, best tumor inhibition ability and the lowest systemic toxicity. The results indicate that EGF DTX/RSV LPNs may be a promising strategy for treatment of NSCLC.

1. Introduction

Lung cancer is the leading cause of cancer death worldwide. In 2017, 222,500 new cases of lung and bronchial cancer and 155,870 deaths are estimated in the United States [1,2]. In the developing countries, an exponential higher rate of lung cancer (approximately 69 percent) has been observed [3]. Among lung cancer cases, non-small cell lung cancer (NSCLC) accounts for more than 80% [4]. Surgery, radiation therapy, and systemic therapy are the most common treatments for patients with NSCLC. Because late diagnosis is a major obstacle to using surgical procedure, systemic therapy (including platinum agents, taxanes, vinorelbine, *etc*) is recommended for patients with stage IV NSCLC [5–7]. Docetaxel (DTX) is an anti-neoplastic agent belonging to the taxoid family. It is recommended in the NCCN guidelines as the treatment for advanced or metastatic NSCLC [8]. However, its low aqueous solubility, drug resistance and severe toxicity such as hypersensitivity reaction, peripheral neuropathy, neutropenia, and stomatitis/mucositis are still major drawbacks for its clinical application [9,10]. In European and American, around 10%–15% of people with NSCLC will have endothelial growth factor receptor (EGFR) mutation positive (EGFR M+); whereas up to 50% of patients with NSCLC have EGFR M + in China [11]. Therefore, special targeted therapy like EGFR targeted therapy is available for the treatment of advanced NSCLC. Current approaches to encapsulate and deliver therapeutic compounds have focused on developing lipid nanoparticles (NPs) and polymeric NPs, resulting in clinically approved therapeutics such as Doxil/Caelyx and Genexol-PM, respectively [12]. Biodegradable core-shell lipid-polymer hybrid nanoparticles (LPNs) consist of a biodegradable hybrophobic polymeric core, a monolayer of phospholipids and an outer layer made of ligand-PEG polymer [13]. LPNs can combine the beneficial properties of lipid and polymeric NPs for controlled drug delivery [14]. In the present study, epidermal growth factor (EGF) conjugated LPNs were fabricated to co-deliver docetaxel (DTX) and resveratrol (RSV).

Resveratrol (RSV, trans-3,5,4′-trihydroxystilbene) is a polyphenol that is most commonly found in grapes, red wine, and peanuts [15]. It is a promising anti-tumor agent due to its ability to inhibit all three major carcinogenesis stages, containing initiation, promotion and progression [16,17]. RSV could act as an activator of the tumor suppressor p53 and silent mating type information regulation 2 homolog 1 (SIRT1) [18,19]. In many studies, RSV has been found to enhance reactive oxygen species (ROS) production in cancer cells inducing cytotoxicity [17,20]. ROS are commonly higher in cancer cells than their normal counterpart cells, including lung caner cells, drug resistant cell with breast cancer MCF-7 cells, prostate cancer cells, and colorectal cancer cells [13,21–23]. Therefore, ROS play a role in tumor microenvironment, drug resistance and targeted therapy [24,25]. In spite of the various merits of RSV aforementioned, its demerits hinder its use in cancer therapy which includes low aqueous solubility, instability in vivo, poor bioavailability and permeability [26]. So NPs that can carry RSV and

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deliver it to the demand site are urgently needed.

Here we report EGFR-targeted LPNs for targeted delivery of DTX and RSV in the mitochondria of tumor cells, which can overcome multi-drug resistance. EGF and stearic acid (SA) are conjugated to polyethylene glycol (PEG) via EDC/NHS chemistry to achieve EGF-PEG-SA. EGF-PEG-SA was then used to prepare EGF-modified DTX and RSV co-encapsulated LPNs (EGF DTX/RSV LPNs).

2. Materials and methods

2.1. Chemicals and reagents

DTX (≥ 97%), RSV (≥ 99%), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), dimethyl sulphoxide (DMSO), didecyldimethylammonium bromide (DDAB), fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), Roswell Park Memorial Institute (RPMI) 1640 medium, and 3-[4, 5-dimethylthiazol-2 yl]-2, 5 diphenyltetrazolium (MTT) were purchased from Sigma Aldrich (St. Louis, MO). NH_2-polyethylene glycol-COOH (Mw 5000, NH_2-PEG_{5000}-COOH) was provided by Beijing Chemgen United Medical Technology Co. Ltd. (Beijing, China). mPEG-PLA (Mw: PEG:5000, PLA:100,000) was obtained from Xi'an Ruixi Biotechnology Co., Ltd (Xi'an, China).

2.2. Synthesis and characterization of EGF-PEG-SA

The conjugation of EGF, PEG, and SA was achieved through chemical crosslinking using a coupling agent EDC (1 mM) and NHS (1 mM) [27]. SA was dissolved in DMSO, EDC and NHS was added and stirred for 1 h at room temperature (RT) (1). NH_2-PEG_{5000}-COOH was dissolved in DMSO and added into (1) under stirring for 12 h at RT to form PEG-SA (2). EGF was dissolved by DMSO in the presence of EDC and NHS and added drop by drop into (2) and stirred at RT for another 12 h to achieve EGF-PEG-SA (Fig. 1A). EGF-PEG-SA was then dialyzed against deionized water for 24 h. ^{1}H NMR of EGF-PEG-SA in dimethyl sulfoxide(DMSO)-d6 is analyzed.

2.3. Preparation of EGF DTX/RSV LPNs

EGF DTX/RSV LPNs (Fig. 1C) were prepared via nanoprecipitation method [28]. Briefly, 100 mg EGF-PEG-SA were dissolved in 20 mL acetone-water solution (10%, v/v) (1). 100 mg mPEG-PLA, 20 mg DTX, and 20 mg RSV were dissolved in 10 mL acetone (2). (1) and (2) was added dropwise into 20 mL 0.5% DDAB water solution (0.5%, w/v). Thereafter, the mixture was stirred at 600 rpm until complete evaporation of acetone. Purification was done by washing the nanoparticles dispersion in water three times using an Amicon Ultra-4 centrifugal filter (Millipore Corporation, Bedford). Non EGF modified DTX and RSV co-encapsulated LPNs (DTX/RSV LPNs) were prepared via the same way using PEG-SA instead of EGF-PEG-SA. Non EGF modified single drug encapsulated LPNs were prepared via the same way and named DTX LPNs and RSV LPNs, respectively. Blank EGF modified LPNs were prepared via the same way without drugs and named EGF LPNs. Coumarin-6-encapsulated all kinds of LPNs were prepared via the same way by adding coumarin-6 into the mixture (2). The final formulations were washed three times by repeating centrifugation step and freeze-dried at −20 °C.

2.4. Physicochemical characterization of EGF DTX/RSV LPNs

A drop of EGF DTX/RSV LPNs suspension was placed onto a copper grid and air drying, followed by negative staining with one drop of 3% aqueous solution of sodium phosphotungstate for contrast enhancement [29]. The air-dried samples were then directly observed on a JEM-1200EX transmission electronic microscopy (TEM) microscope (JEOL Ltd., Tokyo, Japan). Images were recorded digitally to evaluate the morphology of EGF DTX/RSV LPNs. Mean particle size, polydispersity index (PI) and zeta potential of the LPNs were measured with a Coulter Nano-sizer DelsaTM Nano C (Fullerton, CA) [30].

2.5. Drug encapsulation and in vitro release of EGF DTX/RSV LPNs

Drug encapsulation efficiency (EE) of EGF DTX/RSV LPNs was detected by separating the unencapsulated drug from LPNs at 10,000 rpm centrifugation for 10 min. Drug content in clear supernatant was analyzed through high-performance liquid chromatography (HPLC, LC-20AT, Shimadzu, Tokyo, Japan). HPLC was equipped with a LC-20AT pump and SPD-20 A UV detector controlled through Lab-solution software. DTX EE analysis was performed using a Diamonsil 5 μm C18 column (4.6 mm × 250 mm) [31]. The mobile phase was a mixture of acetonitrile and distilled water (6:4, v/v) at a flow rate of 1.0 mL/min at 30 °C. The wavelength was monitored at 230 nm. RSV content was determined using an XBridge 5 μm C18 column (150 × 4.6 mm) [32]. The mobile phase was a mixture of methanol and 0.5% acetic acid (4:6,

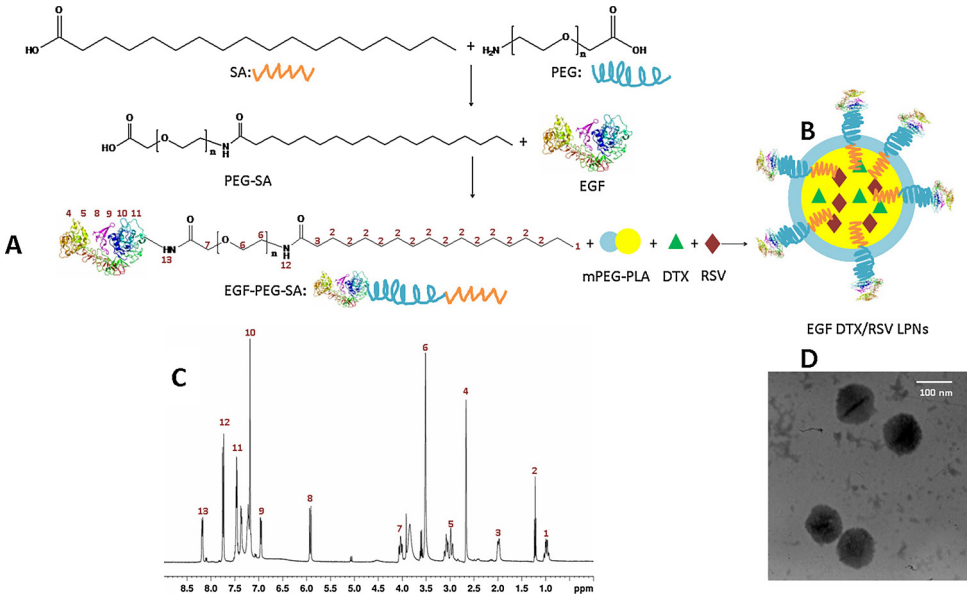

Fig. 1. Synthesis scheme (A) and ^{1}H-NMR spectroscopy (B) of EGF-PEG-SA; schematic diagram (C) and TEM image (D) of EGF DTX/RSV LPNs.