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Transferrin and tocopheryl-polyethylene glycol-succinate dual ligands decorated, cisplatin loaded nano-sized system for the treatment of lung cancer

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ABSTRACT

Nanocarriers decorated with different ligands were used to achieve lung cancer treatment. Surface decoration of nanoparticulate system will assist in targeting the drug to specific tumor cells and tissues. The aim of this research was to develop a dual ligands decorated nanocarriers (NCs), which could increase the cell uptake and anti-tumor efficiency. Two different ligands: Transferrin (Tf) and D-α-tocopheryl polyethylene glycol succinate (TPGS) containing ligands were synthesized. Dual ligands decorated nanocarriers (DL-NCs) was constructed. The *in vitro* cytotoxicity, *in vivo* biodistribution, and *in vivo* antitumor efficacy of the DL-NCs were evaluated. DL-NCs can efficiently deliver cisplatin (CDDP) into lung cancer cells *in vitro* and reduced xenograft tumor size *in vivo*. The encapsulation of CDDP in the DL-NCs significantly improved the cytotoxicity and antitumor efficacy. DL-NCs held great potential for achieving an optimal therapeutic effect in the treatment of lung cancer.

1. Introduction

Lung cancer is the leading cause of cancer-related death worldwide [1]. It is characterized by uncontrolled cell growth in lung tissues, which result in metastasis, the invasion of tissues adjacent to the lesion and infiltration beyond the lungs [2]. The lung cancer treatment includes surgery, radiation therapy, chemotherapy, and/or targeted therapies. Lung cancer patients are usually treated by chemotherapy and targeted drugs [3]. Increasing the therapeutic index and reducing the side effects of chemotherapeutic drugs are major aims of cancer therapy [4]. A number of drug-loaded, receptor-targeted drug delivery systems have showed the possibilities to provide effective treatments to cancer cells which may minimize the adverse effects of drugs to the healthy cells [5–7]. For instance, targeted nanocarriers mediated delivery of therapeutic molecules has the potential to provide safer and more effective therapies for cancer treatment [8].

Several tumor targeting strategies based on nanotechnology have been developed, such as passive tumor targeting *via* the enhanced permeability and retention (EPR) effect, active tumor targeting mediated by receptor targeted ligands, and pH or temperature responded nanocarries [9]. Among these carriers, dual-ligands modified targeted system has obtained an increasing attention due to it is more effective targeting ability based on modification of nanocarriers with two kinds of ligands, thus could provide synergistic targeting effect on tumor cells [10–12].

Transferrin (Tf) is a single polypeptide glycoprotein consisting of about 679 amino acids and has been used as a cancer-targeting agent in multiple delivery systems since the over-expression of transferrin receptor (TfR) on cancer cells makes this glycoprotein an attractive and effective target for site-specific delivery of anti-cancer drug into proliferating cells [13,14]. Cancer cells require more iron for their rapid growth and proliferation which result in up-regulation of transferrin receptors in several malignant tumors including lung, brain, breast and colorectal cancers. Transferrin is selected as targeting ligand and as a platform on account of its possibility to conjugate with a variety of functional moieties and can be utilized for targeting drug delivery.

To address the challenging threat of multidrug resistance (MDR) to lung cancer chemotherapeutic agents, D-α-tocopheryl polyethylene

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glycol succinate (TPGS) was introduced into drug delivery systems [15]. TPGS, formed by etherification reaction of polyethylene glycol (PEG) and vitamin E succinate, could suppress P-glycoprotein (P-gp) meditated MDR in cancer cells by inhibiting P-gp activity. Furthermore, TPGS could enhance the solubility and/or permeability, prolong blood circulation time, improve cellular uptake, increase the bioavailability, and enhanced the cytotoxicity of various drugs [16]. So TPGS has been approved by US FDA and widely used as a solubilizer, emulsifer, and stabilizer and also intensively applied in various drug delivery systems. In this study, TPGS and Tf were used together as dual ligands for the decoration of the system.

Over 85% of all lung cancers is non-small cell lung cancer (NSCLC). Cisplatin (CDDP) remains the leading therapy for advanced NSCLC [17]. CDDP needs to be administered in high doses to obtain the required therapeutic response, thereby leading to severe adverse effects [18]. Low aqueous solubility, high protein binding that reduces drug potency, systemic toxicity, and drug resistance have been reported as major disadvantages of CDDP in cancer therapy. Therefore, strategies for safer and more effective CDDP therapy of lowering the dosage needed without loss of efficacy are desired [19].

In this study, Tf containing lipid ligand was synthesized and applied along with TPGS containing polymeric ligand for the dual ligands decoration of the CDDP loaded nano-sized system. Human NSCLC cell line (A549 cells) was used to prepare CDDP resistance A549 cells (A549/CDDP cells). A549/CDDP cells and cancer cells bearing mice were used for the evaluation of the anticancer efficiency of the system.

2. Material and methods

2.1. Chemicals and reagents

D-α-tocopheryl polyethylene glycol succinate (TPGS), Tf, CDDP, palmitic acid (PA), 2-iminothiolane (Traut's Reagent), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl), triethylamine (TEA), dimethyl sulfoxide (DMSO), stannous octoate (SO), polysorbate 80, Dulbecco Modified Eagle Medium (DMEM), fetal bovine serum (FBS), and (3-[4,5-dimehyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide (MTT) were purchased from Sigma Aldrich (St. Louis, MO, USA). Maleimide-PEG_{2000}-NH_2 was purchased from Seebio Biotech (Shanghai) Co., Ltd. (Shanghai, China).

2.2. Cells and animals

Human NSCLC cell line (A549 cells) was obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA) and cultured in DMEM with 10% FBS and 1% streptomycin/penicillin at 37 °C with 5% CO_2. A549/CDDP cells were prepared by the following procedure [20]: A549 cells were serially treated with increasing doses of cisplatin with intermittent cell recovery/expansion phases to induce resistance. In the first month, A549 cells were grown in the medium with 0.1 μg/mL CDDP and cultured with 0.5 μg/mL CDDP for the following 2 months. The surviving cells were further maintained in 1.0 μg/mL CDDP for 3 months. Then, the cells were transferred to the medium with 1.5 μg/mL CDDP for 2 months. The final surviving cells were cultured in the presence of 2 μg/mL cisplatin. Balb/c mice (4–6 weeks old, 18–22 g weight) were purchased from Laboratory Animal Center of Wenzhou Medical University (Wenzhou, China). To produce the tumor xenografts, about 10^7 A549/CDDP cells suspended in saline (200 μL) were subcutaneously injected into the right flank of mice. All animal experiments were approved by the Affiliated Wenling Hospital of Wenzhou Medical University (No. 201700010218A).

2.3. Synthesis of Tf-PEG-PA

Tf-PEG-PA ligand was synthesized as described in Fig. 1 A. Maleimide-PEG_{2000}-NH_2 and PA-COOH (1 equivalent of Maleimide-PEG_{2000}-NH_2) was dissolved in DMSO (mixture A). EDC·HCl (1.5 equivalents of PA-COOH) and TEA (1 equivalent of EDC · HCl) were dissolved in DMSO and added dropwise into the mixture A, stirred for 4 h to produce Maleimide-PEG_{2000}-NH-CO-PA. Tf-NH_2 was first modified with 1 equivalent of Traut's reagent and then added to the Maleimide-PEG_{2000}-NH-CO-PA (mixture B). The mixture B was stirred (400 rpm) for 4 h at room temperature to get Tf-PEG-PA. Tf-PEG-PA was dialyzed against Milli-Q water for 36 h to remove DMSO, centrifuged (4 °C, 2000 g, 20 min) and resuspended in PBS (pH 7.4). Nuclear magnetic resonance (^{1}H-NMR) spectroscopy of Tf-PEG-PA was carried out at 300 MHz, after dissolving in $CDCl_3$ as solvent.

2.4. Synthesis of TPGS-PLA

TPGS-PLA ligand was synthesized by ring-opening polymerization of the L-lactide monomer with TPGS in the presence of SO (Fig. 1B) [21]. TPGS and L-lactide (1 equivalent of TPGS) was dissolved in DMSO (mixture A). SO (1 equivalent of TPGS) were dissolved in DMSO and added dropwise into the mixture A, stirred for 4 h to produce TPGS-PLA. TPGS-PLA was dialyzed against Milli-Q water for 36 h to remove DMSO, centrifuged (4 °C, 2000 g, 20 min) and resuspended in PBS (pH 7.4). ^{1}H NMR spectroscopy of TPGS-PLA was carried out at 300 MHz, after dissolving in $CDCl_3$ as solvent.

2.5. Preparation of dual ligands decorated nanocarriers

Tf-PEG-PA and TPGS-PLA dual ligands decorated nanocarriers (DL-NCs, Fig. 2A) were prepared by the solvent displacement method [22]. The organic phase was prepared by mixing CDDP (20 mg), TPGS-PLA (100 mg) and acetone (5 mL). An aliquot of Tf-PEG-PA dissolved in ethanol (5 mL) was added to the organic phase and allowed to mix with magnetic stirring (400 rpm, 10 min). This solution was immediately poured on a 10 mL aqueous solution of a Pluronic F127 (0.25%, w/v) which was under magnetic stirring (1000 rpm, 10 min). The DL-NCs were centrifuged (4 °C, 1000 g, 30 min), resuspended in Milli-Q water, washed three times, and filtered through a 0.45 μm membrane. The obtained DL-NCs were stored at 2–8 °C.

2 A4 °C1,000 g-8 °C Tf-PEG-PA single ligand decorated nanocarriers (Tf-NCs) were prepared by the same method using PLA instead of TPGS-PLA. TPGS-PLA single ligand decorated nanocarriers (TPGS-NCs) were prepared by the same method using PA instead of Tf-PEG-PA. Blank dual ligands decorated nanocarriers free of drugs (Blank-NCs) were prepared by the same method without using CDDP.

2.6. Characterization of NCs

DL-NCs suspension was placed onto a carbon-coated copper grid to form a thin liquid film. The morphology of DL-NCs was observed on a transmission electron microscopy (TEM) after one minute of air-drying. Mean particle size and zeta potential of NCs were determined using a Zetasizer Nano series ZS90 (Malvern Instruments, Malvern, UK). The measurements above were taken at room temperature and analyzed in triplicate for each sample.

The drug loading capacity (DL) and entrapment efficiency (EE) of CDDP loaded NCs were measured by using the HITACHI P-4010 (Hitachi Ltd, Kyoto, Japan) inductively coupled plasma mass spectrometry [23]. Briefly, CDDP loaded NCs were centrifuged (10000 rpm, 4 °C, 20 min) separately, and the supernatants were then determined using the ICP-MS. The DL and EE were calculated as follows:

DL (%) = (The weight of total drug − the weight of free drug) / the weight of drug and NCs × 100.

EE (%) = (The weight of total drug − the weight of free drug) / the weight of total drug × 100.