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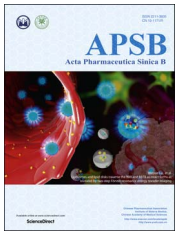

Inhalation treatment of primary lung cancer using liposomal curcumin dry powder inhalers

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KEY WORDS

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Abstract Lung cancer is the leading cause of cancer-related deaths. Traditional chemotherapy causes serious toxicity due to the wide bodily distribution of these drugs. Curcumin is a potential anticancer agent but its low water solubility, poor bioavailability and rapid metabolism significantly limits clinical applications. Here we developed a liposomal curcumin dry powder inhaler (LCD) for inhalation treatment of primary lung cancer. LCDs were obtained from curcumin liposomes after freeze-drying. The LCDs had a mass mean aerodynamic diameter of 5.81 μm and a fine particle fraction of 46.71%, suitable for pulmonary delivery. The uptake of curcumin liposomes by human lung cancer A549 cells was markedly greater and faster than that of free curcumin. The high cytotoxicity on A549 cells and the low cytotoxicity of curcumin liposomes on normal human bronchial BEAS-2B epithelial cells yielded a high selection index partly due to increased cell apoptosis. Curcumin powders, LCDs and gemcitabine were directly sprayed into the lungs of rats with lung cancer through the trachea. LCDs showed higher anticancer effects than the other two medications with regard to pathology and the expression of many cancer-related markers including VEGF, malondialdehyde, TNF-α, caspase-3 and BCL-2. LCDs are a promising medication for inhalation treatment of lung cancer with high therapeutic efficiency.

Abbreviations: BALF, lung bronchoalveolar lavage fluids; CP, curcumin powder; H&E, hematoxylin and eosin; DPI, dry powder inhaler; DMSO, dimethyl sulphoxide; FPF, fine particle fraction; HPLC, high performance liquid chromatography; LCD, liposomal curcumin dry powder inhaler; MDA, malondialdehyde; MMAD, mass mean aerodynamic diameter; NSCLC, non-small cell lung cancer; SEM, scanning electron microscopy; TEM, scanning electron microscopy; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor

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1. Introduction

Lung cancer is one of leading causes of morbidity and mortality among all malignant tumors worldwide. The major causes of lung cancer include smoke, air pollution and ionizing radiation[1]. Small-cell lung cancer (15%–20%) and non-small-cell lung cancer (NSCLC, 80%–85%) are the main types of lung cancer[2,3]. Clinical treatments of primary lung cancer mainly include surgery, radiotherapy and chemotherapy. Chemotherapeutics are commonly combined with the other two treatments. However, oral or injection administration of anticancer drugs, the most commonly applied clinical treatment, results in whole-body exposure to the toxic agents, leading to low drug concentrations in tumor tissues and unwanted drug distribution into normal tissues. In this traditional chemotherapy, serious adverse effects and low therapeutic efficiencies are usually unavoidable[4]. Therefore, the targeted or local delivery of drugs to increase drug concentration in tumor tissues and decrease drug in normal tissues is emergent for the treatment of cancer including lung cancer[5].

Pulmonary drug delivery is a noninvasive administration method by inhalation or spraying through the throat and branchillea. Inhalation therapy is efficient treatment of lung diseases such as asthma, pneumonia and chronic obstructive pulmonary disease (COPD) due to direct drug delivery into the lung[6,7]. Dry powder inhalers (DPIs) are portable solid powder delivery units without propellants. DPIs can directly target drugs into the deep sites of the lung[8]. The stability of loaded drugs is usually better in DPIs than in aerosols and nebulizers[9]. Our previous research demonstrated that oridonin-loaded large porous microparticles had a strong anti-lung cancer effect after pulmonary delivery[10]. Liposomes are phospholipid vesicles that can entrap hydrophobic drugs in their bilayer or hydrophilic drugs in the interior water phase. Liposomes are an efficient formulation for the treatment of cancer because they can enhance drug entry into cells. Generally, intravenous injection of liposomes is the major route of administration[11]. Recently, liposomes have been used for pulmonary delivery to treat lung diseases such as pneumonia[12].

Curcumin is isolated from *Curcuma longa*. It is one of the most studied and most popular natural products of the past decade. Curcumin has been demonstrated to have extensive pharmacological activities including antioxidation[13], anti-inflammation[14–16], anticancer[17], antimicrobial[18], and immunoregulation.[19] Curcumin inhibits the proliferation and migration of A549 lung cancer cells and enhances apoptosis[20,21]. However, curcumin shows low water solubility, poor bioavailability and rapid *in vivo* metabolism[22–24], which seriously limits its clinical applications. A variety of nanotechnologies have been tried to modify the physicochemical properties of curcumin and its distribution *in vivo*[25,26]. However, these technologies remain at the laboratory level without further clinical applications. Nonetheless, topical curcumin formulations may be a good strategy for local treatment of diseases because the major physicochemical disadvantages of curcumin may be avoided[27,28].

In this study, we prepared curcumin liposomes and liposomal curcumin dry powder inhalers (LCDs) for inhalation treatment of primary lung cancer *via* pulmonary delivery. The lung deposition of LCDs was evaluated. The therapeutic efficiency and mechanism of LCDs were explored on rat lung cancer models with comparison to curcumin powders and gemcitabine (a clinical first-line anticancer drug).

2. Materials and methods

2.1. Materials

Curcumin was provided by Sinopharm Reagent Co., Ltd. (Shanghai, China). Soybean lecithin (SPC > 90%) and cholesterol were purchased from Shanghai Taiwei Medicine Co., Ltd. (China) and Sinopharm Reagent Co., Ltd. (Shanghai, China), respectively. Gemcitabine was provided by Jiangsu Hansoh Pharmaceutical Co., Ltd., China. 3-Methylcholanthrene (MCA, TRC, USA), diethylnitrosamine (DEN, Tokyo Chemical Industry, Japan) and iodized oil (Guerbet, French) were used for generating rat primary lung cancer models. All other chemicals and solvents were of analytical grade or high performance liquid chromatographic (HPLC) grade. Pure water prepared with the Heal Force Pure Water System and was always used.

2.2. Animals

Male Sprague–Dawley (SD) rats (190–200 g) were provided by the Beijing Institute of Radiation Medicine (BIRM, Beijing, China). Handling and surgery were according to the Laboratory Animals' Guiding Principles. Lung bronchoalveolar lavage fluids (BALFs) were collected. The lung tissues were excised and then stained with hematoxylin and eosin (H&E).

2.3. Preparation of liposomal curcumin dry powder inhalers

Curcumin-loaded conventional liposomes were prepared by a film method. Briefly, curcumin and the lipids including SPC and cholesterol (5:1, mol/mol) were dissolved in 5 mL of tetrahydrofuran and placed in a round-bottom flask. The solvent was removed under vacuum to obtain a thin film that was hydrated with a phosphate buffered solution (PBS, pH 7.0) at 37 °C for 1 h at 200 rpm (Thermostatic Air Vibrator, THZ-D, Taicang Experimental Instrument Factory, Suzhou, China). Mannitol was added to the liposomes which were further freeze-dried in a lyophilizer (LGJ-30F, Beijing Songyuan Huaxing Technology Develop Co., Ltd., China) for 36 h to obtain liposomal curcumin dry powder inhalers (LCDs).

2.4. Measurement of encapsulation and loading efficiencies in liposomes

Free curcumin was separated from curcumin liposomes by centrifugation at 10,000 rpm (High Speed Centrifuge, TGL-16B, Shanghai Anting Scientific Instrument Factory, Shanghai, China) for 10 min. The supernatant was filtered through a 0.45-μm filter. Free curcumin in the filtrate was analyzed with an HPLC system (Angilent 1260, US): a Dikma Diamonsil C18 column (250 mm × 4.6 mm, 5 μm), a detection wavelength of 425 nm and a mobile phase of acetonitrile/water/acetic acid (60:39:1, *v/v*) at a flow rate of 1 mL/min. Total curcumin was also determined after the LCDs were completely dissolved in ethanol. Encapsulated curcumin was calculated from total curcumin minus free curcumin. The curcumin encapsulation efficiency (EE) and loading efficiency (LE) were calculated with Eqs. (1) and (2).

$$\text{EE}\ (\%) = \frac{\text{Encapsulated curcumin}}{\text{Total curcumin}} \times 100\% \quad (1)$$

$$\text{LE}\ (\%) = \frac{\text{Total curcumin}}{\text{LCDs}} \times 100\% \quad (2)$$

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