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Full Length Article

Albumin nanoparticles with synergistic antitumor efficacy against metastatic lung cancers

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ABSTRACT

Albumin nanoparticles are well-known as effective drug carriers used to deliver hydrophobic chemotherapeutic agents. Albumin nanoparticles encapsulating curcumin and doxorubicin were fabricated using slightly modified nanoparticle albumin-bound (nab™) technology, and the synergistic effects of these two drugs were examined. Albumin nanoparticles encapsulating curcumin, doxorubicin, and both curcumin and doxorubicin were prepared using a high pressure homogenizer. The sizes of albumin nanoparticles were ~130 nm, which was considered to be suitable for the EPR (enhanced permeability and retention) effect. Albumin nanoparticles gradually released drugs over a period of 24 h without burst effect. To confirm the synergistic effect of two drugs, *in vitro* cytotoxicity assay was performed using B16F10 melanoma cells. The cytotoxic effect on B16F10 melanoma cells was highest when co-treated with both curcumin and doxorubicin compared to single treatment of either curcumin and doxorubicin. The combined index calculated by medium-effect equation was 0.6069, indicating a synergistic effect. Results of confocal laser scanning microscopy and fluorescence-activated cell sorting corresponded to results from an *in vitro* cytotoxicity assay, indicating synergistic cytotoxicity induced by both drugs. A C57BL/6 mouse model induced by B16F10 lung metastasis was used to study *in vivo* therapeutic effects. When curcumin and doxorubicin were simultaneously treated, the metastatic melanoma mass in the lungs macroscopically decreased compared to curcumin or doxorubicin alone. Albumin nanoparticles encapsulating two anticancer drugs were shown to have an effective therapeutic result and would be an excellent way to treat resistant lung cancers.

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1. Introduction

Lung cancer, one of the most incurable malignancies, is classified as small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) according to histological features [1]. The 5-year survival for lung cancer patients is extremely low (~18%) because it is rarely detected in the early stage [2,3]. Several chemotherapeutics for lung cancers, such as etoposide, cisplatin, and irinotecan, have been clinically used as first line therapies. Molecular target treatments, such as epidermal growth factor receptor (EGFR) inhibitors [4], anaplastic lymphoma kinase (ALK) inhibitors [5], and VEGF inhibitors [6], can be used appropriately for various gene mutations. However, lung cancer cells rapidly develop drug resistance, which restricts effective treatment by chemotherapeutics and requires higher doses [7]. To date, chemotherapeutic therapy for lung cancer has not shown satisfactory results.

Nanoparticles (NPs) have gained attention as an effective way of diagnosing and treating cancers by their ability to target tumor and encapsulate drugs [8]. The tumor targetability of NPs is due to (i) passive targeting induced by the enhanced permeability and retention (EPR) effect, and (ii) active targeting induced by specific ligand modification, which results in accumulation of drug in cancer tissues [8–10]. NPs in the range of 100–200 nm extravasate through the endothelial fenestrations of tumor tissues [11,12]. After reaching target sites, the biodegradable carrier is degraded and drug is released to exert therapeutic effects. The most well-established

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examples of biodegradable nanoparticles are liposomes, polymeric micelles made of poly(lactic-co-glycolic acid) (PLGA), and nanoparticles consisting of albumin molecules [13–15].

Albumin has been widely used as a nanoparticle carrier due to its many pharmaceutical merits, including biodegradability, non-toxicity/non-immunogenicity, and chemical stability [16]. In particular, albumin effectively targets tumor tissue through albumin-receptor (gp60, 60 kDa glycoprotein)-mediated active transcytosis [10,17]. Gp60 is highly expressed around tumor tissues and has an affinity with albumin [18]. There are several ways to fabricate NPs using albumin, including desolvation [19], self-assembly [20] and nanoparticle albumin bound (nab™) technology [21]. Abrexane®, an albumin NP formulation encapsulating paclitaxel on the market, was prepared by nab™ technology. The nab™ technology is simple and safe because it does not employ a cross-linking agent, glutaraldehyde, which can be toxic to humans [22]. It also does not chemically modify or physically denature the albumin structure, which may result in altered biocompatibility of nanoparticles.

In previous studies, we fabricated doxorubicin (Dox)-loaded PLGA microparticles [23] and self-assembled Dox HSA-NPs for inhaled administration to treat lung cancer [2]. Most chemotherapeutic anticancer agents can be noxious when they directly treat the lungs [24]. The inhalation devices, lung humidity, and degree of airway obstruction in tumor tissues influence the bioavailability of anticancer drugs, which makes it difficult to determine dosage [25]. Here, we developed injectable curcumin and doxorubicin co-loaded albumin nanoparticles for the treatment of lung cancer to take full advantage of both tumor tissue microenvironments and synergistic tumor suppress effect by combined drug therapy. Curcumin (CCM) and Dox are suitable for encapsulation in albumin NPs using a high pressure homogenizer because both drugs are highly hydrophobic and bind well to albumin molecules. Furthermore, an enhanced therapeutic effect is expected due to the tumor targetability of albumin. This study examined the physicochemical properties of albumin NPs, *in vitro* cytotoxicity for B16F10 cells, and *in vivo* anti-tumor effects in a metastatic lung cancer-induced mouse model.

2. Materials and methods

2.1. Materials

Doxorubicin (Dox) was obtained from the Research Laboratories of Korea United Pharm. Inc., (Seoul, Korea). Curcumin and Bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fluorescein isothiocyanate (FITC) Annexin V staining kits and *in situ* cell death detection kits were purchased from BD Pharmingen (Heidelberg, Germany) and Roche Diagnostics (Mannheim, Germany), respectively. Cy5.5 NHS ester dye was purchased from GE Healthcare (Piscataway, NJ, USA). All other reagents were obtained from Sigma-Aldrich unless otherwise specified.

2.2. Animals

C57BL/6 mice (male, seven-week-old) were purchased from Hanlim Experimental Animal Laboratory (Seoul, South Korea). Animals were cared for in accordance with guidelines issued by the National Institutes of Health (NIH) regarding the care and use of laboratory animals (NIH publication 80-23, revised in 1996). Animals were housed in groups of 2–3 under a 12 h light/dark cycle (lights on 6 a.m.), allowed food and water *ad libitum*, and acclimatized for 2 weeks. This study was approved by the Ethical Committee on Animal Experimentation at Sungkyunkwan University.

2.3. Preparation of CCM/Dox BSA-NPs

CCM or Dox BSA-NPs and CCM/Dox BSA-NPs were fabricated by slight modification of nanoparticle albumin-bound (nab™) technology [26]. Briefly, 2 mg CCM, 2 mg Dox, or 1 mg CCM and 1 mg Dox was dissolved in 0.1 ml of a 9:1 solution of chloroform and ethanol, respectively. Fifty milligrams of BSA was dissolved in 5 ml deionized water (DW). The two solutions were mixed and passed through a high-pressure homogenizer (EmulsiFlex-B15 device, Avestin, Ottawa, Ontario, Canada) for nine cycles at 20,000 psi. The resulting dispersion was rotary evaporated for 15 min under reduced pressure at 40 °C to remove chloroform and ethanol. The generated nanoparticles were centrifuged at 6000 rpm, and supernatant was lyophilized and stored at −20 °C until required.

2.4. Characterization of CCM/Dox BSA-NPs

The mean particle sizes and zeta potentials of CCM or Dox BSA-NPs and CCM/Dox BSA-NPs were measured using a Zetasizer Nano-ZS90 (Malvern Instruments, Malvern, UK) and a 633 nm He-Ne laser beam with a 90° scattering angle. Experiments were performed at NP concentration of 1 mg/ml on a BSA weight basis in DW to measure zeta potentials and particle size. The surface morphologies of CCM/Dox BSA-NPs were observed using transmission electron microscopy (TEM) with a model H-7600 microscope (Hitachi, Tokyo, Japan).

The stability of NPs was evaluated based on the particle size maintenance. CCM/Dox BSA-NPs (1 mg) were dispersed in 1 ml DW, and the suspension was kept at room temperature for 24 h. The particle sizes were measured at 0, 1, 3, 6, 9, 12, 15, 18, and 24 h by the method described above.

2.5. Encapsulation efficiency and release of CCM and Dox

To determine the encapsulation efficiency of CCM, 1 mg of lyophilized NPs was mixed with 0.2 ml of a 1:1 solution of acetonitrile (ACN) and DW and thoroughly shaken for 5 min. The solution was further centrifuged at 13,500 rpm to remove BSA. The supernatant was subjected to reverse phase high-performance liquid chromatography (RP-HPLC) using a LiChrospher 100 RP-18 column (250 × 4.0 mm, 5 μm; Merck, Darmstadt, Germany) at 30 °C. Isocratic elution was carried out at a flow rate of 1 ml/min using a 30:70 mixed solution of mobile phase A (0.1% trifluoroacetic acid (TFA) in DW) and mobile phase B (0.1% TFA in ACN). Eluates were monitored at 420 nm. To determine the encapsulation efficiency of Dox, 1 mg of lyophilized NPs was dissolved in 0.5 ml of a 9:1 solution of dimethyl sulfoxide (DMSO) and DW and thoroughly shaken for 5 min. The solution was centrifuged at 13,500 rpm. Dox in the supernatant was directly quantified by spectrophotometry at 515 nm. Data are presented as mean ± SD (n = 3). Drug encapsulation efficiency (%) was calculated as [amount of drug entrapped/amount of drug loaded] × 100.

To investigate CCM and Dox release profiles, 20 mg of CCM/Dox BSA-NPs was suspended in 2 ml DW and dialyzed using a semipermeable membrane with a molecular weight cut-off of 10 kDa (Spectrum Labs, Rancho Dominguez, CA, USA) against 200 ml of 10 mM PBS (pH 7.4) at 37 °C. At 0, 1, 3, 6, 9, 12, 15, 18, and 24 h after incubation, 0.2 ml of sample was withdrawn from the dialysis bag. The concentrations of CCM and Dox in nanoparticles were determined as described above. Cumulative release was expressed as percentage *vs.* initial loading amount at each time point. All samples were prepared and analyzed in duplicate.

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