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Targeting sialic acid residues on lung cancer cells by inhalable boronic acid-decorated albumin nanocomposites for combined chemo/herbal therapy

Mayada M. Elgohary[a,b], Maged W. Helmy[a,c], Elsayeda-Zeinab A. Abdelfattah[d], Doaa M. Ragab[b], Sana M. Mortada[a,b], Jia-You Fang[e,f,g,**], Ahmed O. Elzoghby[a,b,h,i,*]

[a] Cancer Nanotechnology Research Laboratory (CNRL), Faculty of Pharmacy, Alexandria University, Alexandria 21521, Egypt
[b] Department of Industrial Pharmacy, Faculty of Pharmacy, Alexandria University, Alexandria 21521, Egypt
[c] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Damanhour University, Damanhour, Egypt
[d] Medical Research Institute (MRI), Alexandria University, Alexandria 21527, Egypt
[e] Pharmaceutics Laboratory, Graduate Institute of Natural Products, Chang Gung University, Taoyuan 333, Taiwan
[f] Research Center for Industry of Human Ecology and Research Center for Chinese Herbal Medicine, Chang Gung University of Science and Technology, Kweishan, Taoyuan 333, Taiwan
[g] Department of Anesthesiology, Chang Gung Memorial Hospital, Kweishan, Taoyuan 333, Taiwan
[h] Division of Engineering in Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA
[i] Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA 02139, USA

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ABSTRACT

Etoposide (ETP), as a potential treatment for lung cancer, has limited application due to its poor solubility, and systemic side effects. In the current study, we propose inhalable boronate-targeted HSA nanocomposites for combined delivery of ETP and the herbal drug, berberine (BER) for localized therapy of lung cancer. First, ETP was pre-formulated as phospholipid complex (EPC) to enhance drug solubility and facilitate its encapsulation within the hydrophilic albumin nanoparticles (NPs). Second, EPC and BER were then co-loaded with high efficiency into HSA NPs as a synergistic therapy for lung cancer. The NPs displayed suitable size around 200 nm and sequential drug release pattern. Moreover, conjugation of aminophenylboronic acid (APBA) to HSA NPs resulted in enhanced cytotoxicity and internalization into A549 lung cancer cells, compared to non-targeted NPs or free drugs via binding to sialic acid residues over-expressed by cancer cells. Using mannitol as a spray-drying carrier, the developed inhalable nanocomposites demonstrated deep pulmonary deposition, confirmed by small MMAD (2.112 μm) and high FPF (77.86%). In vivo investigations in lung cancer animal models revealed the superior anti-tumor efficacy of the inhalable nanocomposites. Overall, the inhalable APBA-HSA nanocomposites offered an alternative strategy for systemic delivery of ETP and BER in lung cancer therapy.

1. Introduction

Lung cancer is considered to be the most serious type of cancer, as it presents the highest percentage of cancer-related death worldwide. Conventional chemotherapy and radiation are widely used as a protocol of treatment. However, those strategies suffer from non-specificity to cancer cells and serious side effects which limit their application. Therefore, considerable research is being directed towards the development of a novel regimen to overcome these limitations [1].

As a promising alternative to systemic therapy, inhalational chemotherapeutics provide a localized therapy for lung tumors with the benefit of avoiding the side effects of intravenous administration and delivery of high drugs doses [2]. Nanoparticles (NPs) encapsulating anti-cancer drugs possess the advantages of high cellular penetration, avoiding uptake by macrophages, increased drug loading and sustained release behavior [3–5]. However, the small particle size of NPs leads to their quick exhalation thus limiting their application for pulmonary administration [6]. On another avenue, development of inhalable microparticles encapsulating cytotoxic compounds ensures efficient lung deposition [7]. This signifies the rationale of developing nanoparticles-in-microparticles (nanocomposites), as they possess the advantages of both microparticles, to enable effective aerosolization and deep lung

* Correspondence to: Ahmed O. Elzoghby, Division of Engineering in Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA.
** Correspondence to: Jia-You Fang, Graduate Institute of Natural Products, Chang Gung University, Taoyuan 333, Taiwan.
E-mail addresses: fajy@mail.cgu.edu.tw (J.-Y. Fang), aelzoghby@bwh.harvard.edu, ahmed_elzoghby@alexu.edu.eg (A.O. Elzoghby).

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deposition, and nanoparticles in targeting the tumor cells [8].

Etoposide (ETP) is a potent inhibitor of topoisomerase II used for treatment for non-small cell lung cancer (NSCLC) [9]. However, the dose-dependent side effects of ETP treatment include reduced count of white blood cells and platelets, and secondary leukemia [10]. Additionally, resistance to ETP could occur due to up-regulation or mutation of topoisomerase II molecule and the rapid repair of DNA breaks [11]. Cellular extrusion of ETP mediated by transport systems could lead to cross-resistance against other cytostatic agents, as observed for MDR-1–mediated multidrug resistance [12]. Clinical studies revealed that combinatorial cancer treatment significantly reduces individual drug-related toxic effect and evade multi-drug resistance (MDR) via different pathways [13]. In the present study, ETP was combined with the natural isoquinoline alkaloid berberine (BER) as a unique drug combination for effective treatment of lung cancer. BER was previously reported to modulate the MDR through inhibition of P-gp and/or MRP efflux activity in A549 lung cancer cells [14]. Moreover, BER was found to inhibit DNA topoisomerases and protein kinase C, sensitize cancer cells to drugs by increasing their ROS levels and reversal of MDR, thus elevates the cytotoxic effect of combined anticancer drugs [14, 15].

Among promising nanocarriers, human serum albumin (HSA) has attracted great attention in the preparation of NPs due to its biocompatibility, biodegradability, non-immunogenicity, and stability. Moreover, HSA can be chemically modified through its amine and carboxylate moieties for active targeting to cancer cells, beside its targeting abilities through transcytosis pathway by binding to gp60 and SPARC proteins [16, 17]. Unfortunately, hydrophobic anti-cancer drugs are not easily incorporated in hydrophilic albumin NPs except using high energy and shearing technology such as high pressure homogenization (HPH) in case of nab-paclitaxel (Abraxane®) approved by FDA for treatment of advanced and metastatic NSCLC [18]. Therefore, novel pre-formulation approaches may be required to enhance the solubility of hydrophobic drugs and hence facilitate their loading into albumin NPs.

Among those solubility enhancing approaches, complexes between drugs and native phospholipids like soybean phosphatidylcholines, are formed based on hydrogen bonding between the polar parts in both phospholipids and drug [19–21]. The water-insoluble cytotoxic drug, teniposide was pre-formulated as phospholipid complex prior to incorporation into albumin NPs [22]. In addition to the poor water solubility, another challenge lies in non-selective biodistribution of chemotherapeutics leading to systemic toxicity. Therefore, active-targeted nanocarriers were designed to enhance drug accumulation into tumor cells [23, 24]. The presence of over-expressed sialic acid on cancer cell surface is implicated in metastasis, progression, and resistance to chemotherapy [25]. Those sialic acid residues provide a potential binding site for boronic acid group-modified nanocarriers leading to enhanced intra-tumoral transport of nanocarriers through sialic acid recognition [26].

In comparison to the previously reported ETP-albumin NPs prepared by high pressure homogenization (HPH), the desolvation approach does not produce an increase in temperature that may affect the stability of incorporated drugs or induce changes in the protein structure with disruption of some disulfide bonds, thus varying the availability of hydrophobic groups [27, 28]. Moreover, NPs prepared by HPH suffer from low stability and high probability of dis-assembly in the systemic circulation [29]. Concerning injectable APBA-targeted NPs, normal cells may express traces of sialic acid residues for cellular/extracellular interaction which makes the NPs not entirely selective to cancer cells [30]. In addition, higher i.v. doses should be administered compared to those delivered locally via inhalation, thus raising the systemic toxicity and increasing the drug costs. Although nebulized NPs have been widely used for pulmonary administration, they suffer from suspension instability and strong particle-particle interactions, leading to non-adequate nebulization and exhalation of low-inertia NPs [31]. Inhalable NPs powder could solve the stability issue but still suffers from non-suitability for deep lung delivery [6]. Our inhalable nanocomposites offered optimum size for deep lung deposition compared to previously reported inhalable ETP delivery systems [2, 9]. In addition, the NPs would be recovered from microparticles only after reaching deep lung tissues and selectively target cancerous cells offering maximal antitumor efficacy.

So far, the current study is the first to develop inhalable dry powder composed of spray-dried APBA targeted-albumin nanocomposites for combined delivery of ETP and BER to lung cancer. First, to facilitate its incorporation into the hydrophilic HSA matrix without solubilizer, the hydrophobic chemotherapeutic drug, ETP was pre-formulated as phospholipid complex (EPC) by direct lyophilization. Second, for enhancing ETP anti-tumor efficacy, and reducing its therapeutic dose thus minimizing its toxicity and prevent the development of resistance, the water-soluble herbal drug, BER was directly co-encapsulated with EPC into HSA NPs via desolvation method using different crosslinkers. Third, maximal targeting efficacy was hypothesized by sialic acid targeting via anchoring phenylboronic acid to the surface of HSA NPs. Finally, to reduce systemic toxicity, inhalable dry powder nanocomposites were developed via co-spray drying of dual-drug-loaded HSA NPs with inert carriers to prepare micro-sized powders suitable for deep lung deposition. The developed delivery system was thoroughly investigated in vitro and in vivo to prove the anti-tumor superiority of the combined drug nano-delivery compared with the free drug combination.

2. Materials and methods

2.1. Materials

Human serum albumin (HSA), Berberine chloride hydrate (BER), 3-Aminophenyl boronic acid (APBA), tertiary Butyl Alcohol (TBA), N-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDC), *N*-Hydroxysuccinamide (NHS), sodium hydroxide (NaOH), glutaraldehyde solution (GA, 25%), zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$), Sodium Chloride (NaCl), mannitol, maltodextrin, trehalose, L-leucine, 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT), Fluorescein isothiocyanate (FITC), urethane (ethyl carbamate), Haematoxylin solution, Eosin solution (H&E) and Canada balsam were purchased from Sigma-Aldrich (St. Louis, USA). Etoposide (ETP) and genipin (GNP) were purchased from Xian Natural Field Bio-Technique Co., Ltd. (Xian, China). Fat-free soybean phospholipids with 70% phosphatidylcholine (Lipoid S75) were kindly provided by Lipoid GmbH (Ludwigshafen, Germany). Poly (ethylene glycol) 400 (PEG-400) was supplied by Pharonia Pharmaceuticals (Alexandria, Egypt). Absolute ethanol and methanol were purchased from ADWIC, El-Nasr Pharmaceutical Chemicals Co. (Cairo, Egypt). Methanol and acetonitrile HPLC grade were purchased from JT Baker (Phillipsburg, NJ, USA).

2.2. Preparation and characterization of EPC

ETP-PC complex (EPC) was prepared by direct freeze-drying method [32]. Different molar ratios (1:1, 1:2 and 1:4) of ETP and phospholipid (Lipoid S75) were dissolved in tertiary butyl alcohol (TBA, 10 ml). The mixture was maintained at room temperature on a magnetic stirrer until complex formation. The complex was frozen at −80 °C for 4 h then lyophilized for 24 h using mannitol as a cryoprotectant (LyoQuest-55 freeze-dryer, Telstar, Spain). The prepared EPC was evaluated for their physicochemical, and solid state characteristics [33] (Detailed in the Supporting Information). To confirm the enhanced solubility of EPC, the dissolution profiles of EPC prepared with different molar ratios of phospholipid were compared to free ETP. The optimized EPC powder (P1 and P4), equivalent to 5 mg ETP, was added to 50 ml PBS (pH 7.4) and shaken at 100 rpm and 37 °C (Shaking water bath, Maxturdy 30, daihan scientific Co. Ltd., Shanghai, China). Fixed volume samples were