Biochemical and Biophysical Research Communications xxx (2018) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Berberine and zinc oxide-based nanoparticles for the chemo-photothermal therapy of lung adenocarcinoma

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ARTICLE INFO

Article history:
Received 4 May 2018
Accepted 10 May 2018
Available online xxx

Keywords:
Berberine
Chemo-photothermal therapy
Lung cancer
Nanoparticles
Zinc oxide

ABSTRACT

Organic/inorganic hydrid nanoparticles (NPs) composed of berberine (BER) and zinc oxide (ZnO) were developed for the therapy of lung cancers. Without the use of pharmaceutical excipients, NPs were fabricated with only dual anticancer agents (BER and ZnO) by facile blending method. The mean weight ratio between BER and ZnO in BER-ZnO NPs was 39:61 in this study. BER-ZnO NPs dispersed in water exhibited 200–300 nm hydrodynamic size under 5 mg/mL concentration. The exposure of both BER and ZnO in the outer layers of BER-ZnO NPs was identified by X-ray photoelectron spectroscopy analysis. The amorphization of BER and the maintenance of ZnO structure were observed in the results of X-ray powder diffractometer analysis. Improved antiproliferation efficacy, based on the chemo-photothermal therapeutic efficacy, of BER-ZnO NPs in A549 (human lung adenocarcinoma) cells was presented. According to the blood tests in rats after intravenous administration, BER-ZnO NPs did not induce severe hepatotoxicity, renal toxicity, and hemotoxicity. Developed BER-ZnO NPs can be used efficiently and safely for the chemo-photothermal therapy of lung cancers.

1. Introduction

Precise drug delivery to tumor region is very crucial for maximizing therapeutic efficacies and reducing unwanted effects of anticancer agents. A lot of approaches have been investigated for selective delivery of therapeutic cargos to tumor region [1,2]. The physicochemical properties (*i.e.,* particle size) of nanocarriers can provide passive tumor targeting effects, based on the enhanced permeability and retention (EPR) effect [3]. However, due to the absence of tumor-selectivity in passive tumor targeting strategy, active tumor targeting strategies (*i.e.,* introduction of tumor targeting ligands to nanosystems) have been introduced [4]. Various organic and inorganic materials have been used for the preparation of nanocarriers [5–8]. In particular, safety as well as biofunctionality was considered as an important property for the development of intravenous formulations. Several biocompatible and biodegradable organic materials (*i.e.,* hyaluronic acid and poly(lactic-co-glycolic acid)) have been approved by U.S. Food and Drug Administration (FDA) for injection formulations [8,9]. Nanocarriers based on those natural and synthetic polymers and their derivatives have shown substantial drug delivery efficiency to the tumor region [10–12].

On the contrary to organic substances, inorganic materials (*i.e.,* Ag, Au, Cu, Fe, Gd, I, Si, Ti, and Zn) have unique properties for cancer diagnosis and therapy [13]. In particular, the medical applications of zinc, such as common cold, diarrhea, gastroenteritis, and sunburn, have been reported [14,15]. As a source of zinc, zinc oxide (ZnO) has shown distinguished optical, electrical, and mechanical properties [16,17]. Its antibacterial, anticancer, and protection from sunburn effects were demonstrated [17–20]. Although ZnO is considered as "GRAS" (generally recognized as safe) group by the U.S. FDA, its size reduction to nanoparticles (NPs) requires the identification of its safety [20]. Various materials have been mixed or coated with ZnO to render biofunctionalities in the area of drug delivery [21,22].

In our previous study [14], doxorubicin (DOX)-wrapped ZnO NPs were fabricated and their anticancer effects against human colon adenocarcinoma cells have been verified. Anticancer activities of ZnO NPs, based on several mechanisms (*i.e.,* apoptosis and autophagy), were already demonstrated in cancer cells [23,24]. Due to the physicochemical properties (*i.e.,* hydrophilicity) of DOX HCl, only thin layer was coated onto the surface of ZnO NPs in that study [14]. Although the anticancer efficacies were improved compared to DOX or ZnO NPs-treated group, DOX-ZnO NPs have limitations in view of elevating the administration dose of DOX. In this

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https://doi.org/10.1016/j.bbrc.2018.05.063
0006-291X/

investigation, berberine (BER) and ZnO-based NPs were prepared considering the solubility of BER. Without the aid of pharmaceutical excipients, single nanocarrier was fabricated only with dual active ingredients (BER and ZnO). Compared to our previous study [14], the content of organic drug (BER) was significantly improved in BER-ZnO NPs. Single hybrid nanocarrier composed of BER and ZnO may deliver those ingredients simultaneously to tumor region, thereby improving anticancer efficacies. Interestingly, ZnO was also known as one of photothermal therapy (PTT) agents for cancer treatment [25]. Generation of heat, followed by near-infrared (NIR) irradiation, may lead to the thermal ablation of cancer cells [26]. PTT may provide improved temporal-spatial selectivity and minimal invasiveness compared to the other conventional therapeutic methods [26]. Considering the chemotherapeutic efficacies of BER [27], developed hybrid BER-ZnO NPs can be used as a chemo-photothermal agent. Herein, the physicochemical properties of BER-ZnO NPs and their anticancer activities against human lung adenocarcinoma (A549) cells were investigated.

2. Materials and methods

2.1. Materials

BER (berberine chloride hydrate; >98% purity) was purchased from Sigma–Aldrich (Saint Louis, MO, USA). ZnO (nanopowder, 99.95% purity) was acquired from US Research Nanomaterials, Inc. (Houston, TX, USA). RPMI 1640 (developed by Roswell Park Memorial Institute), penicillin, streptomycin, fetal bovine serum (FBS), and phosphate buffered saline (PBS) were acquired from Gibco Life Technologies, Inc. (Grand Island, NY, USA). All other chemicals were of analytical grade.

2.2. Preparation and particle characterization of BER-ZnO NPs

BER-ZnO NPs were fabricated by a facile blending method considering the solubility of BER in water. ZnO was dispersed in distilled water (DW) at 2 mg/mL by using a probe-type sonicator (VC-750; Sonics & Materials, Inc., Newtown, CT, USA). BER was dispersed in DW at 5 mg/mL. Then, they were blended with equal volume ratio and incubated for 30 min at room temperature. After centrifuging at 8000 rpm for 15 min, the supernatant was removed and the pellet of NPs was resuspended in DW. It was lyophilized for further uses.

The hydrodynamic size, polydispersity index, and zeta potential values of BER-ZnO NPs were detected using dynamic light scattering (DLS) and laser Doppler methods (ELS-Z1000; Otsuka Electronics, Tokyo, Japan). The morphology of BER-ZnO NPs was observed by transmission electron microscopy (TEM) (JEM 1010; JEOL, Tokyo, Japan). Freeze-dried BER-ZnO NPs were homogeneously dispersed at 2 mg/mL in DW by probe sonicator (VC-750; Sonics & Materials, Inc.). The dispersion of BER-ZnO NPs was loaded onto the copper grid coated with carbon film and dried at room temperature prior to TEM analysis.

The contents of BER and ZnO in BER-ZnO NPs were quantitatively determined by UV/Vis spectrophotometer and inductively coupled plasma-optical emission spectroscopy (ICP-OES), respectively. The absorbance of BER was detected at 345 nm by using an EMax Precision Microplate Reader (Molecular Devices, Sunnyvale, CA, USA). The content of Zn in BER-ZnO NPs was measured by ICP-OES (Optima 7300 DV, PerkinElmer, Inc., Waltham, MA, USA) in the Central Laboratory of Kangwon National University. BER-ZnO was extracted with HNO_3 prior to ICP-OES analysis.

The concentration-dependent hydrodynamic size of BER-ZnO

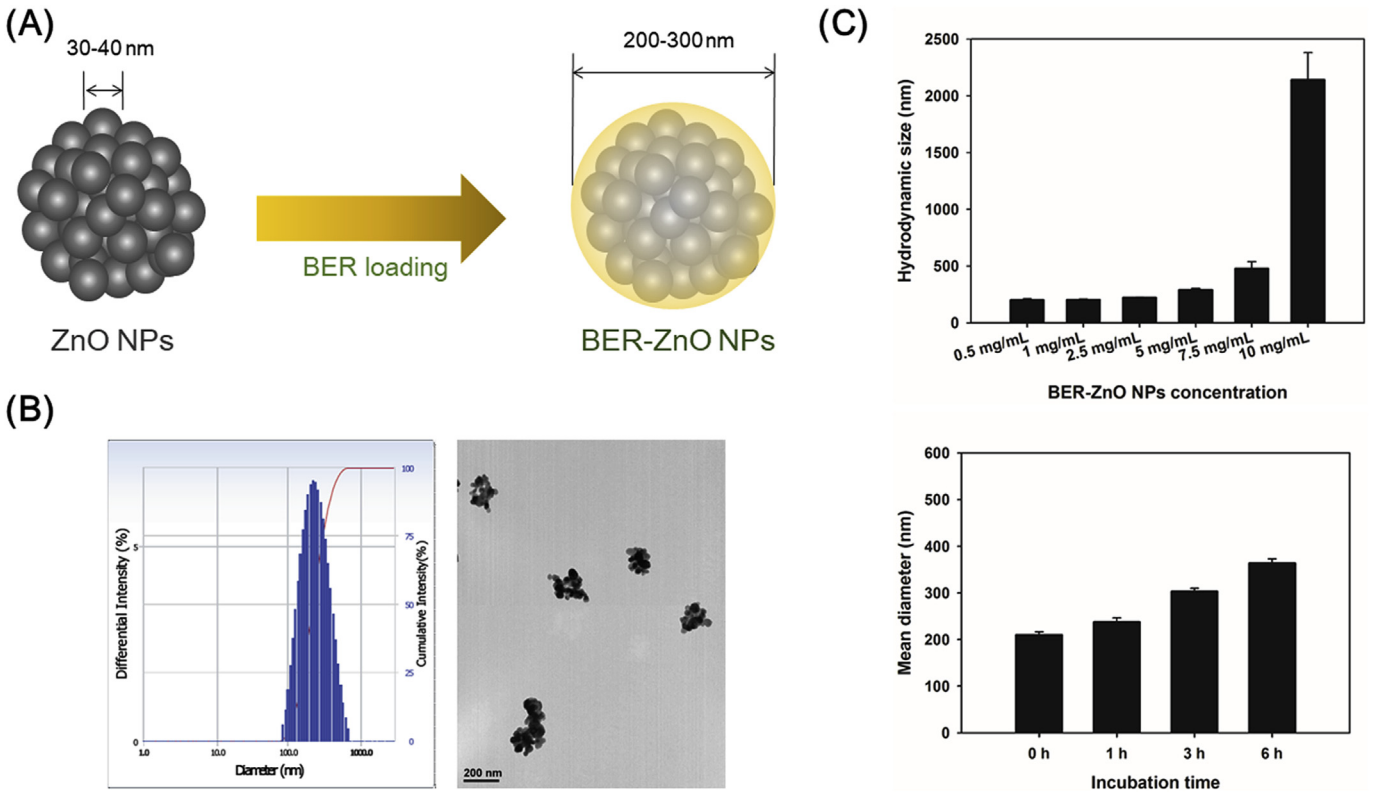

Fig. 1. Preparation and particle characterization of BER-ZnO NPs. (A) Fabrication process of BER-ZnO NPs. (B) Particle characterization of BER-ZnO NPs. Size distribution profile (left), presented as the differential intensity according to the mean diameter, of dispersion of BER-ZnO NPs (2 mg/mL in DW) is shown. TEM image (right) of dispersion of BER-ZnO NPs (2 mg/mL in DW) is presented. The length of scale bar is 200 nm. (C) Particle stability of BER-ZnO NPs. Hydrodynamic size values of dispersion of BER-ZnO NPs according to the concentration and incubation time are presented. Each point indicates the mean ± SD ($n = 3$).

Please cite this article in press as: S. Kim, et al., Berberine and zinc oxide-based nanoparticles for the chemo-photothermal therapy of lung adenocarcinoma, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.05.063