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Research paper

Bufalin loaded biotinylated chitosan nanoparticles: An efficient drug delivery system for targeted chemotherapy against breast carcinoma

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ABSTRACT

Bufalin is a traditional oriental medicine which is known to induce apoptosis in many tumor cells, and it is thus considered as a new anticancer therapeutic. By now, most of the studies of bufalin are in vitro, however in vivo evaluations of its therapeutic efficacy are less and are in great demand for its development toward anticancer drug. One of the problems probably hampering the development of bufalin is the lack of tumor selectivity, which may reduce the therapeutic effect as well as showing side effects. To overcome this drawback, in this study, we designed a tumor-targeted drug delivery system of bufalin based on enhanced permeability and retention (EPR) effect, by using biotinylated chitosan, resulting in bufalin encapsulating nanoparticles (Bu-BCS-NPs) with mean hydrodynamic size of 171.6 nm, as evidenced by dynamic light scattering and transmission electron microscope. Bu-BCS-NPs showed a relative slow and almost linear release of bufalin, and about 36.8% of bufalin was released in 24 h when dissolved in sodium phosphate buffer. Compared to native bufalin, Bu-BCS-NPs exhibited a stronger cytotoxicity against breast cancer MCF-7 cells (IC_{50} of 0.582 μg/ml vs 1.896 μg/ml of native bufalin). Similar results were also obtained in intracellular reactive oxygen species production, apoptosis induction, and decrease in mitochondria membrane potential. These results may contribute to the rapid intracellular uptake of nanoparticles, partly benefiting from the highly expressed biotin receptors in tumor cells. In vivo studies using MCF-7 tumor models in nude mice confirmed the remarkable therapeutic effect of Bu-BCS-NPs. These findings suggest the potential of Bu-BCS-NPs as an anticancer drug with tumor targeting property.

1. Introduction

Targeted therapy is recently among the most attractive strategies for cancer, the major cause of death in most advanced countries in the world. Conventional cancer chemotherapy, which usually utilizes small molecular drugs, is far from successful, mostly due to the lack of tumor selectivity, or the so-called dose-limiting toxicity, resulting severe adverse effects that limits usage. To overcome these drawbacks, targeted anticancer therapy is aiming at a more tumor-selective anticancer effect with less system side effects.

One direction of targeted anticancer therapy is the so-called molecular target therapy, which focuses on specific kinases or receptors that are over-expressed in cancer cells or tissues. However, considering the intrinsic genetic diversity of human solid tumors [1,2], namely high frequency of occurrence of mutant genes, redundant genetic and molecular or metabolic pathways, single gene or receptor concept of molecular target therapy may

Abbreviations: Bu, bufalin; CS, chitosan; BCS, biotinylated chitosan; NPs, nanoparticles; EPR effect, enhanced permeability and retention effect; ROS, reactive oxygen species.

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be invalidated. In fact, recent clinical results of many molecular target drugs have not been successful [3,4].

Another approach that is attracting more and more attentions, is a more general tumor targeting according to the unique anatomical and pathophysiological nature of solid tumor tissues. Namely, most solid tumors have blood vessels with defective architecture and usually produce extensive amounts of various vascular permeability factors, so that macromolecules with molecular weight larger than 40–50 kDa will extravasate from tumor blood vessels and accumulate selectively in tumor tissues, whereas they could not cross normal blood vessels which will result in less side effects. This unique phenomenon was first reported my Matsumura and Maeda in 1986, and was coined as enhanced permeability and retention (EPR) effect [5]. The EPR effect is thus considered to be a landmark principle in targeted cancer chemotherapy and is becoming an increasingly promising paradigm and "gold standard" for anticancer drug development [6–10]. To date, some macromolecular drugs are used in clinic, for example, Doxil that is a liposome formulation of doxorubicin used for treatment of Kaposi sarcoma and other cancers; and more polymeric or micellar drugs are in clinical stage development [9–11].

Along this line, recently we focused on biotin modified chitosan nanoparticles (BCS-NPs) as a drug carrier for tumor targeting. Chitosan is a linear polysaccharide with a number of commercial and possible biomedical uses. Because of its biocompatibility and biodegradability, chitosan has been widely used as polymer materials for the modification and delivery of many anticancer drugs, i.e., chitosan nanoparticles (CS-NPs) [12–15]. Biotin is a water-soluble vitamin, having essential roles involving cell growth, signal transduction and many other cellular functions, it is internalized into the cells through binding to the sodium dependent multivitamin transporter (SMVT) in the cell surface [16,17]. Importantly, many tumor cells highly express this transporter to meet the demand of biotin for rapid tumor growth, and biotinylation is thus a reasonable strategy to enhance the binding/affinity of macromolecular drugs to tumor cells, leading to increasingly effective antitumor therapy [18–21]. It has been reported that biotinylated nanoparticles selectively bound to breast cancer MCF-7 cells resulting in higher intracellular uptake than non-modified nanoparticles [12,22,23]. Therefore, utilization of BCS-NPs would exhibit double tumor-targeting effect, namely, it will selectively accumulate in tumor tissues by EPR effect, and then targetedly bind to and internalized into tumor cells that highly express biotin receptors.

Based on these notions, in this study, we prepared BCS-NPs with the use of bufalin as the antitumor entity. Bufalin is a traditional oriental medicine that is the major digoxin-like immunoreactive component of Chan Su, obtained from the skin and parotid venom glands of toads [24]. Bufalin is a cardioactive C-24 steroid that exhibits a variety of biological activities, especially regulating cardiovascular functions [24,25]. Recently it is also known to induce cell cycle arrest and apoptosis in many cancer cells [24,26], and is thus considered a candidate drug for cancer chemotherapy. However, like many conventional anticancer drugs, the small molecular nature of bufalin hampered its development and application, especially the structural similarity of bufalin to digoxin may cause severe toxic effect if indiscriminately distributed in the body.

Accordingly, the aim of this study was to investigate the efficacy and possibility of BCS-NPs as the drug delivery system for bufalin to increase the tumor selectivity as well as decrease toxic effects. The preparation, physiochemical characterization of bufalin loaded BCS-NPs (Bu-BCS-NPs) are described, the in vitro as well as in vivo antitumor effect are then examined using a breast cancer MCF-7 cell line and its mice xenograft model, by comparison with unmodified native bufalin.

2. Materials and methods

2.1. Chemicals

Bufalin and 1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan (MTT) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Chitosan (CS) with a mean molecular weight of about 1×10^6 Da was from AK Biotech Ltd. (Jinan, China). The deacetylation degree of CS which we used is 91.5%, and the polydispersity index (Mw/Mn) is 1.36 tested by the gel permeation chromatography (GPC) method, as provided by the manufacturer. Sulfosuccinimidobiotin (sulfo-NHS-biotin) were purchased from Pierce Biotechnology, Inc. (Rockford, IL, USA). All other chemicals and reagents were from commercial sources unless otherwise described.

2.2. Cell culture

Human breast cancer cells MCF-7 were kindly provided by Department of Pharmacology, Shenyang Pharmaceutical University. The cells were cultured in RPMI-1640 medium (Sigma) with 10% fetal bovine serum (Invitrogen, Carlsbad, CA) at 37 °C in an atmosphere of 5% CO_2/95% air.

2.3. Synthesis of biotinylated chitosan

Biotinylated CS (BCS) was synthesized according to a method by Yao et al. with some modifications [27]. In brief, 100 mg CS was dissolved in 5 ml of 10% HCl and the pH was adjusted to 6.5. To this solution, 5 mg sulfo-NHS-biotin (0.83 ml) was added dropwise and stirring for 24 h at room temperature. Afterward, the resultant was subjected to dialysis with a membrane of 8000–10000 Da molecular cut-off, against deionized water for 24 h with 3-change of water, followed by lyophilization. According to this protocol, the binding rate of biotin was quantified to be about 2.5 mol/mol CS by using a biotin assay kit (Pierce Biotechnology, Inc.).

In some experiments, to obtain an FITC labeled BCS (FITC-BCS), 100 mg of BCS was dissolved in 5 ml of 0.01 M phosphate-buffered 0.15 M saline (PBS; pH 7.4), to which 0.1 ml of FITC (5 mg/ml in acetone) was added and reacted for 2 h at room temperature avoiding the light. The resulted FITC-BCS was collected after centrifugation (150,000 rpm, 30 min) and lyophilized.

2.4. Preparation of bufalin-BCS nanoparticles (Bu-BCS-NPs)

The Bu-BCS-NPs were prepared by solvent-dialysis method. Briefly, 10 mg bufalin and 50 mg BCS were dissolved in dimethyl sulfoxide, and the solution was subjected to sonication for 5 min. Then, by the use of a dialysis membrane with molecular cut-off of 3500 Da, the solution was dialyzed against deinonized water for 3 days, with the change of water every 6 h. After dialysis, the solution was collected and subjected to sonication for 15 min, after which it was filtered with a syringe filter with a 0.45 μm pore size hydrophilic PVDF membrane (Millex-HV Filter, 0.45 μm, Merck Millipore, Billerica, MA, USA). The filtrate was lyophilized to obtain Bu-BCS-NPs.

In some experiments, nanoparticles without biotinylation (Bu-CS-NPs) and FITC labeled Bu-BCS-NPs were prepared by the same protocol, using CS without biotin modification and FITC-BCS respectively.

2.5. Characterization of Bu-BCS-NPs

2.5.1. Determination of encapsulation efficiency (EE) and drug loading (DL)

The EE and DL of bufalin were determined by HPLC method. Generally, 10 mg of Bu-BCS-NPs was first suspended in 1 ml of