International Journal of Pharmaceutics 515 (2016) 527–534

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Albumin hybrid nanoparticles loaded with tyrosine kinase A inhibitor GNF-5837 for targeted inhibition of breast cancer cell growth and invasion

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ARTICLE INFO

Article history:
Received 19 July 2016
Received in revised form 17 September 2016
Accepted 24 October 2016
Available online 25 October 2016

Keywords:
Albumin hybrid nanoparticles
TrkA inhibitor
Breast cancer
Targeted therapy

ABSTRACT

Novel albumin hybrid nanoparticles (Alb-HNPs) loaded with tyrosine kinase A (TrkA) inhibitor GNF-5837 were prepared and evaluated for antineoplastic efficacy in a panel of breast cancer cell lines. The nanomedicines (GNF-Alb-HNPs, hydrodynamic diameter ~150 nm) were formed through a unique polyelectrolyte complexation process where albumin and GNF-5837 were encapsulated by a stabilizing layer of oppositely charged chitosan and dextran sulfate polysaccharides. GNF-Alb-HNPs showed an excellent colloidal stability and a sustained drug release over more than 24 h. We found that these nanomedicines inhibited TrkA phosphorylation and downstream mitogen-activated protein kinase (MAPK) signaling in breast cancer cells specifically, resulting in anti-proliferative and pro-apoptotic effects. Moreover, the migration and invasion activities of cancer cells were dramatically suppressed and the inhibitory effects were much more prominent with GNF-Alb-HNPS than the drug alone. These results show that the GNF-Alb-HNPs may represent a novel approach for targeted breast cancer therapy.

1. Introduction

The application of nanotechnology products in oncology has greatly impacted cancer diagnosis and therapy in the past decades. Nanoparticles (NPs) in particular have been exploited to circumvent the low bioavailability and rapid degradation/inactivation of drugs (Heath and Davis, 2008; Wang et al., 2012). NPs can also passively accumulate in tumors through the enhanced permeability and retention (EPR) effect, which has been explored to reduce the off-target toxicities of chemotherapeutics with narrow therapeutic index (Greish, 2007; Torchilin, 2011). This passive tumor targeting of NPs is often affected by their particle size and surface property that determine their biological behaviors after systemic administration. NPs of less than 5 nm are subject to rapid renal clearance and those of above 400 nm are prone to elimination by the complement and phagocyte system or hepatic and splenic filtration (Decuzzi et al., 2010; Wang et al., 2011). NPs with a neutral or slightly negative hydrophilic surface generally show superior tumor accumulation as they are able to evade serum opsonization and nonspecific cell interactions (Cho et al., 2009; Chung et al., 2007; Verma and Stellacci, 2010). Polyethylene glycol (PEG) has routinely been used to tailor NP surface for passive tumor targeting (Hu et al., 2013). However, PEG coating can interfere with the interactions of NPs with cancer cells (Hama et al., 2015) and the PEG-specific IgM generated after injection of PEGylated NPs can cause accelerated blood clearance of the subsequent doses (Wang et al., 2007). Natural biomaterials, including hydrophilic polysaccharides dextran sulfate (DS) and chitosan (CS), have attracted considerable attentions for surface coating. In particular, the pH sensitive polyamine backbone of the CS can switch to positive charge at lower pH of tumors and be tuned to increase the internalization of NPs by cancer cells (Li et al., 2015; Yan et al., 2015).

Abbreviations: BCA, bicinchoninic acid; BSA, bovine serum albumin; CS, chitosan; DLS, dynamic light scattering; DMSO, dimethyl sulfoxide; DS, dextran sulfate; EPR, enhanced permeability and retention; FBS, fetal bovine serum; gp, glycoprotein; HPLC, high-performance liquid chromatography; IC_{50}, half-maximal inhibitory concentration; IgM, immunoglobulin M; MAPK, mitogen-activated protein kinase; MW, molecular weight; Nab™, nanoparticle albumin-bound technology; NGF, nerve growth factor; NP, nanoparticle; PBS, phosphate buffered saline; PEC, polyelectrolyte complex; PEG, polyethylene glycol; SEM, scanning electron microscope; SPARC, secreted protein acidic and rich in cysteine; TrkA, tyrosine kinase receptor A.

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http://dx.doi.org/10.1016/j.ijpharm.2016.10.057
0378-5173/

Albumin as the major plasma protein is an ideal material for the formulation of drug delivery systems as it possesses many favorable pharmaceutical properties such as non-toxicity, high stability and drug binding capacity, and long half-life (~19 days) *in vivo*. In fact, albumin is the body's natural carrier for a wide variety of endogenous or exogenous lipophilic ligands, metal ions and drugs (Elsadek and Kratz, 2012; Kratz, 2008). The development of NP albumin-bound technology (nab™) represents a quantum leap in the targeted delivery of chemotherapeutic drugs that were traditionally formulated with poorly tolerated solvents (Fu et al., 2009). The nab™ technology uses the natural characteristics of albumins to reversibly bind and transport the drug to the tumors through their interaction with the endothelial gp60 receptor and SPARC in the tumor microenvironment (Cortes and Saura, 2010). Nab™-paclitaxel (Abraxane®, 130 nm) had already been approved for treatment of metastatic breast cancer. However, the major limitation of nab technology is poor colloidal stability as NPs rapidly dissociate into their constituents of individual albumin-bound drug, albumin or unbound-drug molecules upon systemic administration (Yardley, 2013). The lack of particle stability, thus loss of tumor targeting via the EPR effect, has possibly led to incidence of side effects for nab-based nanomedicines (Gradishar et al., 2005). In particular, healthy tissues also display extensive expression of extracellular or membrane albumin-binding proteins such as cubilin, gp18, gp30, gp60, megalin and SPARC, which can lead to undesired uptake of individual albumin-bound drugs to cause adverse effects in patients (Merlot et al., 2014). Therefore, it would be of great interst to develop novel albumin NPs that can maitain particle stability for passive targeting and enable sustained drug release in tumor microenvironment.

Tyrosine kinase receptor A (TrkA) and its activated form, phospho-TrkA, play a significant role in cancer progression and invasion. We previously showed that TrkA overexpression enhances the tumorigenic properties of breast cancer cells and TrkA signaling pathways could be a potential drug target for breast cancer therapy (Davidson et al., 2004; Lagadec et al., 2009; Romon et al., 2010). Recently, a potent and selective TrkA inhibitor, namely GNF-5837, had been shown to block the inner membrane phosphorylation binding site of the TrkA receptor, leading to strong anti-proliferative effects in Ba/F3 cells and anti-cancer efficacy in a mouse xenograft model derived from rat intestinal epithelial cells expressing TrkA (Albaugh et al., 2012). In that study, GNF-5837 was administered orally with high doses but showed very limited bioavailability (<20%) due to poor absorption deriving from a combination of poor permeability and low aqueous solubility. To overcome poor permeability/solubility and enable systemic administration, Alb-HNPs with desired particle stability were designed and used to encapsulate GNF-5837 to facilitate drug targeting via EPR effect and ehance drug uptake by cancer cells. The GNF-Alb-HNPs were formed via a polyelectrolyte complexation of albumin-bound GNF-5837 with DS and CS. We investigated the cytotoxic specificity of GNF-Alb-HNPs in comparison to the drug alone and its nanoliposomal formulations in a panel of subtype-specific breast cancer cell lines. These nanomedicines were also evaluated with respect to the apoptosis induction and invasion inhibition of cancer cells.

2. Experimental

2.1. Materials

Albumin from bovine serum (BSA) ≥98%, low molecular weight (MW) chitosan (CS), cholesterol, mannitol, methylene blue hydrate, Nonidet™ P-40 and resazurin sodium salt were purchased from Sigma-Aldrich. Dextran sulfate (DS) (average MW 500 kDa), dipalmitoylphosphatidylcholine (DPPC), *N*-(carbonyl methoxypolyethyleneglycol 2000)-1,2-distearoyl-sn-*glycero*-3-phosphoethanolamine (DSPE-mPEG2000) ammonium salt and GNF-5837 were obtained from ICN Biomedicals Inc, Echelon Biosciences, Avanti Polar Lipids and Adooq Bioscience, respectively. All other reagents were of analytical grade and were used without further purification.

2.2. Preparation and characterization of Alb-HNPs

2.2.1. Preparation of Alb-HNPs

Alb-HNPs were prepared via a unique polyelectrolyte complexation process upon pH adjustment. Briefly, BSA and DS were co-dissolved in distilled water at a molar ratio of 10:1 and the pH of solution was adjusted to 5.8 using 0.1 N HCl. A stock solution of CS (1.25 mg/ml) was prepared in 20 mM aqueous acetic acid. All solutions were filtered through 0.22 μm Millipore membranes. For the preparation of GNF-Alb-HNPs, ~1 mg GNF-5837 was dissolved in 100 μl dimethyl sulfoxide (DMSO) and the solution was added dropwise to a BSA-DS solution containing 10 mg BSA. For control Alb-HNPs, the same amount of DMSO without drug was added. Complexations were carried out via one-shot addition of the CS solution to the BSA-DS mixture to reach a BSA/chitosan mass ratio of 10:1 and a final BSA concentration of 5 mg/ml. The resulting suspension of NPs was sequentially subjected to a brief bath-sonication (~1 min) and pH adjustment to 5.8 using 0.1 N NaOH and another brief sonication (~1 min). Finally, 570 μl of 15% mannitol solution was added for each mL of NPs dispersion and the mixture was centrifuged at 15,000 × *g* for 30 min. The supernatant was removed and the pellet was resuspended in 2.5% mannitol solution under mild bath sonication (~2 min) to produce a homogenous dispersion of Alb-HNPs.

2.2.2. Characterization of Alb-HNPs

The hydrodynamic diameter and ζ-potential of NPs were measured using dynamic light scattering (DLS) (Zetasizer Nano-S, Malvern, UK) at 25 °C. The morphology of NPs was characterized on a field emission scanning electron microscope (FESEM) (Zeiss Sigma VP, Germany). SEM imaging samples were prepared by applying 5 μl of diluted NP dispersion on a silicon chip mounted on a standard SEM pin stub followed by overnight drying. The amount of albumin in Alb-HNPs was quantified using the micro bicinchoninic acid (BCA) protein assay kit (Pierce Biotechnology, USA) per the manufacturer's instructions. GNF-5837 incorporation efficiency was determined with high-performance liquid chromatography (HPLC) analysis (see Supplementary data).

2.3. Colloidal stability and release profile of GNF-Alb-HNPs

2.3.1. Colloidal stability of GNF-Alb-HNPs

Real-time DLS measurements were employed to monitor the stability of nanoparticles under two different conditions: (i) 100 times dilution in water and (ii) in the presence of serum. Fetal Bovine Serum (FBS), a common supplement for cell culture, was used to simulate the physiological condition. NPs were mixed with FBS (1:1 v/v) and the mixture was incubated in a water bath at 37 °C with mild stirring for 27 h. The mean particle size was measured at predetermined time points (0, 10, 30 min, 1, 2, 3, 6, 9, 18 and 27 h) to assess the colloidal stability of NPs while in contact with serum components.

2.3.2. Release profile of GNF-Alb-HNPs

The time course release profile of GNF-Alb-HNPs was studied in a release buffer containing 0.2% DMSO, 2.5% mannitol and 50% phosphate buffered saline (PBS, pH 7.4). The GNF-Alb-HNPs were suspended and kept rotating at 37 °C and, at specified time points (0, 0.5, 1, 3, 9, 24 and 48 h), samples were removed and