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Peptide conjugated polymeric nanoparticles as a carrier for targeted delivery of docetaxel

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ABSTRACT

The aim of this research work was to develop Bombesin peptide (BBN) conjugated, docetaxel loaded nanocarrier for the treatment of breast cancer. Docetaxel loaded nanoparticles (DNP) were prepared by solvent evaporation method using sodium cholate as surfactant. BBN was conjugated to DNP surface through covalent bonding. Both DNP and BBN conjugated DNP (BDNP) were characterized by various techniques such as dynamic light scattering, Fourier transform infrared spectroscopy (FTIR), atomic force microscopy (AFM), powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC) and thermogravimetric analysis. The particle diameter and zeta potential of BDNP were 136 ± 3.95 nm and -10.8 ± 2.7 mV, respectively. The change in surface charge and FTIR studies confirmed the formation of amide linkage between BBN and DNP. AFM analysis showed that nanoparticles were spherical in shapes. In nanoparticles, docetaxel was present in its amorphous form as confirmed by DSC and PXRD analysis and was stable during the thermal studies. The formulations showed the sustained release of DTX over the period of 120 h. During cellular toxicity assay in gastrin releasing peptide receptor positive breast cancer cells (MDA-MB-231), BDNP were found to be 12 times more toxic than pure DTX and Taxotere. The IC_{50} value for DTX, Taxotere, DNP and BDNP was >375, >375, 142.23 and 35.53 ng/ml, respectively. The above studies showed that Bombesin conjugated nanocarrier system could be a promising carrier for active targeting of anticancer drugs in GRP receptor over expressing cancer cells.

1. Introduction

Nanoparticle mediated targeted drug delivery has attracted lot of attention in last one decade especially for anticancer drug delivery. Conventional use of anticancer drugs is often hampered by nonspecific toxicity and side effects in non-targeted tissues. Nanoparticle mediated intracellular delivery of anticancer drugs provides the advantages of increased local drug delivery due to enhanced permeability and retention (EPR) effect, specificity, controlled drug release and avoidance of systemic drug toxicity. Apart from passive targeting, nanoparticles also offer an opportunity for active targeting through surface modification [1,2].

Currently various small chemicals such as folic acid [3], beta hydroxybutyric acid [4] and biomolecules like mannose-6-phosphate [5], galactose [6,7], peptides [8], glycoproteins [9], aptamers [10] and monoclonal antibodies [11] are being used for active targeting of anticancer drugs [1,3,12]. However, physiologically occurring or regulatory peptides have several unique advantages that make them attractive as targeting ligands. Peptides are small molecules with high permeability and biocompatibility. The small size of peptide minimizes the overall size of nanoconjugate while still maintaining high surface density (number of peptides per nanoparticle). Further, peptides are usually hydrophilic in nature and cannot cross (less than 0.1% of total injected peptide) the blood brain barrier. This property of peptide gives an extra benefit when peripheral tumors are the desired targets [13–15]. Various peptides such as K237, RGD, LyP-1, and I4R have been conjugated to nanocarriers to target tumor neovasculature, endothelium, lymphatic metastatic tumors and other tumor tissues [8,16–18].

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The primary objective of this study was to develop Bombesin (BBN) conjugated biodegradable polymeric drug delivery system for anticancer drug. BBN, a tetradecapeptide, was first isolated from the skin of the frog bombina bombina by Anastasi et al. [19]. BBN contains a mammalian counterpart called gastrin releasing peptide (GRP). BBN and GRP show structural and functional similarities especially on C-terminus residue. Because of this, BBN and its analogs may be used to target BBN receptor subtype 2 i.e. GRP receptors. GRP receptors have been found over expressed on lung, breast, ovarian and prostate cancer cells [19,20].

Docetaxel (DTX) is a semi-synthetic, taxane derived, highly potent anticancer drug. It has shown broad spectrum anti-tumor activity against prostate, breast, pancreatic, lung, gastric and hepatic carcinomas [21–23]. DTX binds irreversibly with β-actin and stabilizes the microtubule assembly which is responsible for inhibition of cell division and finally cell death [24].

In this study, BBN was conjugated to poly(lactic-co-glycolic acid) (PLGA) nanoparticles for targeted delivery of docetaxel in GRP receptor over expressing breast cancer cells. PLGA is approved by US FDA and European Medicine Agency (EMA) for use in various therapeutic applications in human. Chemically, it is cyclic dimer of two monomers namely lactic acid and glycolic acid. Both monomers are endogenous and by-products of normal metabolic pathways in the body. It provides excellent biocompatibility and biodegradability to PLGA and makes the polymer of choice for drug delivery system development [25]. PLGA nanoparticles provide the advantages of high structural stability, availability of free surface groups for bioconjugation and require less number of excipients than lipid based nanoparticulate systems. On the other hand, they also avoid the problem of rapid agglomeration and surface drug loading of inorganic nanocarriers [26,27]. BBN was covalently coupled to the PLGA nanoparticle surface using EDC/NHS chemistry. The nanoconjugate was physicochemically characterized and studied for in vitro cytotoxicity studies.

2. Materials and methods

2.1. Reagents and chemicals

Docetaxel was obtained as gift sample from TherDose Pharma Pvt Ltd. (Hyderabad, India). Poly(D,L-lactic-co-glycolic acid) (PLGA), 50:50 molecular weight ~ 30,000–60,000, and Bombesin were purchased from Sigma Aldrich (St. Louis, MO, USA). HPLC grade solvents were purchased from Merck specialties (Mumbai, India). MDAMB-231 (breast cancer) cell line was obtained from American Type Culture Collection (ATCC, Manassas, USA). Dulbecco's modified eagle's medium (DMEM), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), trypsin, EDTA were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Fetal bovine serum (FBS) was purchased from Gibco, USA. 96-Well flat bottom tissue culture plates were purchased from Tarson Ltd. (Mumbai, India).

2.2. Preparation of nanoparticles

PLGA nanoparticles were prepared by a modified solvent evaporation (nanoprecipitation) method [28]. Five milligrams of DTX was dissolved in 5 ml of PLGA solution (10 mg/ml in acetone) and then quickly poured in 50 ml distilled water with 0.25% (w/v) sodium cholate. The dispersion was briefly sonicated for 2 min in an ice bath and kept on stirring. After 3 h, the dispersion was centrifuged (15,000 rpm) for 30 min to separate DTX loaded nanoparticles (DNP) from free DTX. Nanoparticles were washed thrice with distilled water, lyophilized and stored at 2–8 °C.

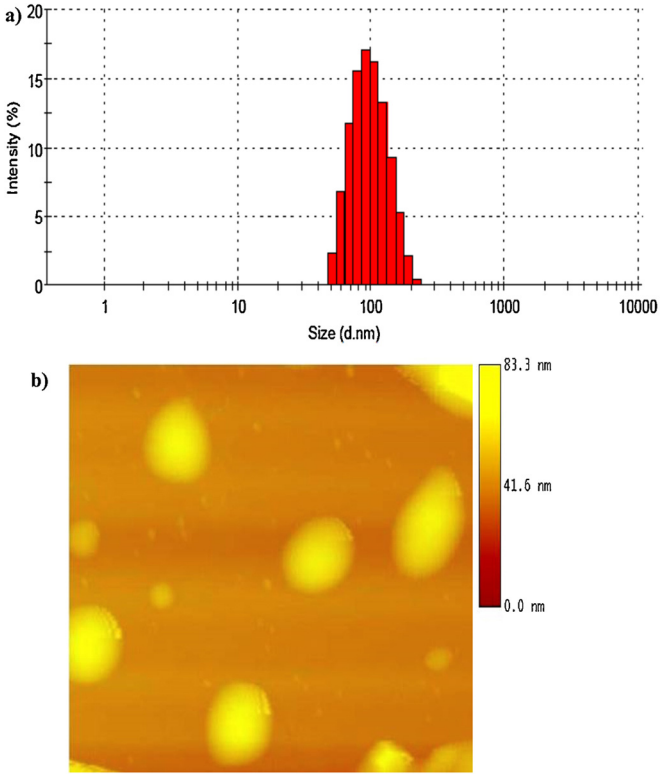

Fig. 1. Particle size and surface morphology of BBN conjugated nanoparticles (BDNP) determined by (a) dynamic light scattering and (b) atomic force microscopy analysis.

2.3. Bioconjugation of BBN to DTX loaded nanoparticles

Twenty milligrams of DNP was dispersed in 5 ml 0.1 M MES buffer (pH 6.2) and incubated with 14.62 mg N-hydroxysuccinimide (NHS) and 191.22 mg N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC). The dispersion was kept on gentle stirring for 1 h at room temperature. To this, 0.5 mg of BBN was added, mixed well and kept for further stirring overnight. BBN conjugated DTX loaded nanoparticles (BDNP) were collected after centrifugation at 10,000 rpm for 10 min and washed thrice with distilled water. Conjugation efficiency was determined by estimating free or unconjugated BBN content in the supernatant.

BBN was quantified using standard Bradford protein assay. A calibration curve of standard range of 0.5–10 μg/ml was prepared using standard BSA stock solution (2 mg/ml). The supernatant was diluted and absorbance was measured at 595 nm using microplate reader (Synergy 4, Biotek, USA).

2.4. Nanoparticle characterization

2.4.1. Particle size and zeta potential

Mean particle size and zeta potential of blank and docetaxel loaded PLGA nanoparticles were measured by proton correlation spectroscopy using Malvern Zetasizer Nano (Malvern Instrument Ltd., Malvern, UK). Samples were diluted 1:9 with distilled water and analyzed at 25 °C with a backscattering angle of 173°.

2.4.2. Surface morphology

Surface morphology of nanoparticles was examined by atomic force microscopy (AFM) using Digital Nanoscope IV (Veeco Instruments, Santa Barbara, CA). A drop of sample was placed on metal slab, air dried for 24 h and scanned for analysis.