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Research paper

DEAE-Dextran coated paclitaxel nanoparticles act as multifunctional nano system for intranuclear delivery to triple negative breast cancer through VEGF and NOTCH1 inhibition

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ABSTRACT

Triple negative breast cancer revolution has identified a plethora of therapeutic targets making it apparent that a single target for its treatment could be rare hence creating an urge to develop robust technologies for combination drug therapy. Paclitaxel, hailed as the most significant advancement in chemotherapy faces several underpinnings due to its low solubility and permeability. Advancing research has demonstrated the role of interferons in cancer. DEAE-Dextran, an emerging molecule with evidence of interferon induction was utilized in the present study to develop a nanoformulation in conjugation with paclitaxel to target multiple therapeutic pathways, with diminution of paclitaxel adverse effects and develop a specific targeted nano system. Evidently, it was demonstrated that DEAE-Dextran coated nanoformulation portrays significant synergistic cytotoxicity in the various cell lines. Moreover, overcoming the activation of ROS by paclitaxel, the combination drug therapy more effectively inhibited ROS through β-interferon induction. The nanoformulation was further conjugated to FITC for internalization studies which subsequently indicated maximum cellular uptake at 60 min post treatment demonstrated by green fluorescence from FITC lighting up the nuclear membrane. Precisely, the mechanistic approach of nuclear-targeted nanoformulation was evaluated by *in vivo* xenograft studies which showed a synergistic release of β-interferon at the target organ. Moreover, the combination nanoformulation inculcated multiple mechanistic approaches through VEGF and NOTCH1 inhibition along with dual β and γ-interferon overexpression. Overall, the combination therapy may be a promising multifunctional nanomaterial for intranuclear drug delivery in TNBC.

1. Introduction

Triple negative breast cancer, the utmost prevailing breast cancers worldwide, possesses an inadequate prognosis and treatment option limited to chemotherapy and radiotherapy. Creating a challenge for researchers, the specific targeting has been focused upon several nanoformulation development techniques for the management of the disease [1,2]. With a better knowledge of the underpinnings of triple negative breast cancer, it has become apparent that a single target for its treatment could be rare [3,4]. Current anti-tumor agents targeted to a molecular entity frequently show limited efficacies, poor bioavailability and development of resistance. Advances in cell signaling pathway research and mechanistic studies of the currently available anti-cancer drugs have identified several synergistic targets which could be concurrently formulated for increased bioavailability and better efficacy towards triple negative breast cancer. As an attractive strategy, combination drug therapies are routinely utilized for a plethora of diseases such as diabetes, AIDS, and cancer [5–7]. These combination therapies enable signaling multiple pathways in tumor cells, maximizing the therapeutic effect, and overcoming the resistance profile [8].

Nanotechnology approaches where a combination therapy is utilized to deliver directly to the tumor cells may result in better therapeutic efficacies, lower adverse effects as a result of the minimized dose of chemotherapeutic agent, and specific tumor targeting [1]. Previous studies have demonstrated the enhanced accumulation of antitumor agents at the target site when conjugated to polymers along with prolonged retention leading to advancement in macromolecular tumor therapeutics [9–11]. However, specific targeting through nanotechnology is influenced by the abnormal tumor microenvironment and vasculature, attracting research attention in targeted drug delivery and

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cancer therapy since the past decade [12,13]. Paclitaxel, hailed as the most significant advancement in chemotherapy since two decades by National Cancer Institute (NCI), faces the challenge of selective tumor targeting due to its low solubility and low permeability [14]. Currently available marketed formulations employ cremophor EL and ethanol in order to overcome the solubility crisis which in addition leads to hypersensitivity, hematologic toxicity and peripheral neuropathy. Abraxane, undoubtedly created history with the use of albumin for easy penetration, however, chronic toxicities still exist due to the chloroform employed during its manufacturing process.

Immunotherapy has recently found its role in cancer and could be a novel approach towards TNBC. Type I IFNs have been precisely studied for its role in the natural immune response to cancer for not more than the past decade. Experimental data strongly shows the existence of a process whereby the immune system, in the absence of any external manipulation, protects the host against oncogenesis and also controls the immunological features of developing tumors [15,16]. The process is known as immunoediting and it comprises of 3 phases; (i) the elimination of malignant cells by the immune system; (ii) establishment of an equilibrium between the genetically unstable malignant cells and the immune system reflecting the immunoediting imposed by the immune system on cancer cells; (iii) escape of neoplastic cell variants with reduced immunogenicity ultimately forming clinically manifest neoplasms. Type I IFNs intervene in all the 3 phases of immunoediting [17,18]. Interferons as such possess limited use due to their stability issues conveying them an expensive drug as well as difficult to store due to its stability issues. In lieu of these drawbacks, interferon inducers could be an alternative in order to bypass the stability dilemma and release interferons in the body to further combat tumor cells [19]. DEAE-Dextran, a polycationic derivative of dextran, offers a wide range of chemical and biological properties, with our recent research declaring it a specific β-interferon inducer. Previous literature has proved its biocompatibility in the development of nanoparticulate system for the delivery of doxorubicin [20,21]. With respect to the dual pharmacological properties, it could synergistically act with paclitaxel as a combination therapy. Moreover, recent advances are employing polymer-drug conjugates in tumor-targeting since polymeric carriers are bio-compatible as well as water soluble. Also, polymers of high molecular weight and hydrophilic nature have been of special interest in tumor targeting due to their prolonged blood circulation and passive targeting to the tumor tissue [13]. In the future, we can expect the emergence of several nanotechnology platforms for drug delivery applications [22]. DEAE-Dextran additionally fits suitable as a nano carrier in order to deliver paclitaxel to the tumor site and hence we have formulated and evaluated for the mechanistic approach of the combination nanoparticles in order to target multiple therapeutic pathways, with diminution of paclitaxel adverse effects and accomplish a specific drug targeted nano system by virtue of DEAE-Dextran coated paclitaxel nanoparticles.

2. Materials and methods

2.1. Materials

Molecular biology grade reagents were commercially purchased. Thiazolyl Blue Tetrazolium Blue (MTT), trypan blue dye, H_2DCFDA, 4′,6-Diamidino-2-phenylindole dihydrochloride (DAPI), DEAE-Dextran and BCA protein estimation kit were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's Modified Eagle's Medium (DMEM), Leibovitz L-15 medium (L-15), Dulbecco's Phosphate buffer saline (DPBS) and fetal bovine serum (FBS) were purchased from Invitrogen (Life Technologies, USA). Estrogen (ab32063), progesterone (ab2765) and HER2 (ab106575) antibodies were purchased from abcam (Abcam, UK). β-interferon (Relibeta) was purchased from Reliance pvt Ltd (Reliance, India). β-interferon ELISA kit was purchased from YH Biosearch Laboratory, China. All other chemicals used were of analytical grade and purchased from Merck (Darmstadt, Germany).

2.1.1. Cell lines and culture

HEK293, MCF7 and MDAMB231 cell lines were generously provided by Zydus Research Centre, India (obtained from ATCC, USA). HEK293 and MCF7 cells were grown in DMEM culture media containing L-glutamine (2 mmol/l). MDAMB231 cells were grown in L-15 culture media. All the media were supplemented with 10% FBS and an antibiotic cocktail containing penicillin (5 mg/ml) and streptomycin (5 mg/ml) (GIBCO, Invitrogen, UK). HEK293 cells were kept in a humidified atmosphere of 95% O_2 and 5% CO_2 in a CO_2 incubator at 37 °C while MDAMB231 cells were kept in 100% O_2 incubator at 37 °C. The exponentially growing cultured cells were used for experiments in the present study.

2.2. Experiment animals

All experiments and protocols described in present study were approved by the Institutional Animal Ethics Committee (IAEC) of Institute of Pharmacy, Nirma University, Ahmedabad as per the guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Ministry of social justice and empowerment, Government of India. Protocol number is IP/PCOL/PHD/17/011. Healthy female Balb/C mice (20-30g) were procured from Zydus cadila pvt ltd, Ahmedabad & Animal vaccination institute, Ahmedabad. Animals have been housed in groups of 6 animals in the animal house of Nirma University, Ahmedabad under controlled conditions of temperature 23 ± 2 °C, relative humidity 55 ± 5%, and photo schedule (12 h light and 12 h dark).

2.3. Experimental design and protocol

2.3.1. Nanoformulation development and characterization

2.3.1.1. Preparation of paclitaxel nanoparticles. Paclitaxel nanoparticles were formulated using the solvent evaporation method. Organic phase was prepared by dissolving paclitaxel in acetone. Aqueous phase was prepared by dissolving PVA in distilled water. Organic phase was then added dropwise into aqueous phase using insulin syringe (26 gauge) at the rate of 0.1 ml/min with continuous stirring on magnetic stirrer at room temperature for 8 h to evaporate acetone. The resulting nano particulate suspension was then utilized for various evaluation parameters.

2.3.1.2. Optimization of PVA. The above procedure was followed for the preparation of paclitaxel nanoparticles keeping the concentration of paclitaxel constant as well as the organic phase constant. The PVA concentration was varied from 0 to 2% drug loading,% encapsulation efficiency were calculated in order to determine the best batch for further optimization.

2.3.1.3. Optimization of organic: aqueous phase. Now, the PVA concentration was kept constant and the same procedure followed as mentioned above. The organic: aqueous phase ratio was varied from 1:5, 1.5:5, 2:5, 2.5:5, 3:5, 3.5:5, 4:5, 4.5:5 and 5:5. The best optimized batch was determined after evaluating % drug load, % encapsulation efficiency, particle size, PDI and zeta potential.

2.3.1.4. Preparation of DEAE-Dextran coated paclitaxel nanoparticles. A solution of DEAE-Dextran with the cross linking agent tripolyphosphate and PVA was dissolved separately. As previously mentioned optimization of paclitaxel formulation, the organic phase was prepared with paclitaxel. Using a 26 gauge needle, paclitaxel was added to the aqueous phase at a rate of 0.1 ml/min. The solution was stirred at 600 rpm for duration of 12 h. Various evaluation parameters were performed in order to validate the best batch for further studies. % drug load, % encapsulation efficiency, zeta potential, particle size and