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Therapeutic effects of dendrosomal solanine on a metastatic breast tumor

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ABSTRACT

Aims: Our previous studies showed that alpha-solanine can inhibit tumor growth in cell culture and animal models of breast cancer. However, solanine is insoluble in common solvents; therefore, we developed a special nanoparticle with high-capacity solubility. The present study is aimed to deliberate the therapeutic effects of dendrosomal solanine (DNS) on a metastatic breast tumor *in vitro* and *in vivo*.
Main methods: After DNS preparation and dosing procedures, forty-five mice were equally divided into five groups to investigate the anti-metastatic effects of DNS on mammary tumor-bearing mice.
Key findings: Compared to solanine, DNS significantly suppressed the proliferation of 4 T1 cells in a dose- and time-dependent manner. DNS showed a remarkable safety rate of up to 10 mg/kg. A significant decrease in white blood-cell count was seen at 20 mg/kg DNS in comparison with control animals. Mice treated with DNS had smaller tumor volume (mm^3) in comparison with control and solanine groups. Moreover, the incidence of the breast tumor metastases was about 67% in the control animals, where as solanine and DNS 1 mg/kg were about 22% and 0%, respectively. Furthermore, the number of metastases per mouse varied from one to three. The tissues of tumor, brain, liver, spleen, and lung showed higher expression levels of Bcl-2 but lower expression levels of Bax, MMP-2, MMP-9, mTOR, and Akt in DNS-treated mice than control and solanine groups.
Significance: The findings suggest that DNS has a more impactful therapeutic effect than solanine on 4 T1-induced breast tumorigenesis *via* influencing the tissue microenvironment.

1. Introduction

Alpha-solanine, a main glycoalkaloid, is found in the nightshade family (Solanaceae) [1]. It is a poisonous compound, even in small amounts, and its symptoms can arise 8–12 h after consumption. It is also one of the plant's natural defenses against insects and disease [2,3]. Studies have shown that alpha-solanine can inhibit tumor growth and metastasis in both cell culture and animal models of breast cancer [4]. This suggests that it can be used as an anti-metastatic compound to prevent migration and invasion of tumors [1]. Several studies have confirmed the anti-cancer effects of solanine by suppressing phosphatidylinositide 3-kinase (PI3k) and serine/threonine-protein kinase (Akt) enzyme activity [5]. According to Hasanain et al. (2015) lack of these pathways can lead to inhibition of other signal transduction pathways such as mammalian target of rapamycin (mTOR) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) [5], which can cause reduction of cell proliferation, invasion, and metastasis [6,7]. The main mechanism of solanine is unknown. However, previous studies suggest that solanine can cause cell membrane depolarization by activating caspase-3, thereby initiating apoptotic responses through the opening of mitochondrial permeability transition pores (MPTP) [4,8]. As a result, solanine may be a preventive and therapeutic candidate for cancer.

The glycoalkaloids, such as solanine, are easily dissolved in alcohol but are practically insoluble in water, ether, and chloroform [9]. In this regard, our team has succeeded in developing a robust nanoparticle with the high-capacity solubility (national patent, NO: 79290). This method involves the synthesis of non-ionic biocompatible polymeric carriers (dendrosomes), which introduce the biocompatible plant-fatty-acid-based carriers. The polymeric nanocarrier is a neutral, amphipathic, and biodegradable nanoparticle. Our previous experimental data showed that the polymeric carriers have the required characteristics for suitable carriers [10,11]. These carriers are capable of sustaining solanine for at least one year in an aqueous phosphate buffer. They can also enhance the solubility of alpha-solanine and increase its possible

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effectiveness as an anti-cancer agent. In the present study, our aim is to investigate the role of dendrosomal solanine (DNS) on development and spread of the breast tumor in a metastatic animal model.

2. Materials and methods

2.1. Materials

Alpha-solanine (>99%), doxorubicin hydrochloride (DOX), methyl thiazoltetra zolium (MTT), phosphate-buffered saline (PBS), ketamine, and xylazine were purchased from Sigma Aldrich Co. (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Technologies. The mouse mammary (4 T1) carcinoma cell line and normal fibroblast cells were procured from Pasteur Institute of Iran (Pasteur Institute, Tehran, Iran). Materials for the polymeric nanocarrier (OM2000) preparation including oleoyl chloride and methoxy polyethylene glycol 2000 (mPEG 2000) were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). The polymeric nanocarrier was locally produced in our lab (Patent Number: 79290) [12].

All animal studies have been conducted according to relevant national and international guidelines of the Weatherall report and Institutional Animal Care and Use Committee (IACUC) of Tehran University of Medical Sciences and Dezful University of Medical Sciences. Inbred female BALB/c mice (6–8 weeks old, purchased from Iran Pasteur Institute) were maintained under 12-hour dark and light cycle, with free access to food and water.

2.2. The study design

Studies were conducted in four series of experiments including (i) DNS preparation, (ii) DNS effects on the cell viability of mammary carcinoma cells, (iii) *in vivo* toxicity of DNS, and (iv) the therapeutic effects of DNS on metastatic breast cancer [13].

2.3. Polymeric nanocarrier synthesis

Polymeric nanoparticle (PNP, OM2000) was synthesized by esterification of oleoyl chloride (3.01 g, 0.01 mol) and methoxy polyethylene glycol 2000 (20 g, 0.01 mol) in the presence of triethylamine (1.2 g, 0.012 mol) and chloroform as solvents at 25 °C for 2 h [12]. Triethylamine hydrochloride was filtered from organic phase. Chloroform was then evaporated and OM2000 dried in a 40 °C vacuum oven for 4 h with 96% purity. Fourier transform infrared (FTIR) spectroscopic measurements were done using a Perkin-Elmer spectrometer in potassium bromide (KBr) pellets. The ^{1}H NMR spectrums were carried out using Bruker 400 MHz in DMSO-d_5.

2.4. Critical micelle concentration determination

Critical micelle concentration (CMC) of PNP was determined by detecting shifts in the pyrene fluorescence absorbance spectra. In this regard, 3 mL of pyrene solution (6×10^{-6} M) in acetone were added to a glass test tube and evaporated to remove the solvent. Then, 5 mL of PNP (0.005–1 mg/mL) was conjugated with phosphate-buffered saline (PBS; 0.01 M, pH 7.4) and added to pyrene to reach a final concentration of 6×10^{-7} M. The solutions were vortexed and conditioned overnight at 37 °C. Fluorescence excitation spectra of pyrene (300–350 nm) was measured at an emission wave length of 390 nm with slit widths of 2.5 and 5.0 nm (Perkin-Elmer Fluorimeter, USA) for excitation and emission, respectively. The fluorescence excitation shifts from 334 to 339 nm were used to determine CMC of the polymeric nanocarrier [12].

2.5. DNS preparation

Alpha-solanine (5 mg) was dissolved in acetone and added to nanocarrier solution (100 mg in 3 mL water). It was then mixed and rotated with rotary evaporator for acetone evaporation. DNS was filtered using 0.22 μm syringe filter to remove non-encapsulated solanine. The complex was then lyophilized [4]. They were supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin. All cells were grown at 37 °C in a 5% carbon dioxide, humidified atmosphere. Cell viability was assessed by MTT assay according to the manufacturer's instruction [13]. The cells were plated onto 96-well plates and incubated for 24, 48, and 72 h in the presence of various concentrations of DNS, solanine, avoid carrier, and Doxorubicin (Dox) as a positive control group. Media containing the treatment agents was carefully removed afterwards, and the cells were washed twice with phosphate buffered saline (PBS). The medium was then completely removed and 200 μL dimethyl sulfoxide was added to each well. Absorbance, which is directly proportional to the cell viability, was subsequently measured at 570 nm in each well, and presented as the percentage of cell viability of treated cells against control cells using an enzyme-linked immunosorbent assay plate reader.

2.6. In vivo toxicity

Thirty-five BALB/c mice were equally divided into five groups in order to study the toxicity of DNS with doses of 1, 5, 10 and 20 mg/kg (B.W, i.p) for 7 consecutive days together with the control group. The dose with no adverse reactions during 24 h was assigned as a survival dose. Survived animals were weighed on a daily basis and euthanized one week later. Abnormal hematological and blood chemical indices, and the body weight changes were amongst the toxicity signs.

2.7. Hematology and blood chemistry tests

Animals were decapitated under general anesthesia to evaluate hematology and clinical chemistry parameters. Blood samples were taken and added into the ethylene-diamine-tetra-acetic-acid (EDTA)-coated tubes for hematology and heparin-coated tubes for clinical chemistry tests. Total white blood cell (WBC), red blood cell (RBC) count as well as platelets (Plt), hemoglobin (Hgb), and hematocrit (Hct) were measured by using an animal blood counter (Celltac; Nihon Kohden, Tokyo, Japan). Plasma urea nitrogen (BUN), creatine (Cr), and glucose (Glu) were determined by using CCX System (CCX *WB*; Nova Biomedical, USA). Plasma alkaline phosphatase (ALP), albumin (ALB), alanine transaminase (ALT), and aspartate transaminase (AST) were also measured (Autoanalyser Model Biotecnica, *BT* 3500, Rome, Italy) [12].

2.8. Tumorgenicity

4 T1 cells were trypsinized and re-suspended in 10-fold excess culture medium. After centrifugation, cells were re-suspended in PBS, and 1×10^{6} cells were injected (0.1 mL, s.c) using a 21-gauge needle in the left flank of BALB/c mice under ketamine and xylazine (10 mg/kg, i.p) anesthesia [14]. The tumors were measured about two weeks after cell injection.

2.9. DNS effects on a metastatic breast tumor

Forty-five mice were equally divided into five groups: 1 and 5 mg/kg doses of DNS, solanine (5 mg/kg), and the positive and negative groups. In the positive control group, Dox (5 mg/kg) and Cyclophosphamide (Cytoxan, 2 mg/mouse) were divided into three doses and intraperitoneally coadministered weekly for three consecutive weeks. Dendrosome carrier was also given to the negative control group. DNS was given for 14 days after tumor injection from day 14 up to day 28. All animals