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The protective and therapeutic effects of alpha-solanine on mice breast cancer

Maryam Mohsenikia [a], Ali Mohammad Alizadeh [b,*], Saeed Khodayari [a], Hamid Khodayari [c], Seyed Amin kouhpayeh [d], Aliasghar Karimi [d], Mina Zamani [b], Saleh Azizian [b], Mohammad Ali Mohagheghi [b]

[a] Young Researchers and Elite Club, Islamic Azad University, Tehran Medical Branch, Tehran, Iran
[b] Cancer Research Center, Tehran University of Medical Sciences, Tehran, Iran
[c] Islamic Azad University, Tehran Pharmaceutical Sciences Branch, Tehran, Iran
[d] Department of Pharmacology, Fasa University of Medical Sciences, Fasa, Iran

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ABSTRACT

Alpha-solanine, a naturally steroidal glycoalkaloid, is found in leaves and fruits of plants as a defensive agent against fungi, bacteria and insects. Herein, we investigated solanine toxicity *in vitro* and *in vivo*, and assessed its protective and the therapeutic effects on a typical animal model of breast cancer. The study conducted in three series of experiments to obtain (i) solanine effects on cell viability of mammary carcinoma cells, (ii) *in vivo* toxicity of solanine, and (iv) the protective and therapeutic effects of solanine on animal model of breast cancer. Alpha-solanine significantly suppressed proliferation of mouse mammary carcinoma cells both *in vitro* and *in vivo* ($P<0.05$). Under the dosing procedure, 5 mg/kg solanine has been chosen for assessing its protective and therapeutic effects in mice breast cancer. Tumor take rate in the solanine–treated group was zero compared with a 75% rate in its respective control group ($P<0.05$). The average tumor size and weight were significantly lower in solanine-treated animals than its respective control ones ($P<0.05$). Proapoptotic Bax protein expression increased in breast tumor by solanine compared with its respective control group ($P<0.05$). Antiapoptotic Bcl-2 protein expression found to be lower in solanine-treated animals ($P<0.05$). Proliferative and angiogenic parameters greatly decreased in solanine-treated mice ($P<0.05$). Data provide evidence that solanine exerts a significant chemoprotective and chemotherapeutic effects on an animal model of breast cancer through apoptosis induction, cell proliferation and angiogenesis inhibition. These findings reveal a new therapeutic potential for solanine in cancer.

1. Introduction

Recently, investigations in the field of traditional medicine and on the efficacy of medicinal plants and their ingredients on different sorts of diseases have received much attention by the researchers, and different therapies are applied either as monotherapy and/or in combination with conventional medicine (Ben-Arye et al., 2012). The mycotoxins and alkaloids play an important role in incidence and/or treatment of diseases in animal and human (Alizadeh et al., 2012c; Mahmoodi et al., 2012). Glycoalkaloids (GAs) are natural toxic compounds found in a number of vegetables and plants that their concentration may alter following unfavorable storage conditions, such as mechanical damage, temperature, and light (Machado et al., 2007). Solanine, a glycoalkaloid material, found in the plants of the Nightshade (*Solanaceae*) family including *Solanum tuberosum* (potato), *S.lycopersicum* (tomato), and *S.melongena* (eggplant), is considered as a natural defense for its containing plant since it has pesticidal and fungicidal influences (Chaube and Swinyard, 1976). Glycoalkaloids reported to have slow clearance, and can accumulate in the body following regular and continuous consumption (Mensinga et al., 2005). Signs and symptoms of solanine toxicity may appear 8–12 h after consumption; however, higher doses may lead to earlier clinical presentations (Khodayari et al., 2013). Moreover, Nightshade has been used for the treatment of gastrointestinal cancer in Chinese traditional medicine (Gan et al., 2010). Several reports showed that solanine suppresses the growth of neoplastic cells, and induces apoptosis in cancerous cells *e.g.* cancers of skin, liver, prostate, and colon through various cellular and molecular pathways, abilities which are useful against cancerous cells (Lu et al., 2010a, 2010b; Ji et al., 2008; JI and GAO, 2012). Solanine was also shown to have anti-metastasis activity on

* Correspondence to: Cancer Research Center, Tehran University of Medical Sciences, Keshavarz Blvd Street, Imam Khomeini Hospital, Tehran 1419733141, Iran. Tel./fax: +98 21 61192501.
E-mail address: aalizadeh@razi.tums.ac.ir (A.M. Alizadeh).

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different cancers *e.g.* prostatic cancer and melanoma *in vitro* (Lu et al., 2010a, 2010b; Ji et al., 2008; JI and GAO, 2012; Rosenkranz and Wink, 2007). While *in vitro* observations shed light on the potential of solanine in live biological systems, a thorough investigation on animals is needed to relate the observed *in vitro* impacts of solanine with *in vivo* outcomes. Herein, we investigated the toxicity effects of solanine with a series of different doses to identify the toxic doses in mice. Clinical observations, hematological/blood chemistry tests, and histological examinations were conducted to evaluate *in vivo* toxicity. In addition, the protective and the therapeutic effects of solanine were assessed on mammary carcinoma cells, and a typical animal model of breast cancer through apoptosis, proliferation and angiogenesis pathways.

2. Materials and methods

2.1. Materials

Alpha-solanine (> 99%), Doxorubicin hydrochloride, Cyclophosphamide, Ketamine and Xylazine were purchased from Sigma Aldrich Co. (St Louis, MO, USA). The mouse mammary (4T1), human hepatocellular (HuH-7) carcinoma cell lines and normal human fibroblast cells were purchased from Pasteur Institute of Iran (Pasteur Institute, Tehran, Iran). Polyclonal mouse antiRat/Rabbit Bax, Bcl-2 and CD31 antibodies (DAKO Corporation, USA), and a rabbit monoclonal antibody against Ki-67 (Thermo Fisher Scientific Inc., San Jose, CA) were also provided.

2.2. The study design

The study was conducted on three series of experiments to obtain (i) solanine effects on cell viability of mammary carcinoma cells, (ii) *in vivo* toxicity of solanine in BALB/c mice, and (iii) the protective and the therapeutic effects of solanine in a typical animal model of breast cancer.

2.3. Animals

All the animal studies were 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. Animals were housed in pens exceeding the stipulated sizes requirements. All inbred female BALB/c mice (6–8 weeks old, purchased from Iran Pasteur Institute) maintained in large group houses under 12-h dark and light cycles, and gave access to food and water *ad libitum*.

2.4. The preparation of alpha-solanine stock

Alpha-solanine was dissolved in H_2O containing 0.45% saline and 0.25% acetic acid (pH 3.0) and kept in $-20\ ^{\circ}C$.

2.5. MTT assay

The 4T1, HuH-7 and normal fibroblast cell lines were grown in Dulbecco's modified Eagle's medium (DMEM; GIBCO, USA) containing 10% fetal bovine serum (FBS; GIBCO, USA) at a 37 °C temperature in a humid atmosphere of 5% CO2. The cell viability was measured by MTT (3–4, 5-dimethylthiazol-2-yl 2, 5-iphenyltetrazolium bromide) assay (Mosmann, 1983). Briefly, identical numbers of cells in 200 μl DMEM containing 10% FBS were seeded in triplicate on 96-well plates and incubated overnight. These cells subsequently treated with various concentrations (10, 20, 30, 40 and 50 μM) of solanine, and Doxorubicin (Dox) as a positive control for 24 and 48 h. Afterwards, we added 20 μl of MTT (5 mg/ml) to each well and incubated for an additional 4 h followed by adding 200 μl of dimethyl sulfoxide. We determined the relative cell viability by using a 96-well plate reader (TECAN, Switzerland) at 540 nm. IC50, the concentration of cells growth inhibitor was reduced by half, determined by standard curve method (Mosmann, 1983).

2.6. The dosing procedure

Forty-two mice were used to study the toxic effects of solanine. In order to obtain the desired concentration with maximal toxicity of solanine, we chose the starting dose administered to mice to be 200 mg/kg, and continued increasing the dose to the amount in which no dying or symptoms of poisoning occurred. If major adverse reactions in animals identified at a certain dose (toxic dose) within 24 h days, a decreased dose (usually the mean value between toxic dose and last tolerated dose) was applied to a new group of mice. Animals (one or more) may have shown the onset of major adverse reactions even before all animals (six) in the same group injected; when this occurred, no more animal was injected. If the dose reduced to a level that no major adverse reactions observed in all animals in a specific group for 24 h, then this dose was identified as a survival dose. Organ damage histological findings, abnormal hematological/blood chemical indices, reduced organ weight ratios, and body weight changes were amongst the signs favoring major toxicity. Therefore, doses of 200, 100, 40, 20, 10, 5 and 1 mg/kg (B.W, i.p) of alpha-solanine were injected, and the animals were euthanized one week after injection. The animals were under observation for abnormal sequels. Animals that survived post solanine treatment weighed on a daily basis and euthanized one week later. Hematology, blood chemistry, and pathology tests were carried out. Vital organs including heart, liver, spleen, lung, brain, and kidney excised and weighed separately.

2.7. Hematology, blood chemistry, and pathology tests

At the end of the treatment, we decapitated the animals under general anesthesia. Blood samples were taken and added into ethylene-diamine-tetra-acetic-acid (EDTA)-coated tubes for hematology and heparin-coated tubes for clinical chemistry. Total leukocyte count (WBC), erythrocyte count (RBC), platelets (Plt), hemoglobin (Hgb), hematocrit (Hct), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), neutrophils, lymphocytes, eosinophils and monocytes measured by using an animal blood counter (Celltac; Nihon Kohden, Tokyo, Japan). Plasma urea nitrogen (BUN), creatine (Cr), sodium (Na), potassium (K), chloride (Cl), bicarbonate (HCO^{3-}), calcium (Ca), magnesium (Mg), lactate (Lac), osmolarity (Osm) and glucose (Glu) determined by using CCX System (CCX WB; Nova Biomedical, USA). Plasma alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST) also measured by means of an Autoanalyser System (Autoanalyser Model Biotecnica, BT 3500, Rome, Italy). Liver, kidney, brain, lung, spleen and heart tissue samples were fixed and preserved in 4% buffered formaldehyde for at least 24 h. We prepared the tissue blocks and the slides were evaluated for histopathological changes.

2.8. The protective effects of solanine on an animal model of breast cancer

Sixteen mice divided into two equal control and treated groups for the study of the solanine protective effects on mice breast cancer. We administered solanine for 24 days, from 3 days before