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ORIGINAL ARTICLE

Dose-Response Relationship Study of Selenium Nanoparticles as an Immunostimulatory Agent in Cancer-bearing Mice

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Backgrounds and Aims. Oral administration of selenium nanoparticles has an immunomodulatory effect on individuals with cancer. In the present study we aimed to compare the cancer preventive effect via administration of different doses of selenium nanoparticles in mice with cancer.

Methods. Forty 6- to 8-week-old inbred female BALB/c mice were used and divided into four test and control groups; each group contained ten mice. Group 1 (administered PBS) was used as the control and the test groups 2, 3, and 4 were daily administered 50, 100, and 200 μg of selenium nanoparticles, respectively, for 60 days. After 60 days, tumor induction was carried out and 10 days later serum samples were collected to measure the cytokines. Tumor growth and life span of the mice were also monitored during the study.

Results. The results showed a significant increase in serum IFN-γ and the ratio of IFN-γ/IL-4 in all administered doses compared to control. In addition, in mice that received higher doses of selenium nanoparticles (200 μg/day), lower tumor volume and extended life span were observed compared to control. Administration of selenium nanoparticles in normal mice without tumor challenge caused a nonsignificant increase in cytokine production, indicating that selenium supplementation has no effect on the immune response in the absence of tumor challenge.

Conclusions. The 200-μg dose of selenium nanoparticles can induce more efficient responses against breast tumors.

Key Words: Selenium nanoparticles, Breast tumor, Tumor growth, Life span, Preventive strategy.

Introduction

Breast cancer is the most common type of cancer and the second leading cause of cancer-related deaths among women (1). The high global incidence and mortality rates have led not only to the development of different generations of drugs but also to new preventive and therapeutic approaches to control this type of cancer (1). Among the newly emerging approaches, nanotechnology holds promise for direct anticancer treatments as well as indirect advanced drug delivery. Exploration of nanomaterials with chemopreventive traits may lead to amazing new horizons in the discovery of novel anticancer agents, with an expanded window between efficacy and toxic doses. In fact, anticancer materials formulated at the nanoscale exhibit considerable bioactivity with lower toxicity (2). Nanochemoprevention could be developed as an inexpensive, tolerable, and readily important approach for controlling and managing cancer. Moreover, nanoparticles have exhibited enhanced efficacy with decreased side effects owing to properties such as active cellular uptake (3). Cancer chemoprevention is defined as the use of natural, synthetic, or biologic compounds to reverse, suppress, or prevent

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carcinogenic progression to invasive cancer. In addition, the term chemoprevention includes prevention or inhibition of the recurrence and development of new cancers in high-risk individuals (4).

Selenium (Se), an essential trace element for animals and humans, has been demonstrated to affect the functions of several specific intracellular selenoproteins by being a component of their essential constituent selenocysteine (SeCys) (5). Selenium deficiency is associated with mammary carcinogenesis due to this element's role as a redox-modulating factor in regulating cell proliferation, differentiation, and apoptosis (5). Additionally, selenium has immune-enhancing and antioxidant effects (6). Interest in this area was stimulated by Clark et al. who found that supplementation of alcohol with Se-enriched brewer's yeast with selenomethionine (SeMet) decreased the total cancer mortality almost to 50% (7). Ip et al. showed that Se compounds are good candidates as a chemopreventive agent for breast cancer. In animal studies, Se ions have been shown to have antitumor activity but at levels greater than nutritional need (8). Thus, Se has high potential to be used as a tumor-selective drug, and recent investigations have shed light on the application of Se compounds treatment (8).

Contrastingly, Chen et al. recently reported that long-term oral supplementation (90 days) of organic (selenomethionine) or inorganic Se compounds (selenite or selanate) has a nonsignificant effect on the survival rate of mice inoculated with a low level of 4T1 cells (5×10^4) (9). They also reported that only the organic Se complex reduced the tumor size and increased some proinflammatory cytokines when the study was terminated (30 days after cancer cell inoculation) (9). Whereas it was previously observed that metalloid Se nanoparticles (Se NPs) with different properties compared to organic and inorganic Se due to its nanoscale nature can significantly stimulate the immune system and simultaneously reduce tumor size in inbred Balb/C mice inoculated with a higher count of 4T1 cells (1×10^6). Se NPs are categorized as metalloids and exhibit lower toxicity compared to ionic Se compounds such as selenium dioxide (10). In the present study, we specifically evaluated the effect of long-term (60 days) preventive supplementation of Se NPs at different doses on the tumor size and survival rate of mice with breast cancer to further evaluate and confirm that this type of Se (Se NPs) can successfully be used as an immunostimulatory agent to increase the immune capacity of mice against cancer cells and lead to enhance the life span of cancer-bearing mice.

Materials and Methods

Experimental Animals

Forty 6- to 8-week-old inbred female BALB/c mice weighing ~25-30 g were purchased from the Pasture Institute of Iran (Tehran, Iran). The experimental mice were divided into four groups. Each group contained ten mice, which were housed at a temperature of 24°C and humidity of 55 ± 10%, with a 12 h light/dark cycle. Also there were two additional groups each contained ten mice to study the effect of Se NPs administration on the immune response of normal mice (sham group). They were fed with water and standard mice pellets ad libitum. During the experiment, the animals' weight and general appearance were regularly monitored each day.

Ethics Approval

In this study all experimental procedures using animals were carried out according to the guidelines of the Tehran University of Medical Science (Tehran, Iran) for the care and use of laboratory animals.

Se NP Preparation

Lactobacillus brevis (from our laboratory collection) was cultivated in 100 ml of DeMan–Rogosa–Sharpe (MRS) broth (Merck, Germany) and incubated at 37°C. After overnight incubation, 1 ml of selenium dioxide stock solution (200 mg/L) was added in 100 ml of lactobacillus broth culture and incubated for 72 h. The cells were collected by centrifugation at 4000 ×g for 15 min, and the resultant pellet washed three times with phosphate buffer saline (PBS) was used for extracting the Se NPs. The resulting pellet was frozen by adding liquid nitrogen and disrupted with a mortar and pestle. Se NPs were isolated from the cell debris with an N-octyl alcohol water extraction system (11). Isolated Se NPs were examined at 100 kV with a Philips EM-208 transmission electron microscope (TEM) (FEI Ltd., Eindhoven, The Netherlands) to evaluate the size of the biogenic Se NPs. The zeta potential of the purified NPs was determinedby using a Zetasizer MS2000 (Malvern Instruments, UK). To determine the elemental composition of the NPs, energy dispersive X-ray spectrum (EDX) microanalysis (Vega Tescan, Brno, Czech Republic) was also performed. Finally, red elemental Se NPs were suspended in sterile PBS and stored at 4°C.

Administration Schedule

All experimental mice were gavaged PBS (''control'') and biogenic Se NPs at different doses of 50, 100, and 200 μg/day for 60 days.

Tumor Cell Injection

On the 60^{th} day of the experiment, the 4T1 cell line (ATCC CRL-2539) was used, and 200 μl of RPMI containing 1×10^6 cell/ml was injected sub-subcutaneously near the mice mammary glands. The mice were monitored until the tumor nodule appeared.