Restorative Effect of Vitamin E on some Immunological Parameters of Sub-Lethal γ-Irradiated BALB/c Mice

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ABSTRACT

Elevated amount of the free radicals due to ionizing radiation causes deteriorating damage on immune system. Therefore, we made attempts to investigate the protective effect of vitamin E (vit-E), a biological antioxidant in BALB/c mice, so as to find an affordable prophylactic supplementation for individuals who are at risk. Several groups of mice were selected and exposed to sub-lethal γ-irradiation with or without vit-E supplementation. At the end of exposure, mice were immunized by either live attenuated *Brucella melitensis* vaccine or sheep red blood cell (SRBC). Consequently, the following parameters were assayed: specific antibody response, delayed type hypersensitivity and lymphocyte proliferation. We showed that vit-E supplementation restored the immune response in γ-irradiated mice. These findings might have implications for individuals who are at risk of exposure to ionizing radiation. *Iran. Biomed. J. 4: 51-55, 2000*

INTRODUCTION

During the normal cellular metabolism free radicals so called reactive oxygen species (ROS), are produced by oxidation during normal cellular metabolism. It is the primary means by which humans and other animals derive energy [1]. Oxidation catalysis provides the stable electrons that are necessary for oxidation. As electrons are transferred from oxidant catalyst to oxygen, a variety of new oxygen species are formed, each of them are characterized by an unpaired set of electrons in their outer orbital. Therefore, one free radical can induce a destructive process by removing electrons from stable compounds, forming many ROS and transforming stable compounds into a variety of free radicals [2].

Other sources of the free radicals including: inflammation [3], exposure to certain chemicals, radiation [4], ultraviolet light, alcohol [5] and high fat dietary [6] have been reported. ROS are toxic via their effects on the cellular compounds such as proteins [7], membrane lipids [8] and DNA [9]. The damage caused by ROS tends to accumulate over time and it is a major reason facilitating cancer development in human subjects [2]. Since ROS are produced abundantly by a variety of pathways, humans and animals have evolved defense mechanisms against these free radicals. Antioxidants are small molecules that act as scavengers of ROS and prevent them from causing further cellular damage [10]. Antioxidants can increase immune responses by controlling the amount of the free radicals generated in a cell [11]. Vit-E is one of the biological antioxidants [12] acting as an immunopotentiator agent [13]. Its protective effect has been shown in a variety of diseases [14]. In addition, it is documented in laboratory animals that T and B cell proliferation are correlated with dietary and serum vit-E levels [15] however radioprotective effect of vit-E on immune response of exposed subjects has not been extensively studied. Radiation damage is due to the free radical production [16, 17], and it has been documented that it induces vit-E deficiency and damages the immune system [18]. In this report, we try to evaluate the effect of vit-E supplementation on immunosuppression induced by sub-lethal g-radiation in BALB/c mice.

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MATERIALS AND METHODS

All materials were purchased from Sigma (St. Louis, MO, USA) unless otherwise stated.

Animal and diet. Female BALB/c mice (16-18 g) at 6-8 weeks old obtained from Razi Institute of Iran (Tehran, Iran), were maintained on a regular mice chow diet for a week. The mice were housed, five animals per cage, in transparent plastic box with chip bedding and a stainless steel wire lid. The room temperature was kept at 20-22° C with a constant humidity and a 12:12 h light-dark cycle. Following this adjustment period, mice were divided into different groups and maintained on vit-E (1g/kg of diet) or regular chow diet. vit-E was added to the mice chow 3 weeks before exposure.

Ionizing irradiation. Whole body irradiation was performed by ionizing radiation using ^{60}Co-g -rays from a Gamma cell 220 Machine with a dose-rate of 0.1 Gy/s. It has previously been shown that 3 Gy (1 Gy/day) **-**irradiation is tolerable for mice but decreases some immunological parameters (data not shown).

Immunization. Two different antigens were selected including live attenuated *Brucella melitensis* Rev 1 vaccine and SRBC (Razi Institute, Hessarak, Karaj, Iran). After the completion of **-**irradiation, mice were either received intraperitoneally injection of Rev 1 vaccine (5×10^5 CFU) or 10^8 SRBC. Four weeks after immunization, the following immunological parameters were assessed:

Humoral response. Based on type of Ag, two methods were used to determine specific antibody response in the sera. Anti *Brucella* antibody was determined by means of lab-made dot-ELISA and hemagglutination was used for anti SRBC antibody.

Dot-ELISA. Bacteria were fixed in 1% formalin in phosphate-buffered saline, washed three times with PBS, pH 7, then dotted on the dull side of 5mm diameter nitrocellulose filter discs (0.22 m m pore size, Millipore Inc./Bedford, Mass.) using a 10 m l Hamilton syringe. 1m l volumes of bacteria contains 2.5 to 5×10^4 organisms. Ag discs were then dried for 10 min at 56° C and stored at -20° C until used. The dot-ELISA was performed at room temperature

and all solutions were prepared in TBS. Ag discs were placed in 96-well flat bottom microtitre plate. The discs were blocked for 1h with 75m l of 5% (w/v) bovine serum albumin (BSA-TBS). After aspirating the blocking solution, 50m l of increasing dilutions of sera diluted in 1% BSA-TBS, were added to each well and incubated for 30 min. Then the discs were washed three times by 0.05% (V/V) Tween 20 in TBS. Peroxidase-conjugated affinity-purified anti mouse Ab (50 ml) was added to each well and the plates were incubated for 30 min. At the end of incubation, the peroxidase-conjugated antibody was aspirated off and the wells were washed again. A perceptible chromogenic substrate, 4-chloro-1-naphtol in TBS activated with hydrogen peroxide, was added to each test-well and incubated for 30 min. Antigen discs were then washed three times with TBS, dried, and read visually. Discs showing clearly defined blue dots were considered positive.

Hemagglutination. The sera from SRBC immunized mice were diluted in PBS at micro-hemagglutination trays (V-shaped). Then, the same volume of 2% v/v SRBC was added to diluted sera and left the tray at room temperature for 1 h. The plates were read on a white surface. The last agglutinated well was reported as the titer of anti SRBC.

Cellular response. To assess the specific cellular response, two methods were used including delayed type hypersensitivity (DTH, in vivo) and lymphocyte transformation test (LTT, in vitro).

DTH. DTH to *Brucella* and SRBC antigens were assessed by the footpad swelling assessment. The right hind footpad of mice were received 50 m l of formalin fixed *Brucella* (1×10^8 CFU) or SRBC antigens (1×10^8 Cell) intradermally. As a negative control, in the left footpad of mice 50 m l of PBS/formalin were injected and footpad thickness increase was measured after 4, 24 and 48 h with a dial-caliper. DTH at 24 h was expressed as absolute footpad thickness increase in 10^{-2} mm.

LTT. Spleen cells suspensions were provided as follows: the mice were sacrificed by cervical dislocation and the spleens were collected. Mononuclear cells were obtained by gently teasing with tweezers in complete medium (CM). Cell

suspensions were washed twice with cold CM. SRBC were depleted by hypotonic lysis in lysing buffer, and the remaining nucleated cells were washed twice in tissue culture medium which was composed of RPMI 1640 supplemented with l-glutamine (2mM), penicillin (100IU/ml), streptomycin (100 mg/ml), and 2-ME (50m l). The cells were then adjusted to 2×10^6 /ml in a complete tissue culture medium supplemented with 10% FCS. For the proliferation assay, 100 µl of the cell suspension containing 2×10^5 cells were dispensed into round bottomed, 96-well microtiter plates (Nunclon, Delta, Denmark) plus appropriately diluted Ag in the complete tissue culture medium to a final volume of 200 µl. The cultures were incubated at 37° C in a humidified atmosphere containing 5% CO_2 in air for 72 h. For the last 18 h 1µCi of tritiated thymidine (Radiochemical Center, Amersham, UK) was added and the cultures were terminated by harvesting on a MASH automatic harvester (Pharmacia). Results are expressed as the difference (cpm) in uptake of thymidine between cultures stimulated with Ag and those containing medium only.

Statistical analysis. Statistical significance ($P<0.05$) was assessed by using the student t-test in analysis of at least four determinations.

RESULTS

Humoral responses of mice immunized by bacterial vaccine, B. melitensis. As shown in Fig. 1a, the Ab titer of -irradiated compared with non-irradiated mice were completely decreased ($P<0.05$). However the group of mice which was subjected to -irradiation and received vit-E showed a remarkable increase in Ab titer in such way that no statistically difference was observed between this group and the control mice.

Cellular response. Comparable results were obtained when DTH was assessed confirming that vit-E supplementation was able to restore cellular response in exposed mice (Fig. 1b). In addition, there was clear decrease in the percentage of footpad thickness increment of exposed mice without vit-E supplementation (Fig. 1b). However, the group received vit-E and exposed to -irradiation demonstrated an elevated DTH response to the specific antigen and no statistical difference was
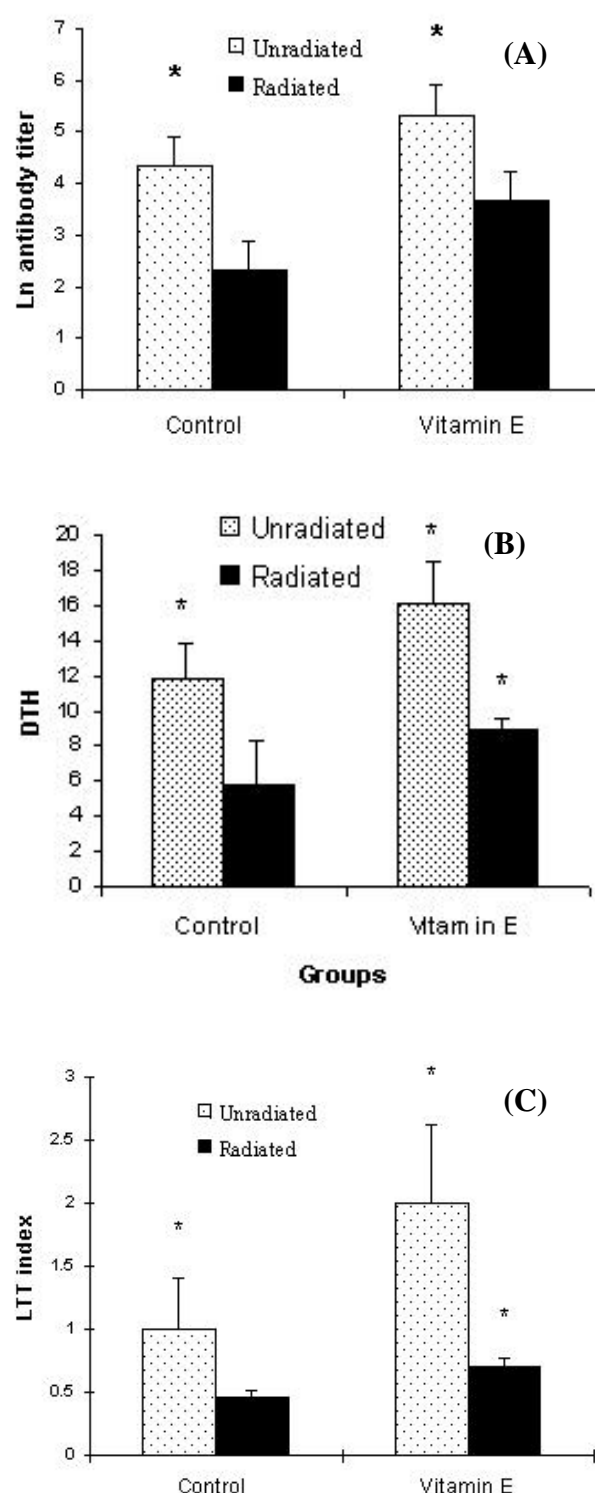

Fig. 1. Effect of vit-E supplementation of -irradiated mice immunized by *Brucella* antigen. (A) antibody production, (B) delayed type hyper-sensitivity (DTH) response, (C) lymphocyte trans-formation test (LTT) indices. * Significantly different from control ($P<0.05$).

observed between this group and the control group (Fig. 1b).

Lymphocyte transformation test. LTT confirmed the DTH results (Fig. 1c) since vit-E supplementation increased stimulation indices (SI) of unexposed mice. Furthermore, vit-E was able to protect mice against deteriorative effect of -irradiation on cellular response (SI), since the exposed group receiving vit-E supplementation showed a significant difference to exposed mice and no statistical difference was observed between mice receiving vit-E and the control mice.

The results of mice immunized by SRBC. In order to show that the prophylactic effect of vit-E is not dependent on the type of antigen used for immunization, the same groups of mice were selected but the immunization was performed by SRBC. Figure 2a-b showed the results of humoral and cellular response against SRBC in different groups of mice. Similarly, protective effect of vit-E on immune response in the exposed mice was observed.

DISCUSSION

It has been shown that radiation induces lipid peroxidation coupled with deficit of essential antioxidants including vit-E in children [19]. Furthermore, the serum level of vit-E decreases in the individuals exposed to ionizing radiation [16, 17]. There have been reliable reviews about the toxicological safety of oral intake of vit-E in human subjects [20]. On the other hand, it has been shown that serum level of vit-E decreases in individuals exposed to ionizing radiation [16, 17]. Since damage caused by ROS tends to accumulate over time, it would be of considerable clinical interest to demonstrate the protective effect of vit-E on immune responses of -irradiated mice so as to find a protective supplementation for individuals at risk.
It has recently been reported that vit-E decreases mitotic accumulation in -irradiated human tumor, but not in normal cells [21], therefore vit-E would protect individuals subjected to whole body -irradiation and at the same time makes the tumor cells susceptible to the effect of radiation. As individuals subjected to the whole body radiation
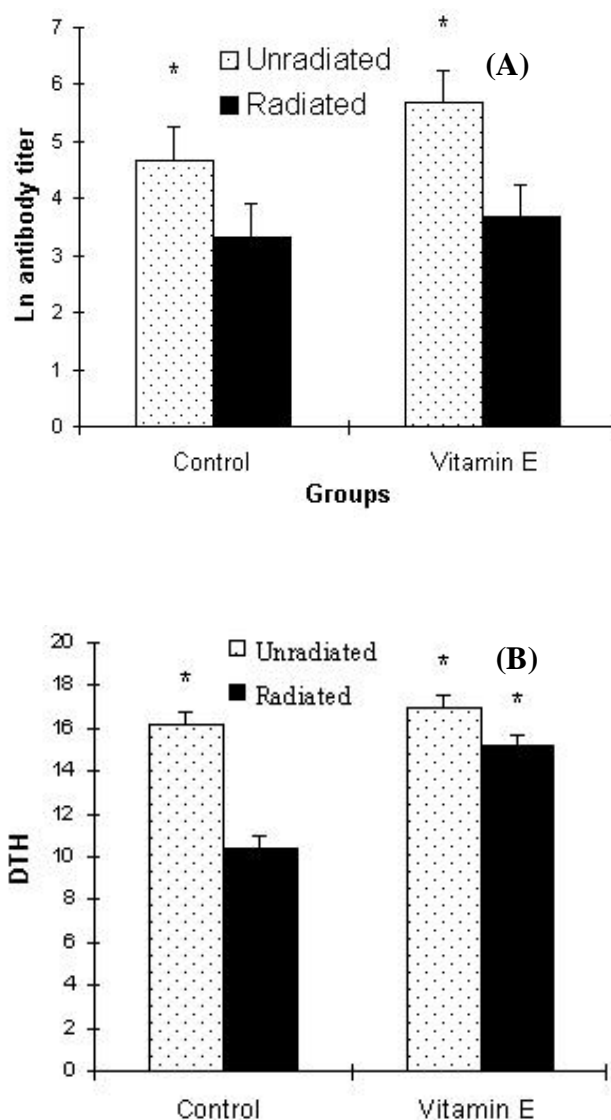

Fig. 2. Effect of vit-E supplementation of -irradiated mice immunized by SRBC. (A) antibody production, (B) delayed type hypersensitivity (DTH) response. * Significantly different from control (*P<0.05*).

suffer from immuno-suppression and are susceptible to the infectious disease, it would be worth finding a safe prophylactic supplementation for these subjects.
Our results clearly demonstrated that vit-E is able to block the deteriorative effect of -irradiation on humoral and cellular responses of normal mice. Therefore, we would propose a chemoprophylatic application of vit-E. In addition, it would be interesting to evaluate the effect of vit-E on tumor regression after -irradiation.

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