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

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ABSTRACT

Elevated amount of the free radicals due to ionizing radiation cause deteriorating damage on immune system. Therefore, we made attempt to investigate the protective effect of vitamin E (vit-E), a biologic antioxidant in BALB/c mice, so as to find an affordable prophylact supplementation for individuals who are at risk. Several groups of mic were selected and exposed to sub-lethal g -irradiation with or witho vit-E supplementation. At the end of exposure, mice were immunize by either live attenuated Brucella melitensis vaccine or sheep red bloo cell (SRBC). Consequently, the following parameters were assaye specific antibody response, delayed type hypersensitivity an lymphocyte proliferation. We showed that vit-E supplementatio restored the immune response in g -irradiated mice. These findin might have implications for individuals who are at risk of exposure t ionizing radiation. Iran. Biomed. J. 4: 51-55, 2000

Keywords: Vitamin E, Immune response, g -Irradiation, BALB/c mice

INTRODUCTION

During the normal cellular metabolism free radicals so called reactiv oxygen species (ROS), are produced by oxidation during normal cellul

metabolism. It is the primary means by which humans and other anima derive energy [1]. Oxidation catalysis provides the stable electrons that a necessary for oxidation. As electrons are transferred from oxidant cataly to oxygen, a variety of new oxygen species are formed, each of them a characterized by an unpaired set of electrons in their outer orbita Therefore, one free radical can induce a destructive process by removir electrons from stable compounds, forming many ROS and transformir stable compounds into a variety of free radicals [2].

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

MATERIALS AND METHODS

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

Animal and diet. Female BALB/c mice (16-18g) at 6-8 weeks o obtained from Razi Institute of Iran (Tehran, Iran), were maintained on regular mice chow diet for a week. The mice were housed, five animals p

cage, in transparent plastic box with chip bedding and a stainless steel wi lid. The room temperature was kept at 20-22 C with a constant humidi and a 12:12 h light-dark cycle. Following this adjustment period, mice we divided into different groups and maintained on vit-E (1g/kg of diet) regular chow diet. vit-E was added to the mice chow 3 weeks befo exposure.

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

Immunization. Two different antigens were selected including li attenuated Brucella melitensis Rev 1 vaccine and SRBC (Razi Institut Hessarak, Karaj, Iran). After the completion of g -irradiation, mice we either received intraperitoneally injection of Rev 1 vaccine (5 ´ 10^5 CFU) 10^8 SRBC. Four weeks after immunization, the following immunologic parameters were assessed:

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

Dot-ELISA. Bacteria were fixed in 1% formalin in phosphate-buffer saline, washed three times with PBS, pH 7, then dotted on the dull side 5mm diameter nitrocellulose filter discs (0.22 m m pore size, Millipo Inc./Bedford, Mass.) using a 10 m l Hamilton syringe. 1m l volumes bacteria contains 2.5 to 5´ 10^4 organisms. Ag discs were then dried for 1 min at 56° C and stored at -20° C until used. The dot-ELISA was performe at room temperature and all solutions were prepared in TBS. Ag discs we placed in 96-well flat bottom microtitre plate. The discs were blocked f 1h with 75m l of 5% (w/v) bovine serum albumin (BSA-TBS). Aft aspirating the blocking solution, 50m l of increasing dilutions of se diluted in 1% BSA-TBS, were added to each well and incubated for 30 mi Then the discs were washed three times by 0.05% (V/V) Tween 20 in TB Peroxidase-conjugated affinity-purified anti mouse Ab (50ml) was adde to each well and the plates were incubated for 30 min. At the end

incubation, the peroxidase-conjugated antibody was aspirated off and th wells were washed again. A perceptible chromogenic substrate, 4-chloro-1 naphtol in TBS activated with hydrogen peroxide, was added to each tes well and incubated for 30 min. Antigen discs were then washed three time with TBS, dried, and read visually. Discs showing clearly defined blue do were considered positive.

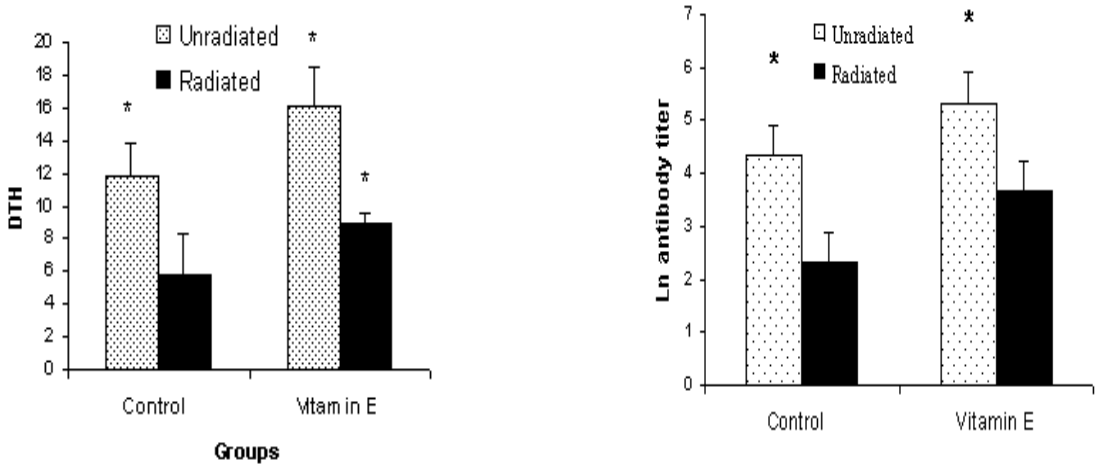
Hemagglutination. The sera from SRBC immunized mice were diluted 1 PBS at micro-hemagglutination trays (V-shaped). Then, the same volum of 2% v/v SRBC was added to diluted sera and left the tray at roo temperature for 1 h. The plates were read on a white surface. The la agglutinated well was reported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

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

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

LTT. Spleen cells suspensions were provided as follows: the mice we sacrificed by cervical dislocation and the spleens were collecte Mononuclear cells were obtained by gently teasing with tweezers 1 complete medium (CM). Cell suspensions were washed twice with co CM. SRBC were depleted by hypotonic lysis in lysing buffer, and th remaining nucleated cells were washed twice in tissue culture mediu which was composed of RPMI 1640 supplemented with l-glutamin (2mM), penicillin (100IU/ml), streptomycin (100 mg/ml), and 2-ME (50 l). The cells were then adjusted to 2 ′ 10^6 /ml in a complete tissue cultu medium supplemented with 10% FCS. For the proliferation assay, 100 m of the cell suspension containing 2 ′ 10^5 cells were dispensed into rou bottomed, 96-well microtiter plates (Nunclon, Delta, Denmark) plu appropriately diluted Ag in the complete tissue culture medium to a fin

volume of 200 m l. The cultures were incubated at 37° C in a humidifi atmosphere containing 5% CO_2 in air for 72 h. For the last 18 h 1m Ci tritiated thymidine (Radiochemical Center, Amersham, UK) was added a the cultures were terminated by harvesting on a MASH automatic harvest (Pharmacia). Results are expressed as the difference (D cpm) in uptake thymidine between cultures stimulated with Ag and those containi medium only.



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

RESULTS

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

Cellular response. Comparable results were obtained when DTH w assessed confirming that vit-E supplementation was able to restore cellul response in exposed mice (Fig. 1b). In addition, there was clear decrease the percentage of footpad thickness increment of exposed mice without v E supplementation (Fig. 1b). However, the group received vit-E a exposed to g -irradiation demonstrated an

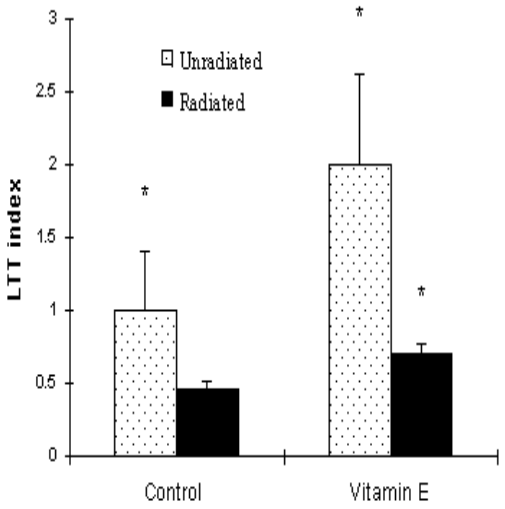


Fig. 1. Effect of vit-E supplementation of gamma-irradiated mice immunized t Brucella antigen. (A) antibody production, (B) delayed type hyper-sensitivity (DTI response, (C) lymphocyte trans-formation test (LTT) indices. * Significantly differe from control (P<0.05).

elevated DTH response to the specific antigen and no statistical differenc was observed
between this group and the control group (Fig. 1b).

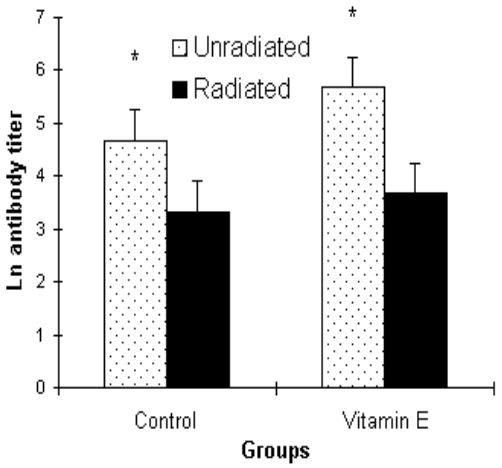
Lymphocyte transformation test. LTT confirmed the DTH results (Fi 1c) since vit-E supplementation increased stimulation indices (SI) unexposed mice. Furthermore, vit-E was able to protect mice again deteriorative effect of g -irradiation on cellular response (SI), since t exposed group receiving vit-E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

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

DISCUSSION

It has been shown that radiation induces lipid peroxidation coupled wi deficit of essential antioxidants including vit-E in children [19 Furthermore, the serum level of vit-E decreases in the individuals expose to ionizing radiation [16, 17]. There have been reliable reviews about th toxicological safety of oral intake of vit-E in human subjects [20]. On th other hand, it has been shown that serum level of vit-E decreases individuals exposed to ionizing radiation [16, 17]. Since damage caused b ROS tends to accumulate over time, it would be of considerable clinic interest to demonstrate the protective effect of vit-E on immune respons of g -irradiated mice so as to find a protective supplementation f 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 wou protect individuals subjected to whole body g -irradiation and at the sam time makes the tumor cells susceptible to the effect of radiation. A individuals subjected to the whole body radiation suffer from immuno-



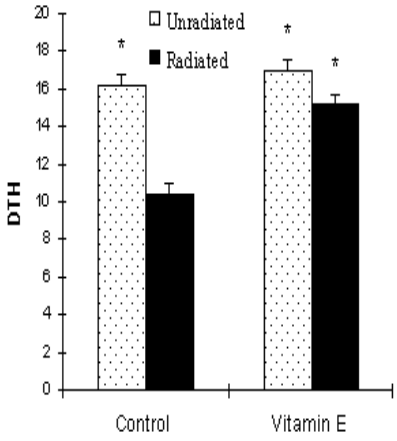


Fig. 2. Effect of vit-E supplementation of gamma-irradiated mice immunized t SRBC. (A) antibody production, (B) delayed type hypersensitivity (DTH) response. Significantly different from control (*P<0.05*).

suppression and are susceptible to the infectious disease, it would be worth findi a safe prophylactic supplementation for these subjects.

Our results clearly demonstrated that vit-E is able to block the deteriorati effect of g -irradiation on humoral and cellular responses of normal mic Therefore, we would propose a chemoprophylatic application of vit-E. addition, it would be interesting to evaluate the effect of vit-E on tum regression after g -irradiation.

REFERENCES

1. Taylor, A.E., Matalon, S., Ward, P.A. (1986) Chemistry and cytotoxicity of reactive oxygen metabolits. eds. The Williams Wilkins company, Baltimore, Maryland.
2. Freeman, B.A. and Crapo, J.D. (1982) Biology of disease: Free radicals and tissue injury. *Lab. Invest. 47: 412-426.*
3. Sies, H. (1985) Oxygen-centered free radicals as mediators of inflammation. Academic press, London.
4. Johns, H.E. (1983) The physics of radiology. Thomas publication, Springfield, Illinois, USA. pp. 514-552.
5. Seitz, H and K, Simanowski, U.A. (1988) Alcohol and carcinogenesis. *Annu. Rev. Nutr. 8: 99-119.*

6. Cinader, B. Clandinin, M.T., Hosokawa, T., Robblee, N.M. (1983) Dietary fat alters the fatty acid composition of lymphocyte membranes and the rate at whicl suppressor capacity is lost. *Immunol. Lett. 6: 331-337.*
7. Davies K.J.A. and Goldberg, A.L. (1987) Oxygen radicals stimulate intracellula proteolysis and lipid peroxidation independent mechanism in erythrocytes. *J. Biol. Chem. 262: 8227-8234.*
8. Aikens, J. and Dix, T.A. (1991) Perhydroxyl radical (HOO°) initiated lipid peroxidation: the role of fatty acid hydroperoxides. *J. Biol. Chem. 266: 15091-15098.*
9. Slater, T.F., Cheeseman, K.H., Davies, M.J., Proudfoot, K., Xin, W. (1987) Free radical mechanisms in relation to tissue injury. *Proc. Nutr. Soc. 46: 1-12.*
10. Bendich, A. (1990) Antioxidant nutrients and immune functions introduction. *Adv. Exp. Med. Biol. 262:1-12.*
11. Noroselova, E.G., Safonova, M.V., Gordon, R. and Semiletova, N.V. (1995) Immune function of spleen lymphocytes of rats subjected to chronic irradiation and antioxidant (Ubiquinone Q-4). *Int. J. Radiat. Biol. 67: 469-476.*
12. Packer, L. and Fuches, J. (1993) Vitamin E in health and disease. Marcel Dekke New York.
13. Tengerdy, R.D. (1990) Immunity and disease resistance in antioxidant supplement. *Adv. Exp. Med. Biol. 262:103-110.*
14. Garewal, S.H. (1994) Chemoprevention of oral cancer beta-carotene and vitami E in leukoplakia. *Eur. J. Cancer Prev. 3: 101-107.*
15. Bendich, A., Gabriel, E. and Machlin, L.J. (1986) Dietary vitamin E requiremen for optimum immune responses in the rat. *J. Nutr. 116: 675-681.*
16. Baraboi, V.A., Oleinik, S.A., Blium, I.A., Khmeleskii., I.U., V. (1994) Pro-oxidant and antioxidant homeostasis in guinea pigs following fractionated x-ray irradiation at low doses and the correction of disorders with an antioxidant complex. *Radiats. Biol. Radioecol. 34: 240-246.*
17. Shindo, Y., Witt, E., Han, D., Packer, L. (1994) Dose-response effects of acute ultraviolet irradiation on antioxidants and molecular markers of oxidation in murine epidermis and dermis. *J. Invest. Dermatol. 102 : 470-475.*
18. Wang Y., Huang D.S., Wood, S., and Watson, R.R. (1995) Modulation of immune function and cytokine production by various levels of vit-E supplementation during murine AIDS. *Immunopharmacology 29 : 225-233.*
19. Neyfah, E.A., Alimbekova, A.I., Ivanenko, G.F. (1998) Radiation-induced lipoperoxidation stress in children coupled with deficit of essential antioxidants. *Biochemistry (mosc) 63: 977-987.*
20. Kappus, H. and Diplock, A.T. (1992) Tolerance and safety of vitamin E: a toxicological position report. *Free. Radic. Biol. Med. 13: 55-74.*

21. Jha, M.N., Bedford, J.S., Cole, W.C., Edward-Prasad, J. and Prasad, K.N. (1999 Vit-E (d-alpha-tocopheryl succinate) decreases mitotic accumulation in gamma-irradiated human tumor, but not in normal cells. *Nutr. Cancer 35: 189-194.*
22. Dekruyff, R.H., Fang, Y. and Umetsa, D.T. (1995) IL-4 based helper activity of CD4+T cells is radiation sensitive. *Cell Immunol. 160: 248-256.*