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Vitamin E Modulates Radiation-induced Oxidative Damage in Mice Fed a High-Lipid Diet

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The Vitamin E (VE) effect was examined on oxidative damage to DNA, lipids, and protein in mice that were fed various levels of lipid diets after total body irradiation (TBI) with X-rays at 2 Gy. No increase of 8-hydroxydeoxyguanosine (8OHdG) by TBI was observed in the +VE group; however, in the case of the −VE group, a significantly higher 8OHdG level was observed in the high-lipid group than in the low- or basal-lipid group. In the groups with TBI, the concentration of thiobarbituric reactive substances (TBARS) only significantly increased in the high-lipid (−VE) group. These changes in TBARS, due to TBI, were not detected in other groups. The contents of protein carbonyls only increased in the (−VE) group. The contents of protein carbonyls was significantly different between the (+VE) and the (−VE) groups, regardless of the lipid levels. The concentrations of GSH, vitamins C and E in the liver were lower, and the concentration of non-heme iron in the liver was higher in the high-lipid group than in the low- and basal-lipid groups. These concentrations in the high-lipid group were significantly different between the (+VE) and the (−VE) groups. These results strongly suggest that mice that are fed a high-lipid diet are susceptible to TBI-induced oxidative damage. Also, decreases in the GSH levels and an increase in the iron level are involved in the mechanism of this susceptibility.

Keywords: Dietary lipid, Free radical, Oxidative damage, Vitamin E

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Abbreviations: TBI, total body irradiation; TBARS, thiobarbituric reactive substances; 8OHdG, 8-hydroxydeoxyguanosine; VE, vitamin E

Introduction

The induction of oxidative damage in the body is the result of oxidative stress that exceeds the antioxidant capacity, which is dependent on the levels of antioxidants and antioxidative enzymes. Oxidative modification of DNA, protein, and lipids by reactive oxygen species (ROS) plays a role in aging and diseases.

Vitamins E (VE), an important lipid-soluble antioxidant, prevents the formation of lipid peroxides. Lipid peroxides have been shown to induce the oxidative damage of DNA *in vitro* (Carmen and Oyvind, 2001; Lee *et al.*, 2001; Lee and Lim, 2001). This DNA damage may produce mutations that cause permanent genetic alterations when the cell replicates its DNA. This increases the risk of cancer (Helzlsouer *et al.*, 2000; Michaud *et al.*, 2000; Levi *et al.*, 2001). These results suggest that there is an association between a low intake of VE with a subsequent risk of cancer.

There have been many studies on the relationship between various levels of VE and oxidative damage in the body (Umegaki *et al.*, 1993; Cho *et al.*, 1995; Umegaki *et al.*, 1997). However, the effects of low levels of VE or VE deficiency on DNA damage in the liver and bone marrow were not observed in these studies, despite their exhibiting effects of low levels of VE or VE deficiency on lipids or protein damage. Moreover, rodent diets that contain adequate amounts of VE do not significantly enhance the VE accumulation in mice or rats. In a preliminary experiment, the 8OHdG level did not differ between the low VE and basal VE groups.

In this study, mice were fed a low-lipid (−VE), low-lipid (+VE, 15 mg α-tocopherol/kg diet), basal-lipid (−VE), basal-lipid (+VE, 15 mg α-tocopherol/kg diet), high-lipid (−VE), or high-lipid (+VE, 15 mg α-tocopherol/kg diet) diet, based on the AIN93G formula (Reeves *et al.*, 1993). The mice were then examined for TBI-induced oxidative damage to DNA, lipids, and protein in the liver. Furthermore, changes in the non-heme iron concentration were evaluated because iron is

involved in oxidative damage (Toyokuni, 1996; Abalea *et al*., 1998).

To evaluate the contributions of antioxidants to oxidative damage, the changes in the concentrations of vitamins C and E, and GSH were also examined.

Materials and Methods

Materials Corn starch, vitamin-free casein, cellulose, beef oil, mineral mixture (AIN-93G) and vitamin E-free vitamin mixture (AIN-93G), and α-tocopherol acetate were purchased from Oriental Yeast Co. (Tokyo, Japan). Other chemicals were obtained from Wako Pure Chemical Inc. (Osaka, Japan).

Animals and diets Male ICR mice (4-wk-old) were purchased from Japan Clea (Tokyo, Japan). The animals were housed two per cage in a room with a constant temperature at 23 ± 1ºC and a 12 h light: 12 h dark cycle. Various diets [a 1% lipid (–VE), 1% lipid (+VE; 15 mg α-tocopherol acetate/kg diet), 7% lipid (–VE), 7% lipid (+VE; 15 mg α-tocopherol acetate/kg diet), a 15% lipid (–VE), or 15% lipid (+VE; 15 mg α-tocopherol acetate/kg diet)] were prepared, based on the AIN93G formula (Reeves *et al*., 1993). The mice were fed the 7% lipid diet for 3 days to allow them to adapt to the semi-purified diet, then divided into 12 groups (6 mice per group), each of which was fed 1 of the 6 different diets, then subjected to 1 of 2 irradiation doses (0 or 2 Gy). The initial body weight of each group was adjusted to 23.8 ± 0.1 g (mean ± SEM). Throughout the study, the mice were given free access to food and water. Food intakes in the 1%, 7%, and 15% lipid groups were 3.8, 3.6, and 3.5 g/mouse/day, respectively. Minerals and vitamins for all of the diets were set at 35 g/kg and 10 g/kg, respectively. Levels of dietary lipid, vitamins, minerals, and other nutrients were set, based on the recommendation of AIN93G (Reeves *et al*., 1993). In order to make all of the diets isocaloric, the 1% lipid diet was prepared by substituting the carbohydrates for a lipid-deficient portion when compared to the 7% lipid diet. The 15% lipid diet was prepared by substituting the carbohydrates for a lipid-sufficient portion when compared to the 7% lipid diet. Since humans eat about 0.5 kg of food on a dry wt basis daily, and the Recommended Dietary Allowances (RDA) for VE is 8-10 mg/person/day, about 20 mg (relevant range of 5-30 mg) α-tocopherol/kg diet is in the appropriate range for experimental animals (Tappel and Tappel, 1997). In the present study, 15 mg α-tocopherol/kg diet was set in the +VE diet. The diets were divided into several portions, sealed in bags, and stored at −30ºC until use. The food fed to the mice was replaced every day. After 2 weeks of consumption of the indicated diet, the mice were subjected to TBI with X-rays using an X-ray unit (OM-150RS, Tokyo, Japan) at a dose rate of 0.4 Gy/min (140 kV, 9 mA). The beam was filtered through Cu (0.1 mm) and Al (0.2 mm). The mice were anesthetized with pentobarbital, sacrificed 48 h post radiation exposure, then the liver and spleen were immediately removed, frozen, and stored at −80ºC until use.

All of the procedures were performed in accordance with National Institute of Health and Nutrition guidelines for the care and use of laboratory animals.

Analytical methods Vitamin E (α-tocopherol) and vitamin C (dehydroascorbic acid) were extracted and analyzed by HPLC with an electrochemical detector, as described elsewhere (Shin *et al*., 2002). For the GSH analysis, the liver samples (100 mg) were mixed with 0.5 ml of 0.1 N formic acid in tubes, and the resulting mixtures were centrifuged at 17,000 × *g* for 30 min at 4ºC. The GSH in the supernatant was analyzed by the method using *o*-phthalaldehyde (Mokrasch and Teschke, 1984).

The TBARS concentration in the liver was measured using the colorimetric method (Kikugawa *et al*., 1992). The concentration of protein carbonyls was determined by the method of Evans *et al*. (1999).

DNA damage was assessed by a 8OHdG assay. An analysis of 8OHdG in the liver was performed as follows. The DNA that was extracted using a DNA extraction kit was digested with nuclease P1 and acid phosphatase, according to the method of Yamaguchi *et al*. (1996). The 8OHdG and deoxyguanosine (dG) contents in the deoxynucleotide mixture were analyzed by HPLC (Shimadzu LC-6A, Shimadzu Co., Kyoto, Japan) with an ECD (Coulochem II, ESA, Chelmsford, USA) that was equipped with analytical cells (detector 1, 180 mV; detector 2, 380 mV), and an ultraviolet detector (Shimadzu SPD-10A, AT 280 nm). The separating conditions were as follows: column, Beckman Ultrasphere ODS (4.6 × 250 nm); column temperature, 23ºC; mobile phase, 10 mM NaH_2PO_4 containing 8% methanol; flow rate, 1 ml/min. The 8OHdG levels in the DNA are expressed as the number of 8OHdG per 10^5 dG.

Non-heme iron was measured using the method of Torrance and Bothwell (1980). Protein was determined using a BCA protein assay kit (Pierce, Rockford, USA).

Statistical analysis The data are presented as means with standard errors (SEM) for the individual groups. Statistical analyses of the data for the groups were carried out using ANOVA, followed by a post hoc test of Fisher's Protected Least Significant Difference. All of the statistical analyses were performed using the computer program Stat View 4.5 (Abacus Concepts, Inc., Berkeley, USA).

Results

Body, liver, and spleen weights As shown in Table 1, the final body weight was lower in the low-lipid group than in the other groups. The relative liver weight to body weight did not differ among the groups (data not shown). However, the relative spleen weight was lower in the high-lipid group with TBI (Table 2).

Changes in oxidative damage Oxidative damage to DNA, lipids, and protein was evaluated by measuring the levels of 8OHdG, TBARS, and protein carbonyls (Tables 3-5). An increase of 8OHdG by TBI was observed in the high-lipid (−VE) group. These changes, due to TBI, were not detected in the other groups (Table 3). In the groups without TBI, the TBARS concentration tended to be higher in the −VE groups than in the +VE groups, regardless of the level of dietary lipid

Table 1. Body weights of mice fed various diets for 2 weeks and then subjected to TBI with X-rays (g)

	X-rays	Dietary lipid level		
	(Gy)	Low groups	Basal groups	High groups
−VE groups	0	32.7 ± 1.1 (100)*	36.7 ± 0.9 (100)	36.4 ± 0.9 (100)
	2	32.0 ± 0.9 (98)*	35.8 ± 0.6 (98)	35.6 ± 1.3 (98)
+VE groups	0	33.1 ± 1.3 (100)*	36.7 ± 0.8 (100)	36.2 ± 1.1 (100)
	2	32.2 ± 1.0 (97)*	36.5 ± 1.2 (99)	36.2 ± 1.2 (100)

Male ICR mice (4 weeks old) were subjected to TBI at a dose of 0 or 2 Gy, and then sacrificed at 48 h after exposure. Values are means ± SEM for 6 mice per group. The number in parenthesis indicates the % of the 0 Gy value of each lipid-level group. *: Significant dietary lipid effect (versus Basal group with the same TBI dose, $p < 0.05$) †: Significant TBI effect (versus unirradiated (0 Gy) group within the same lipid group, $p<0.05$) !: Significant dietary vitamin E effect (versus Basal group with the same TBI dose, $p<0.05$)

Table 2. Relative spleen weights of mice fed various diets for 2 weeks and then subjected to TBI with X-rays (g/100 g BW)

	X-rays	Dietary lipid level		
	(Gy)	Low groups	Basal groups	High groups
−VE groups	0	0.23 ± 0.01 (100)	0.27 ± 0.03 (100)	0.25 ± 0.03 (100)
	2	0.18 ± 0.02 (78)	0.22 ± 0.02 (82)	0.16 ± 0.02 (64)*†
+VE groups	0	0.26 ± 0.01 (100)	0.25 ± 0.04 (100)	0.23 ± 0.01 (100)
	2	0.21 ± 0.01 (81)	0.20 ± 0.02 (80)	0.16 ± 0.01 (70)†

Refer to Table 1 footnote for details.

Table 3. 8OHdG/10^5 dG in livers of mice fed various diets for 2 weeks and then subjected to TBI with X-rays

	X-rays	Dietary lipid level		
	(Gy)	Low groups	Basal groups	High groups
−VE groups	0	0.98 ± 0.11 (100)	0.96 ± 0.09 (100)	1.37 ± 0.14 (100)*!
	2	1.06 ± 0.09 (108)	0.99 ± 0.06 (103)	1.43 ± 0.12 (104)*
+VE groups	0	1.04 ± 0.10 (100)	0.98 ± 0.08 (100)	1.05 ± 0.12 (100)
	2	1.01 ± 0.09 (97)	1.01 ± 0.07 (103)	1.24 ± 0.10 (118)

Refer to Table 1 footnote for details.

Table 4. TBARS in livers of mice fed various diets for 2 weeks and then subjected to TBI with X-rays (nmol/mg protein)

	X-rays	Dietary lipid level		
	(Gy)	Low groups	Basal groups	High groups
−VE groups	0	0.92 ± 0.05 (100)	0.90 ± 0.06 (100)	1.07 ± 0.08 (100)
	2	1.25 ± 0.04 (136)!	1.13 ± 0.09 (126)!	1.65 ± 0.13 (154)*†!
+VE groups	0	0.79 ± 0.03 (100)	0.83 ± 0.07 (100)	0.93 ± 0.07 (100)
	2	0.82 ± 0.03 (104)	0.81 ± 0.04 (98)	1.24 ± 0.10 (133)*

Refer to Table 1 footnote for details.

(Table 4). In the groups with TBI, the TBARS concentration significantly increased only in the high-lipid (−VE) group. These changes in TBARS, due to TBI, were not detected in the other groups. The contents of protein carbonyls increased in the high-lipid (−VE) group with TBI (Table 5).

Changes in the concentrations of GSH To evaluate the contributions of antioxidants to radiation-induced oxidative damage, the concentrations of GSH, vitamins C and E were analyzed (Table 6-8). In the −VE groups, the concentrations of GSH, vitamins C and E in the high-lipid groups tended to be lower than those in the low- or basal-lipid group. The level of antioxidants was significantly reduced in the high-lipid (−VE) group with TBI.

Changes in the concentrations of non-heme iron The

Table 5. Protein carbonyls in livers of mice fed various diets for 2 weeks and then subjected to TBI with X-rays (nmol/mg protein)

	X-rays	Dietary lipid level		
	(Gy)	Low groups	Basal groups	High groups
−VE groups	0	1.89 ± 0.16 (100)!	1.88 ± 0.13 (100)!	1.71 ± 0.11 (100)!
	2	1.93 ± 0.14 (102)!	1.80 ± 0.16 (96)!	2.18 ± 0.18 (128)*†!
+VE groups	0	1.50 ± 0.09 (100)	1.51 ± 0.15 (100)	1.33 ± 0.06 (100)
	2	1.57 ± 0.16 (105)	1.39 ± 0.07 (92)	1.52 ± 0.08 (114)

Refer to Table 1 footnote for details.

Table 6. GSH in livers of mice fed various diets for 2 weeks and then subjected to TBI with X-rays (nmol/mg protein)

	X-rays	Dietary lipid level		
	(Gy)	Low groups	Basal groups	High groups
−VE groups	0	35.4 ± 3.2 (100)	36.1 ± 2.9 (100)	33.7 ± 3.4 (100)*
	2	34.5 ± 4.7 (103)	35.0 ± 3.2 (97)	31.5 ± 3.3 (94)*!
+VE groups	0	35.1 ± 6.1 (100)	36.5 ± 1.9 (100)	34.3 ± 2.7 (100)
	2	35.0 ± 2.6 (99)	37.9 ± 3.3 (104)	34.9 ± 3.2 (102)

Refer to Table 1 footnote for details.

Table 7. Vitamin C in livers of mice fed various diets for 2 weeks and then subjected to TBI with X-rays (nmol/mg protein)

	X-rays	Dietary lipid level		
	(Gy)	Low groups	Basal groups	High groups
−VE groups	0	24.2 ± 2.1 (100)	25.1 ± 2.6 (100)	20.8 ± 2.1 (100)*
	2	22.1 ± 1.5 (91)	22.9 ± 2.3 (91)	17.8 ± 1.8 (86)*!
+VE groups	0	24.6 ± 1.7 (100)	25.7 ± 1.9 (100)	23.7 ± 2.8 (100)
	2	24.1 ± 3.0 (98)	26.1 ± 3.1 (102)	22.5 ± 1.7 (95)*

Refer to Table 1 footnote for details.

Table 8. Vitamin E in livers of mice fed various diets for 2 weeks and then subjected to TBI with X-rays (pmol/mg protein)

	X-rays	Dietary lipid level		
	(Gy)	Low groups	Basal groups	High groups
−VE groups	0	168 ± 13 (100)!	171 ± 11 (100)!	136 ± 10 (100)*!
	2	142 ± 11 (85)!	148 ± 9 (87)!	97 ± 9 (71)*†!
+VE groups	0	820 ± 46 (100)	835 ± 61 (100)	790 ± 24 (100)
	2	767 ± 28 (94)	758 ± 45 (91)	602 ± 37 (76)†

Refer to Table 1 footnote for details.

concentrations of non-heme iron in the high-lipid (−VE) groups were significantly higher than those in the low- and basal-groups (Table 9).

Discussion

Oxidative damage is the mismatched redox equilibrium between the production of reactive oxygen species (ROS) and the ability of the cell to defend against them. Oxidative damage, therefore, occurs when the production of ROS increases and scavenging of ROS decreases. Ionizing radiation such as X-rays is thought to produce free radicals in the cell. These can cause a number of diseases, and are involved in the detrimental effect of ionizing radiation.

Lipids are the essential components of cell membranes and lipoproteins. Lipid peroxidation is an important effect of radiation on membranes, apart from DNA, are critical targets of radiation action. VE serves a vital role in the scavenging of lipid peroxides, and may often prevent or delay the onset of cancer (Levi *et al.*, 2001).

Decreases in the levels of vitamins C and E in guinea pigs

Table 9 Non-heme iron in livers of mice fed various diets for 2 weeks and then subjected to TBI with X-rays (μg/mg protein)

	X-rays	Dietary lipid level		
	(Gy)	Low groups	Basal groups	High groups
−VE groups	0	2.5 ± 0.1 (100)	2.7 ± 0.4 (100)	3.9 ± 0.4 (100)*!
	2	3.0 ± 0.3 (120)	3.2 ± 0.3 (119)	4.0 ± 0.2 (103)!
+VE groups	0	2.7 ± 0.2 (100)	2.5 ± 0.2 (100)	3.1 ± 0.2 (100)*
	2	3.0 ± 0.2 (111)	2.9 ± 0.6 (116)	3.1 ± 0.4 (100)

Refer to Table 1 footnote for details.

do not cause increases in 8OHdG in DNA (Cadenas *et al.*, 1997). Also, feeding a low VE diet to rats or mice does not induce oxidative DNA damage in the liver or bone marrow (Umegaki *et al.*, 1993; 1997). Cho *et al.* (1995), also reported that there was no VE deficiency or oxidative DNA damage in the livers of rats that were fed fish oil. Moreover, rodent diets that contained adequate amounts of VE did not significantly enhance VE accumulation in mice or rats. Therefore, there were none of the expected effects from dietary VE that were detected in the preliminary experiment. Based on these considerations, this study compared the effects of feeding (+VE) and (−VE) among a 1%, 7%, and 15% lipid diet groups.

DNA is the dominant target of irradiation. DNA damage may produce mutations that cause permanent genetic alterations when the cell replicates its DNA, therefore, increasing the risk of cancer. The present study shows that dietary VE prevented TBI-induced DNA damage in the high-lipid group. In the present study, the consumption of the VE-free diet significantly increased the 8OHdG levels in the DNA from mice that were fed the high-lipid diet with TBI, but these changes were not detected in the DNA from mice that were fed the low- or basal-lipid diet. The contents of protein carbonyls were increased in the high-lipid (−VE) group with TBI. The −VE consumption also significantly increased the TBARS level (a useful marker of oxidative lipid) in mice that were fed a high-lipid diet with TBI. These findings indicate that mice that are fed a high-lipid diet are more susceptible to TBI-induced oxidative damage by (−VE) consumption than mice that are fed a low- or basal-lipid diet.

The induction of oxidative damage in the body is the result of oxidative stress that exceeds the antioxidant capacity, which is dependent on levels of antioxidants and antioxidative enzymes. In this study, low levels of antioxidants, such as vitamins E and C and GSH in the high-lipid group, were related to the susceptibility to TBI-induced oxidative damage.

Iron is a very common metal that is widely utilized by living organisms in a large number of biological processes. Iron has redox potential, and is known to induce oxidative damage in biomolecules (Gordon *et al.*, 1995). In this study, the concentrations of non-heme iron in the high-lipid (−VE) groups were significantly higher than those in the low- and basal-groups.

The spleen is susceptible to ionizing radiation. In the case of TBI at 2 Gy, the decrease in the relative spleen weight was more marked in the high-lipid group, indicating that this group was more susceptible to TBI than the other groups. This suggests that higher levels of radiation-induced oxidative damage occurred in the high-lipid group than in the other groups.

These results suggest that low levels of antioxidants were involved in the appearance of oxidative DNA damage, lipid peroxidation, and protein oxidation in mice that were fed a high-lipid diet with TBI, due to the consumption of a VE -free diet. Interestingly, higher levels of non-heme iron were detected in the high-lipid group than in the low- or basal-lipid group, regardless of the level of VE or TBI.

In the present study, the VE effect on radiation-induced oxidative damage was observed in mice that were fed a high-lipid diet, but not in mice that were fed a low- or basal-lipid diet. Finally, these results suggest that both the low levels of antioxidants and high levels of iron were involved in the appearance of oxidative damage in mice that were fed a high-lipid diet with TBI, due to (−VE) consumption.

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