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Vitamin E Prevents Increase in Oxidative Damage to Lipids and DNA in Liver of ODS Rats Given Total Body X-ray Irradiation

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We examined the effects of dietary vitamin E (VE) on oxidative damage to DNA and lipids in the liver a few days after total body irradiation (TBI). ODS rats, which lack vitamin C synthesis, were fed either a low VE diet (4.3 mg VE/kg) or a basal VE diet (75.6 mg VE/kg) for 5 weeks while vitamin C was supplied in the drinking water. The VE level in the liver of the low VE group was lower and the levels of lipid peroxides were higher compared to those of the basal VE group: the relative levels in the two groups were 1:30 for VE, 18:1 for 4-hydroxynonenal (HNE), and 10:1 for hexanal (HA). The level of 8-hydroxydeoxyguanosine (8OHdG), a marker of oxidative DNA damage, did not differ between the low VE and the basal VE groups. When the rats received TBI at the dose of 3 Gy and were killed on day 6, the levels of HNE, HA and 8OHdG increased by 2.2-, 2-, and 1.5-times, respectively, in the low VE group, but TBI did not cause such increases in the basal VE group. Changes in antioxidative enzymes (glutathione peroxidase, catalase, and Cu/Zn-SOD) in the liver could not explain the different responses of the two diet groups to TBI-induced oxidative damage. The concentrations of vitamin C and glutathione in the liver did not differ between the two groups. These results suggest that dietary VE can prevent the oxidative damage to DNA and lipids in the liver which appear a few days after TBI at dose of 3 Gy.

Keywords: Radiation; Vitamin E; 4-Hydroxynonenal; 8-Hydroxydeoxyguanosine; Antioxidant enzyme

INTRODUCTION

Total body irradiation (TBI) at a dose of about 3 Gy is performed several times preceding bone marrow transplantation to kill the bone marrow cells of the recipient. However, the irradiation also damages non-target cells and/or tissues. In fact, it has been shown that after bone marrow transplantation, the transplant recipients have a significantly elevated risk of developing a new solid cancer.[1] Accordingly, it is important to induce complete damage to bone marrow cells with little damage to the other tissues in the body. Since irradiation of the body results in oxidative stress due to the formation of oxygen radicals,[2] it may be possible to use antioxidants or antioxidative enzymes to control damage due to irradiation, resulting in successful radiotherapy.

In previous studies, we observed that TBI of rats and mice at a dose of about 3 Gy decreased the concentration of vitamin E (VE) and increased the lipid peroxides in bone marrow and plasma.[3,4] However, the changes were delayed for several days in the plasma compared to the bone marrow.[4] Similar delayed onset of lipid peroxidation in the liver and spleen has also been reported by other investigators.[5] Breath ethane, a marker of n-3 fatty acid oxidation, has been shown to increase on day 2 after TBI in humans.[6] We also reported that feeding rats and mice a diet containing insufficient or excess VE did not modify TBI-induced chromosomal damage or lipid peroxidation in the bone marrow.[4,7] In contrast, Przbyszewski *et al.*[8] reported that VE treatment prevents the increase in lipid peroxide in the serum and heart of rats given gamma-irradiation.

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These findings suggest that VE does not prevent oxidative damage to DNA and lipids in bone marrow, but does prevent it in other tissues such as plasma and liver, in which the damage appears a few days after TBI. However, this putative beneficial effect of VE has not been proven yet.

VE is an important antioxidant that prevents lipid peroxidation, and vitamin C also plays an important role in preventing lipid peroxidation.[9] Rats synthesize vitamin C and the synthesis is enhanced when the rats are subjected to oxidative stress.[10,11] This vitamin C synthesis in response to oxidative stress might complicate evaluations of the antioxidant effects of VE in rats. For clarification of the effects of VE, it is therefore better to use ODS rats, which lack vitamin C synthesis, like humans. The type of diet to be used must also be considered: the generally used normal rat diets contain an adequate amount of VE and further addition of VE to the diet would not cause a marked accumulation of VE in the body.[12] The use of such diets would not show a clear effect of VE. Rather, to clarify the effects of dietary VE in rats, the effects of a normal diet and a low VE diet should be compared.

In this study, we fed ODS rats either a low VE diet or a basal VE diet, both based on AIN93G formula,[13] and then examined the TBI-induced oxidation of lipids and DNA in the liver, which appeared a few days after the TBI. We also evaluated the changes of antioxidative enzymes in the liver caused by feeding of the low VE diet and TBI.

MATERIALS AND METHODS

Materials

4-Hydroxy-2-nonenal and acid phosphatase were obtained from Sigma Chemical (St. Louis, MO). Nuclease P1 was obtained from Seikagaku Kogyo (Tokyo, Japan). Tert-butyl hydroperoxide was obtained from Nacalai Tesque (Kyoto, Japan). Other chemicals were purchased from Wako Pure Chemical Ind. (Osaka, Japan).

Experimental Animals

Male ODS rats (4 weeks old) purchased from Japan Clea (Tokyo, Japan) were divided into two groups of 12 rats each: a low VE diet and a basal VE diet group. The composition of the basal VE diet (AIN-93G)[13] was as follows: 52.9% corn starch; 20% vitamin-free casein; 10% sucrose; 5% cellulose; 3.5% mineral mixture (AIN-93G); 1% vitamin mixture (AIN-93G); 0.3% L-cystine; 0.25% choline bitartrate; 0.014% butylated hydroxytoluene; and 7% soybean oil. The low VE diet was prepared by substituting a vitamin mixture lacking VE (AIN-93G) in the basal diet. Thus, the same soybean oil which contained VE as in the basal diet was used to avoid the influence of oxidized oil. The actual concentrations of α-tocopherol in the basal VE diet and the low VE diet were 75.6 and 4.3 mg/kg, respectively. The rats were kept in individual cages with ad libitum access to the diet and drinking water containing 0.1% ascorbic acid throughout the experimental period. After 5 weeks, six rats of each group were subjected to TBI with X-rays at the dose of 3 Gy in a chamber without anesthesia and they were sacrificed on day 6 after the TBI. The irradiation was performed using a soft X-ray unit (OM-150RS, Ohmic, Tokyo, Japan) at a dose rate of 0.4 Gy/min (140 KV, 9 mA) with Cu (0.5 mm) and Al (0.1 mm) filters. Rats with or without TBI treatment were anesthetized with pentobarbital and sacrificed. The organs were immediately removed, frozen in liquid nitrogen and stored at −80°C until use.

Analytical Methods

Ascorbic acid and α-tocopherol were extracted and analyzed by HPLC with an electrochemical detector (ECD) as described elsewhere.[3,14] Glutathione was measured by the method of Mokrasch and Teschke[15] using o-phthalaldehyde. 4-Hydroxynonenal and hexanal were measured by HPLC with a fluorescence detector as reported previously.[4]

Analysis of 8OHdG was performed as follows. DNA extracted using a DNA extraction kit was digested with P1 nuclease and acid phosphatase according to the method of Yamaguchi et al.[16] The 8OHdG and deoxyguanosine (dG) contents in the deoxynucleotide mixture were analyzed by HPLC (Shimadzu LC10AD, Shimazdu, Kyoto, Japan) with an ECD (Coulochem II, ESA, MA, USA) equipped with analytical cells (detector 1, 180 mV; detector 2, 380 mV) and an ultraviolet detector (Shimadzu SPD-10A, at 280 nm). The separation conditions were as follows: column, Beckman Ultrasphere ODS (4.6 × 250 mm); column temperature, 23°C; mobile phase, 10 mM NaH_2PO_4 containing 8% methanol; the flow rate, 1 ml/min. The 8OHdG levels in the DNA are expressed as the number of 8OHdG per 10^5 dG.

Total RNA was prepared from the liver of each rat using TRIzol reagent (Life Technologies Inc., Grand Island, NY, USA), and the concentration was determined using a spectrophotometer. Total RNA was loaded at 15 μg per lane on a 1% agarose/formaldehyde gel and transferred onto a nylon membrane (Hybond N^+, Amersham, Arlington Heights, IL, USA). Membranes were hybridized with [α-^{32}P]dCTP-labeled cDNA probes for rat glutathione peroxidase (GPx), rat catalase (CAT), rat Cu/Zn-SOD (superoxide dismutase) and rat Mn-SOD. The blots were exposed to X-ray film (Fuji Photo Film, Tokyo, Japan) at −80°C using an

intensifying screen. Signals were quantified with a FUJIX Bio-Imaging Analyzer BAS2000 (Fuji Photo Film).

The activities of GPx were measured by monitoring NADPH oxidation at 340 nm using tert-butyl hydroperoxide and hydrogen peroxide as substrates.[17] CAT activity was measured by the decrease in H_2O_2 concentration.[18] SOD enzyme activity was measured by the NBT (nitroblue tetrazolium) reduction method using a SOD activity detection kit (Wako Pure Chemical Ind., Japan). Protein was determined using a BCA protein assay kit (Pierce, Rockford, IL, USA).

Statistical Analysis

Data are presented as the mean ± SE. Comparison between groups was carried out using ANOVA (one-way) followed by a post hoc test (Fisher's protected least significant difference). A P value less than 0.05 was considered significant. All statistical analyses were performed using the computer program Stat View 4.5 (Abacus Concepts, Inc., Berkeley, CA, USA).

RESULTS

Body and liver weights did not significantly differ among the groups (Table I). In both diet groups, the spleen weight decreased in rats which were given TBI at a dose of 3 Gy and sacrificed on day 6 after the TBI. In the groups with TBI, the concentrations of HNE and hexanal in the liver were higher by 18- and 10-fold, respectively, in the low VE group than in the basal VE group, indicating that lipid peroxidation was increased by consumption of the low VE diet (Fig. 1). The level of 8OHdG in DNA, a marker of oxidative DNA damage, was not different between the low VE and basal VE groups without TBI. The concentrations of HNE, hexanal, and 8OHdG in the liver in the low VE diet group were increased by 2.2-, 2.1-, and 1.6-fold, respectively with TBI. In contrast, no increases in HNE, hexanal or 8OHdG were caused by TBI in the basal VE group.

The concentrations of ascorbic acid, dehydroascorbic acid, VE and glutathione (reduced form, GSH; oxidized form, GSSG) in the liver are shown in Table II. The concentration of VE in the low VE group was only 1/30 of that in the basal VE group. The VE concentration was decreased by 13% by TBI in the basal VE group, but was not decreased in the low VE group. Neither consumption of the low VE diet nor TBI caused changes in the levels of ascorbic acid, glutathione or dehydroascorbic acid, or in the GSSG/GSH ratio.

The activities of various antioxidative enzymes (CAT, GPx, Mn-SOD, Cu/Zn-SOD) are shown in Table III. In the low VE group, CAT activity was lower and SOD activity was higher compared to the levels in the basal VE group. GPx activity tended to be increased by TBI. Like the enzyme activities, the levels of the m-RNAs of antioxidative enzymes were not markedly changed by either consumption of the low VE diet or TBI (data not shown).

DISCUSSION

TBI induces oxidative damage to biomolecules in various tissues, but in plasma and liver the occurrence of such damage seems to be delayed until several days after the TBI.[4–6,8] In this study, we examined the effect of dietary VE on oxidative damage to DNA and lipids in the liver of rats on day 6 after TBI. Rats synthesize vitamin C in their body, and the synthesis increases when they are subjected to oxidative stress.[11] Moreover, the generally used rodent diets contain adequate amounts of VE, and further addition of VE to the diets does not markedly enhance the accumulation of VE in the bodies of the rats, and consequently the expected effects of dietary VE are blunted.[12] Based on these considerations, in the present study we used ODS rats, which can not synthesize vitamin C, and compared the effects of feeding a basal VE diet and a low VE diet. As shown in Table I, the VE level in the liver of the low VE group was 1/30 of that of the basal VE group, suggesting that these experimental conditions were appropriate for evaluating the effects of VE on TBI-induced oxidative stress.

In the low VE group, the concentrations of HNE and HA, and the level of 8OHdG in DNA were all

TABLE I Body weight and relative tissue weight in ODS rats fed either basal VE diet or low VE diet and with or without TBI at 3 Gy*

	Basal VE diet†		Low VE diet†	
	Control	+TBI	Control	+TBI
Body weight (g)	282 ± 9.1^{b}	252 ± 7.9^{a}	271 ± 7.0^{ab}	258 ± 4.5^{a}
Relative tissue weight (% of body weight)				
Liver	3.89 ± 0.03^{a}	4.21 ± 0.09^{b}	3.82 ± 0.06^{a}	4.18 ± 0.06^{b}
Spleen	0.21 ± 0.006^{a}	0.10 ± 0.003^{b}	0.21 ± 0.006^{a}	0.11 ± 0.002^{b}

* Male ODS rats (4-weeks-old) fed either a basal VE diet or a low VE diet for 6 weeks were subjected or not subjected to TBI with X-rays at a dose of 3 Gy, and then sacrificed on day 6. Data are mean ± SEM for 6 rats.
† Values with different superscripts within the same row are significantly different ($P < 0.05$).

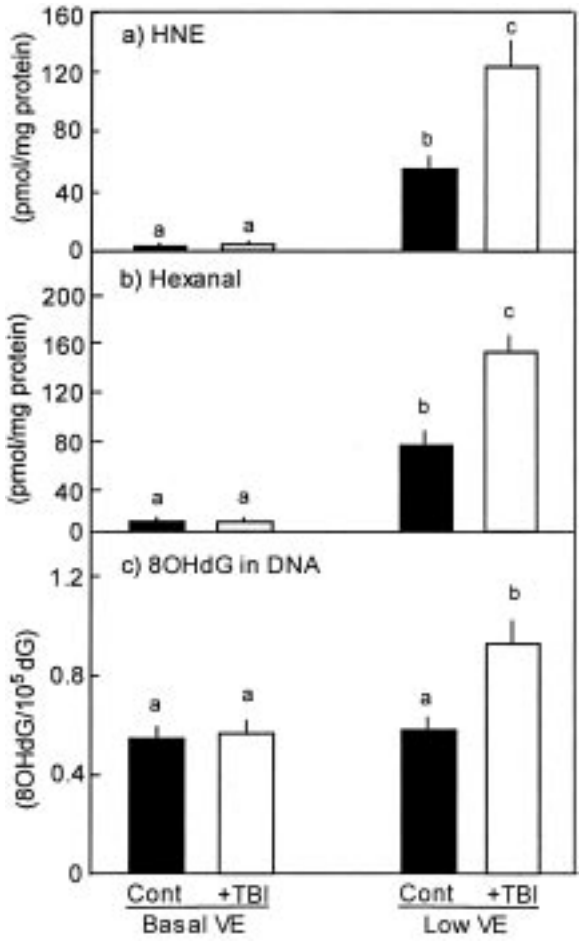

FIGURE 1 Levels of HNE, hexanal and 8OHdG in liver from ODS rats fed either basal VE diet or low VE diet and with or without TBI at 3 Gy. Values are means for six rats. Values not sharing a letter are significantly different ($P < 0.05$).

increased by TBI (Fig. 1). In contrast, no such increases in the markers of oxidative damage were detected in the basal VE group after TBI. Several factors are involved in the occurrence of oxidative damage, including antioxidative enzymes and antioxidants. In this study, the levels of antioxidative enzymes (CAT, GPx, Cu/Zn-SOD) were not markedly changed by TBI in either diet group (Table III). The levels of antioxidants other than VE were not significantly changed by either consumption of the low VE diet or TBI (Table II). Therefore, it is reasonable to conclude that the different susceptibility to TBI-induced oxidative damage of DNA and lipids between the low VE and basal VE groups was related to the concentration of VE. The decrease of VE in the liver of the basal VE group after TBI (Table II) may have been the result of scavenging radicals formed due to TBI. These results suggest that VE provides effective protection against TBI-induced oxidative damage in the liver that is detected a few days after the irradiation.

Generally, oxidative damage of biomolecules takes place as the result of a predominance of oxidative stress over antioxidant activities. However, feeding of a low VE diet alone to ODS rats induced oxidative damage to lipids in the liver, but not to DNA, when the damage was evaluated by the increases in HNE or HA, and in 8OHdG, respectively (Fig. 1). This finding indicates that susceptibility toward oxidative stress is different between DNA and lipids, and that DNA is better protected against oxidative stress. The failure to detect DNA damage by feeding of the low VE diet alone is consistent with the results of our previous studies, in which DNA damage was evaluated by measuring 8OHdG in the hepatic DNA of rats or by a micronucleus assay in the bone marrow of mice.[7,19] It has been shown that nuclear DNA is efficiently repaired[20] and that the repair system for 8OHdG in DNA is activated when the body undergoes oxidative stress.[21] Unlike X-ray irradiation, the consumption of a low VE diet gradually induces oxidative stress, and thus induction of the 8OHdG repair system, if it takes place, is enough to protect DNA against the oxidative stress. In contrast, 3 Gy of TBI suddenly induces marked oxidative stress in the body. HNE has been shown to be highly toxic and to inactivate various enzymes.[22,23] In this study, the concentration of HNE in the liver of the low VE group was 18-times higher without TBI and 34-times higher with TBI, respectively, than that in the liver of the basal VE group without and with TBI (Fig. 1). This high concentration of HNE in the low VE group

TABLE II Concentrations of vitamin C, vitamin E and glutathione in the liver of in ODS rats fed either basal VE diet or low VE diet and with or without TBI at 3 Gy*

	Basal VE diet†		Low VE diet†	
	Control	+TBI	Control	+TBI
Ascorbic acid (nmol/mg protein)	4.64 ± 0.33	4.16 ± 0.36	4.61 ± 0.40	4.83 ± 0.22
Dehydroascorbic acid (nmol/mg protein)	0.36 ± 0.07	0.27 ± 0.09	0.25 ± 0.04	0.25 ± 0.02
Vitamin E (pmol/mg protein)	463 ± 21.3[b]	403 ± 33.3[c]	14.0 ± 1.19[a]	13.8 ± 0.66[a]
Glutathione‡ (GSH+GSSG) (nmol/mg protein)	48.0 ± 1.3	43.4 ± 2.7	47.9 ± 2.3	51.1 ± 1.6
GSSG/GSH (%)	5.94 ± 0.15	5.83 ± 0.24	5.88 ± 0.14	5.93 ± 0.10

* Male ODS rats (4-weeks-old) fed either a basal VE diet or a low VE diet for 6 weeks were subjected or not subjected to TBI with X-rays at a dose of 3 Gy, and then sacrificed on day 6. Data are mean±SEM for 6 rats.
† Values with different superscripts within the same line are significantly different ($P < 0.05$).
‡ Glutathione: reduced form (GSH) and oxidized form (GSSG).

TABLE III Activities of antioxidant enzymes in the liver of ODS rats fed either basal VE diet or low VE diet and with or without TBI at 3 Gy*

	Basal VE diet†		Low VE diet†	
	Control	+TBI	Control	+TBI
Catalase (units / mg protein)	292 ± 9.0[a]	298 ± 13.1[a]	251 ± 9.4[b]	295 ± 8.5[a]
Glutathione peroxidase (units / mg protein)				
Substrate t-BuOOH	1034 ± 22.7	1119 ± 38.3	1037 ± 21.9	1098 ± 37.8
Substrate H_2O_2	440 ± 9.7	496 ± 29.7	463 ± 18.9	481 ± 22.6
SOD (units / mgP)	365 ± 5.7[a]	373 ± 7.8[a]	396 ± 4.1[b]	362 ± 9.5[ab]

* Male ODS rats (4-weeks-old) fed either a basal VE diet or a low VE diet for 6 weeks were subjected or not subjected to TBI with X-rays at a dose of 3 Gy, and then sacrificed on day 6. Data are mean ± SEM for 6 rats.
† Values with different superscripts within the same line are significantly different ($P < 0.05$).

with TBI may inactivate 8OHdG repair enzymes, resulting in the high level of 8OHdG in this group. In situations, where oxidative stress is suddenly induced, the antioxidative enzyme system in the body is not likely to operate immediately, and thus dietary VE seems to act more efficiently by preventing increases in HNE as well as 8OHdG.

Preceding bone marrow transplantation, TBI is performed several times at a dose of about 3 Gy to kill the bone marrow cells of the recipient. This TBI damages not only abnormal bone marrow cells but also normal tissue and / or cells. In fact, an increase in lipid peroxide and a decrease in antioxidant vitamins have been reported in humans who received TBI.[24] It has also been reported that a transplant patient with a high concentration of lipid peroxides in the plasma underwent an unsuccessful transplantation[25] and that there is a high risk of new cancer in patients receiving bone marrow transplantation.[1] In our previous study, we showed that feeding rats and mice a diet containing insufficient or excess VE did not modify TBI-induced chromosomal damage or lipid peroxidation in the bone marrow.[4,7] On the other hand, the present study showed that dietary VE prevented TBI-induced oxidative damage in DNA and lipids of the liver. These findings suggest that VE treatment might improve the results of bone marrow transplantation, because VE treatment before transplantation does not interfere with the killing of abnormal bone marrow cells of the recipient but prevents oxidative damage in other tissues. Przybyszewski *et al.*[8] also reported that increases in lipid peroxides in the serum and heart about 4 days after fractionated gamma-irradiation was diminished by VE treatment. Recent evidence indicates that oxidative damage to DNA is related to various cancers in the body.[26] Accordingly, the prevention of the TBI-induced increase in 8OHdG in hepatic DNA detected here in the low VE diet group is noteworthy. These results strongly suggest that adequate intake of VE in the daily diet is important for successful bone marrow transplantation.

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