Adequate Intakes of Vitamin E and Protein Prevent Increases of Oxidative Damage to DNA, Lipids, and Protein Induced by Total Body Irradiation in Mice

Sung Jae Shin and Kazuhiko Yamada

Abstract: We examined the influence of the level of dietary protein or vitamin E (VE) on oxidative damage to DNA, lipids, and protein in the liver after total body irradiation (TBI) with X-rays at 1 or 4 Gy. Levels of 8-hydroxydeoxyguanosine, thiobarbituric acid-reactive substances, and protein carbonyls in the liver did not differ among the groups that did not receive TBI. However, oxidative damage to lipids and protein was increased by TBI only in the 1% protein group. DNA damage, lipid peroxidation, or protein oxidation in the liver was increased by TBI in a dose-dependent manner, and the damage was consistently higher in the 1% than in the 20% protein group. In the 1% protein group, a greater decrease in relative spleen weight by TBI was also observed. Concentrations of antioxidants (vitamins C and E and glutathione) in the liver were lower and the concentration of nonheme iron in the liver was higher in the 1% than in the 20% protein group. Mice fed a 1% protein diet became susceptible to TBI-induced oxidative damage, and decreases in antioxidant levels and an increase in iron level were involved in the mechanism of this susceptibility. These results suggest that dietary VE and protein can prevent oxidative damage to DNA, lipid, and protein in mice subjected to TBI. Consumption of a VE-free diet significantly increased 8-hydroxydeoxyguanosine levels in DNA from mice fed the 1% protein diet with TBI, but such changes were not detected in DNA from mice fed the 20% protein diet.

Introduction

Total body irradiation (TBI) is performed before bone marrow transplantation to kill the bone marrow cells of the recipient. However, the irradiation also damages nontarget cells or tissues. 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 the bone marrow cells with as little damage as possible to the other tissues in the body. Because irradiation of the body results in oxidative stress due to the formation of oxygen radicals (2), it may be possible to use antioxidants to control radiation-induced oxidative damage, resulting in successful radiotherapy.

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. Vitamin E (VE), an important lipid-soluble antioxidant, prevents the formation of lipid peroxides, which have been shown to induce oxidative damage of DNA in vitro (3). There have been many studies on the relation between various levels of VE and oxidative damage in the body (4,5). 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. In a preliminary experiment, we found that the 8-hydroxydeoxyguanosine (8-OHdG) level did not differ between the low-VE and basal-VE groups. Mouri et al. (6) reported that the content of VE in red blood cells tended to decline when the dietary casein level was <10% in rats. Huang and Shaw (7) also showed a reduced bioavailability of VE in rats fed a low-protein diet (8%). Using 3H-labeled α-tocopherol, Rajaram et al. (8) showed reduced absorption, transport, and distribution of VE in rats fed a 5% casein diet. Therefore, we hypothesized that, in mice fed a low-protein diet with TBI, enhanced oxidative damage, especially DNA damage, could occur due to VE deficiency or a low level of VE.

We fed mice a 1% protein diet without VE, a 1% protein diet with VE, a 20% protein diet without VE, or a 20% protein diet with VE based on the AIN-93G formula (9). Animals were subjected to TBI with X-rays at 0, 1, or 4 Gy, and then we examined the TBI-induced oxidative damage to DNA, lipids, and protein in the liver. Furthermore, changes in the nonheme iron concentration were evaluated, because iron is involved in oxidative damage (10,11).

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

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Materials and Methods

Materials

Cornstarch, vitamin-free casein, cellulose, mineral mixture (AIN-93G) and VE-free vitamin mixture (AIN-93G), and α-tocopherol acetate were purchased from Oriental Yeast (Tokyo, Japan). Other chemicals were obtained from Wako Pure Chemical Industries (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 of 23 ± 1°C and a 12:12-h light-dark cycle. Various diets, i.e., 1% protein without VE (1% protein – VE), 1% protein with VE (15 mg α-tocopherol acetate/kg diet; 1% protein + VE), 20% protein without VE (20% protein – VE), or 20% protein with VE (15 mg α-tocopherol acetate/kg diet; 20% protein + VE), were prepared on the basis of the AIN-93G formula (9). The mice were fed a 20% protein diet for 3 days to allow them to adapt to the semipurified diet and then divided into 12 groups (6 mice/group), each of which was fed one of the four different diets and subjected to one of three irradiation doses (0, 1, or 4 Gy). Throughout the study, the mice were given free access to food and water. The diets were divided into several portions, sealed in bags, and stored at −30°C until use. The food fed to the mice was replaced each day. Daily food intakes in the 1% and 20% protein groups were 4.2 and 4.1 g/mouse, respectively. Minerals and vitamins for all diets were set at 35 and 10 g/kg, respectively. Levels of dietary protein, vitamins, minerals, and other nutrients were based on the AIN-93G diet (9). To make all diets isocaloric, the 1% protein diet was prepared by substituting the carbohydrates for a protein-deficient portion compared with the 20% protein diet. It has been reported that the ratio of protein in human diets, in the case of the Japanese population, is ~28%, although it differs depending on such factors as age and gender (12). Because humans eat ~0.5 kg of food on a dry weight basis daily and the Recommended Dietary Allowance for VE is 8–10 mg/day, ~20 mg (relevant range 5–30 mg) α-tocopherol/kg diet is in the appropriate range for experimental animals (13). In the present study, 15 mg α-tocopherol/kg diet was set in the +VE diet. After 2 wk of consumption of the indicated diet, a soft X-ray unit (model OM-150RS, Ohnic, Tokyo, Japan) was used to subject the mice to TBI with X-rays at a dose rate of 0.4 Gy/min (140 kV, 9 mA). The irradiation doses used in the present study were set at 0, 1, or 4 Gy, because TBI at ~3 Gy is performed before bone marrow transplantation to kill the bone marrow cells of the recipient (1). The beam was filtered through Cu (0.1 mm) and Al (0.2 mm).

The mice were anesthetized with pentobarbital sodium, and then the liver and spleen were immediately removed, frozen, and stored at −80°C until use.

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

Analytic Methods

VE (α-tocopherol) and vitamin C (dehydroascorbic acid) were extracted and analyzed by high-performance liquid chromatography with an electrochemical detector (Shiseido, Tokyo, Japan), as described elsewhere (14). For analysis of GSH, 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 4°C; the GSH in the supernatant was analyzed by the method of Mokrasch and Teschke (15) using o-phthaldehyde.

The concentration of thiobarbituric-reactive substances (TBARS) in the liver was measured using a colorimetric method (16). The concentration of protein carbonyls was determined by the method of Evans et al. (17).

DNA damage was assessed by 8-OHdG assay. Analysis of 8-OHdG in the liver was performed as follows. DNA extracted using a DNA extraction kit was digested with nuclease P1 and acid phosphatase according to the method of Yamaguchi et al. (18). The 8-OHdG and deoxyguanosine contents in the deoxynucleotide mixture were analyzed by high-performance liquid chromatography (model LC10AD, Shimadzu, Kyoto, Japan) with an electrochemical detector (Coulochem II, ESA, Chelmsford, MA) equipped with analytical cells (Detector 1, 180 mV; Detector 2, 380 mV) and an ultraviolet detector (model SPD-10A, Shimadzu; AT 280 nm). The separating conditions were as follows: Beckman Ultrasphere ODS column (4.6 × 250 nm), column temperature of 23°C, mobile phase of 10 mM NaH2PO4 containing 8% methanol, and flow rate of 1 l/min. The 8-OHdG levels in the DNA are expressed as the number of 8-OHdG per 10^9 deoxyguanosine.

Nonheme iron was measured using the method of Torrance and Bothwell (19). Protein was determined using a bicinchoninic acid protein assay kit (Pierce, Rockford, IL).

Statistical Analysis

Values are means ± SE. Statistical analyses of the data for the groups were carried out using analysis of variance followed by a post hoc test of Fisher’s protected least significant difference. All statistical analyses were performed using the computer program Stat View 4.5 (Abacus Concepts, Berkeley, CA).

Results

Body, Liver, and Spleen Weights

The final body and spleen weights relative to the body weight were lower in the 1% than in the 20% protein groups. However, the weight of the liver relative to the body weight did not differ among the groups. The weights were unaffected by the VE content of the diets (data not shown).

Changes in Oxidative Damage

Oxidative damage to DNA, lipids, and proteins was evaluated by measuring the levels of 8-OHdG, TBARS, and protein carbonyls (Tables 1–3). In the +VE groups, no increase
in 8-OHdG by TBI was observed, although the 1% protein group showed a slightly higher level than the 20% protein group (Table 1). In the –VE groups, the 8-OHdG levels were significantly increased by TBI in the 1% protein group.

In the groups without TBI, the concentration of TBARS was higher in the –VE than in the +VE groups, regardless of the level of dietary protein (Table 2). In the groups with TBI, the concentration of TBARS increased in an irradiation dose-dependent manner only in the 1% protein – VE group, and such changes in TBARS due to TBI were not detected in the other groups (Table 2). The protein carbonyl contents were higher in the 1% than in the 20% protein groups, especially the 20% protein + VE group (Table 3). Similar to the TBARS, increases in the protein carbonyl contents due to TBI were detected only in the 1% protein groups.

Changes in the Concentrations of Antioxidants

To evaluate the contributions of antioxidants to radiation-induced oxidative damage, the concentrations of vitamins C and E and GSH were analyzed (Tables 4–6). In the –VE groups, the concentrations of vitamins C and E and GSH were significantly lower in the 1% than in the 20% protein group without TBI (Table 4). In the 1% protein group, the concentration of VE was reduced by 4 Gy of TBI.

### Table 1. 8-OHdG per 10⁵ dG in Liver of Mice Fed for 2 Weeks After TBI<br><br>Table 2. TBARS in Liver of Mice Fed for 2 Weeks After TBI<br><br>Table 3. Protein Carbonyls in Liver of Mice Fed for 2 Weeks After TBI<br><br>Table 4. Vitamin C in Liver of Mice Fed for 2 Weeks After TBI
in the –VE and +VE groups. GSH was significantly reduced in the 1% protein – VE group.

Changes in the Concentrations of Nonheme Iron

The concentrations of nonheme iron were significantly higher in the 1% than in the 20% protein groups, regardless of TBI (Table 7). The concentration of nonheme iron was not markedly changed by TBI.

Discussion

Oxidative damage is the mismatched redox equilibrium between the production of reactive oxygen species (ROS) and the ability of the cell to defend itself against ROS (20). Oxidative damage thus occurs when the production of ROS increases and scavenging of ROS decreases. Ionizing radiation, such as X-rays, is believed to produce ROS in the cell, and this in turn attacks DNA and degrades it. ROS produced by ionizing radiation have been shown to damage DNA, resulting in various cancers (3,4).

It has been shown that decreases in the levels of vitamins C and E in guinea pigs do not cause increases in 8-OHdG in DNA (21). It has also been reported that feeding a low-VE diet to rats or mice does not induce oxidative DNA damage in the liver or bone marrow (22,23). Cho et al. (24) also reported the lack of any effect of a VE deficiency on oxidative DNA damage in the liver of rats fed fish oil (24). Moreover, rodent diets containing adequate amounts of VE do not significantly enhance the VE accumulation in mice or rats. Therefore, we could not detect any of the expected effects from dietary VE in a preliminary experiment. On the basis of these considerations, in this study we compared the effects of feeding +VE and -VE between a 1% and a 20% protein diet group.

Table 6. GSH in Liver of Mice Fed for 2 Weeks After TBIa,b

<table>
<thead>
<tr>
<th>X-Ray Dose, Gy</th>
<th>Dietary Protein</th>
<th>1%</th>
<th>20%</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>–VE</td>
<td>256 ± 41 (100)*,‡ 195 ± 18 (100)‡</td>
<td>191 ± 52 (75)*,‡ 146 ± 7 (75)‡</td>
</tr>
<tr>
<td></td>
<td>+VE</td>
<td>151 ± 19 (59)*,‡ 155 ± 18 (80)‡</td>
<td>439 ± 74 (33)*,‡ 740 ± 88 (73)</td>
</tr>
</tbody>
</table>

a: Values are means ± SE in nmol/mg protein; values in parentheses are percentages for 0-Gy value of each protein level.
b: Statistical significance is as follows: *, significant effect of dietary protein vs. 20% group with the same TBI dose, P < 0.05; †, significant TBI effect vs. unirradiated (0-Gy) group within the same protein group, P < 0.05; ‡, significant effect of dietary VE vs. +VE group with the same TBI dose, P < 0.05.

Table 7. Nonheme Iron in Liver of Mice Fed for 2 Weeks After TBIa,b

<table>
<thead>
<tr>
<th>X-Ray Dose, Gy</th>
<th>Dietary Protein</th>
<th>1%</th>
<th>20%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>–VE</td>
<td>9.8 ± 0.7 (100)* 5.6 ± 0.4 (100)</td>
<td>11.8 ± 1.1 (120)* 4.3 ± 0.4 (76)</td>
</tr>
<tr>
<td></td>
<td>+VE</td>
<td>10.7 ± 0.3 (109)* 4.6 ± 0.3 (82)</td>
<td>11.4 ± 0.3 (114)* 5.7 ± 0.6 (118)</td>
</tr>
</tbody>
</table>

a: Values are means ± SE in nmol/mg protein; values in parentheses are percentages for 0-Gy value of each protein level.
b: Statistical significance is as follows: *, significant effect of dietary protein vs. 20% group with the same TBI dose, P < 0.05.
liver microsomal detoxification enzymes' activities and inducibility were shown to be lowered by low-protein diets (25).

In this study, the low levels of antioxidants such as vitamins C and E and GSH in the 1% protein group may have been related to an elevated susceptibility to TBI-induced oxidative damage. In particular, the lower levels of antioxidants were observed in the 1% protein – VE group. In the present study, we could not clarify the mechanism of the decrease in antioxidants in the 1% protein group. However, several studies have shown changes in VE levels and oxidative damage in lipids in rats fed different protein diets. Moura et al. (6) reported that the content of VE in red blood cells tended to decline when the dietary casein level was <10% in the rats. They further reported that a low protein intake may make it difficult to maintain an adequate cellular α-tocopherol level due to a probable decrease in cellular α-tocopherol-binding protein. Huang and Shaw (7) also showed a reduced bioavailability of VE in rats fed a low-protein diet (8%). Using 3H-labeled α-tocopherol, Rajaram et al. (8) showed lowered absorption, transport, and distribution of VE in rats fed a 5% casein diet. These findings are in agreement with our present findings and suggest that consumption of a low-protein diet, in particular, a low-protein diet devoid of VE, causes sensitivity to oxidative damage due to a reduced availability of antioxidants.

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 (11,12). Increases in the concentration of iron and saturation of iron-binding capacity in the plasma have been reported in human patients receiving TBI for bone marrow transplantation (26). In this study, the nonheme iron levels were also consistently higher in the 1% than in the 20% protein group, and the high levels of nonheme iron in the 1% protein group may have been related to an elevated susceptibility to TBI-induced oxidative damage. However, nonheme iron was unaffected by the level of VE. The clarification of increased iron in mice fed a low-protein diet is very important to control TBI-induced oxidative damage, and further study is needed.

Our results suggested that low levels of antioxidants were involved in the appearance of oxidative DNA damage, lipid peroxidation, and protein oxidation in mice fed a low-protein diet with TBI due to consumption of a VE-free diet. Interestingly, higher levels of nonheme iron were detected in the 1% than in the 20% protein group, regardless of the level of VE or TBI.

DNA is the dominant target of irradiation, and DNA damage may produce mutations that cause permanent genetic alterations when the cell replicates its DNA and, thus, increases risk of cancer. It has been reported that a transplant patient with a high concentration of lipid peroxides in the plasma underwent an unsuccessful transplantation (27) and that there is a high risk of new cancer developing in patients receiving bone marrow transplantation (1). The present study showed that dietary VE prevented TBI-induced DNA damage, lipid peroxidation, and protein oxidation in the 1% protein group, and these findings suggest that VE or protein treatment might improve the results of bone marrow transplantation, because VE or protein 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. (28) also reported that increases in lipid peroxides in the serum and heart after γ-irradiation were diminished by VE treatment. Our results, therefore, support those of a recent study indicating correlations between a low level of vitamin consumption and subsequent risk of cancer (29).

In conclusion, our findings suggested that adequate VE and protein intakes could prevent TBI-induced oxidative damage and are important as potential cotherapy for successful bone marrow transplantation with respect to reducing the risk of secondary tumors.

Acknowledgments and Notes

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