Influence of Dietary Vitamin E on the
8-Hydroxydeoxyguanosine Levels
in Rat Liver DNA

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(Received March 22, 1993)

Summary Rats were fed either a low vitamin E (VE) diet (−VE), a basal VE diet (+VE, 4.5 mg%), or a high VE diet (+VE, 45 mg%) for 7 weeks. VE content, oxidative DNA damage and lipid peroxide levels in their livers were measured. When purified lard was used as a dietary fat, VE content decreased in the low VE group to one-thirtieth of that in the basal VE group; in the high VE group it increased to 4.5-fold that in the basal VE group. Corresponding to the VE levels, lipid peroxide levels increased to 2.7-fold in the low VE group and decreased to two-thirds in the high VE group. The level of 8-hydroxydeoxyguanosine (8-OHdG) in DNA, a marker of oxidative DNA damage, was about 0.6 per 10^5 deoxyguanosine in the basal VE group and comparable values were found in the low VE and the high VE groups. When either soybean oil or safflower oil was used as a dietary fat, VE content and lipid peroxide levels in the liver were also markedly changed in both the low VE group and the high VE group. 8-OHdG levels in DNA of the low VE with safflower oil group tended to be higher than that of the basal and high VE groups. However, no significant difference was observed among them. These results suggest that the change in VE has little influence on the level of oxidative DNA damage in the liver.

Key Words lipid peroxidation, vitamin E, 8-hydroxydeoxyguanosine, DNA damage, rat liver

Increasing evidence has indicated that oxidative DNA damage is associated with cancer and aging (1–3). This damage seems to be produced by the oxidative stress in the body due to decreased levels of antioxidants such as vitamins and enzymes. As a marker of oxidative DNA damage, 8-hydroxydeoxyguanosine (8-OHdG) in DNA and urine has been analyzed (4–8).

Oxidative DNA damage has been observed when chemical carcinogens, a choline-deficient diet or radiation have been administered, and such conditions have increased lipid peroxide levels as well (4–9). Lipid peroxidation products and their decomposition products are known to be highly reactive, and interact with DNA.
In contrast, it is well known that vitamin E (VE) is a strong antioxidant and the decrease of VE in tissue enhances lipid peroxide levels therein. In addition, it has been reported that VE prevented cancer normally induced by chemical carcinogens (11). These findings indicate that the change of VE content in tissue may have some influence not only on lipid peroxide levels, but also on the level of oxidative DNA damage, thereby modifying the incidence of cancer and the process of aging. At the same time, little is known whether VE content itself actually influences the level of oxidative DNA damage in the tissue or not.

In this study, we fed rats either a low VE diet, a basal VE diet, or a high VE diet for 7 weeks, and examined the changes in VE content, and the levels of lipid peroxides and oxidative DNA damage in the liver. In addition, the effects of dietary fats with different polyunsaturated fatty acids on these changes were also examined.

**METHODS**

1) **Materials.** Proteinase K and RNase T1 were obtained from Boehringer-Mannheim. RNase A, alkaline phosphatase and deoxyguanosine (dG) were purchased from Sigma, and Nuclease P1 was purchased from Yamasa Shoyu Co., Ltd. (Tokyo), and VE (DL-α-tocopheryl acetate) was purchased from Kawai Pharmaceutical Science Co. (Tokyo). Standard 8-hydroxydeoxyguanosine (8-OHdG) was kindly supplied by Dr. H. Kasai, University of Occupational and Environmental Health.

2) **Diet and animals.** Purified lard, VE-deficient soybean oil and safflower oil were used as dietary fats. VE in soybean oil and safflower oil was eliminated according to the method of Mohri et al. (12). Major fatty acid components of the fats were: C16 (29%), C18 (14%), C18:1 (42%), and C18:2 (9%) in lard; C16 (10%), C18:1 (23%), C18:2 (55%), and C18:3 (10%) in soybean oil; C16 (7%), C18:1 (12%), and C18:2 (77%) in safflower oil. Low VE diet, basal VE diet and high VE diet were prepared from one of the three fats. As a whole, 9 kinds of semi-synthetic diets were made. The composition of low VE diet was: 50% sucrose, 20% casein, 15% cornstarch, 5% VE eliminated fat, 5% cellulose, 3.5% salt mixture (AIN76), 1% vitamin mixture (AIN76) without VE, 0.3% dl-methionine, 0.2% choline bitartrate. Basal VE diet and high VE diet were prepared by adding 4.5 and 45 mg% of VE to the low VE diet.

Male Sprague-Dawley rats (4-week-old) purchased from Japan Clea (Tokyo, Japan) were fed one of the 9 semi-synthetic diets, as described above, for 7 weeks. The rats were given free access to the diets throughout the experimental period. Then after overnight fasting, rats were anesthetized with ether, and killed by an incision to the abdominal aorta. The liver was immediately removed, rinsed with saline, and stored at −80°C until the analysis.

3) **Measurement of 8-OHdG.** The whole DNA in the liver was extracted by the method of Gupta (13). Portions of the isolated DNA were digested to
nucleoside by Nuclease P1 and alkaline phosphatase according to the method of Kasai et al. (8). Contents of 8-OHdG and dG in the deoxynucleoside mixture were simultaneously analyzed using high-performance liquid chromatography (Hitachi L6000) with an electrochemical detector (IRICA sigma 875, applied voltage 600 mV) and UV detector (Hitachi L-4200, at 280 nm), respectively. The separating conditions were as follows: column, IRICA RP18 (4 × 250 mm); column temperature, 40°C; mobile phase, 50 mM potassium phosphate (pH 5.5) containing 10% methanol; and flow rate, 0.6 ml/min. The level of 8-OHdG in DNA was expressed as numbers of 8-OHdG per 10⁵ dG.

4) **Measurement of VE and lipid peroxide.** VE content in liver was analyzed by high-performance liquid chromatography with a fluorometric detector (Hitachi F-1050, excitation at 298 nm and emission at 325 nm) by the method of Ueda and Igarashi (14). Lipid peroxides were measured by the colorimetric method using thiobarbituric acid (15).

5) **Statistical analysis.** Data are presented in terms of the mean ± standard error (SEM). Comparison between groups was made by one-way analysis of variance followed by Duncan’s multiple range test.

**RESULTS**

When purified lard was used as a dietary fat, VE content in liver in the low VE group decreased to one-thirtieth of that in the basal VE group; in the high VE

![Graphs showing](image-url)

Fig. 1. Content of α-tocopherol (a), levels of lipid peroxides (b) and 8-OHdG in DNA (c) of livers from rats fed various VE diets with purified lard for 7 weeks. Each column and vertical bar indicates M ± SEM for 6–7 rats. Columns with different alphabets are significantly different from one another (p < 0.05). Abbreviations used: Low, low VE diet group; Basal, basal VE diet group; High, high VE diet group; TBARS, thiobarbituric acid reacting substances; MDA, malondialdehyde; 8-OHdG, 8-hydroxydeoxyguanosine; dG, deoxyguanosine.

Vol. 39, No. 4, 1993
group, it increased 4.5-fold that in the basal VE group (Fig. 1).

Corresponding to the changes of VE levels, lipid peroxide levels (thiobarbituric acid reacting substances, TBARS) in liver increased to 2.7 times in the low VE group and decreased to two-thirds in the high VE group when compared with the levels in the basal VE group. 8-OHdG levels in hepatic DNA of the basal VE group was about 0.6 per 10^5 dG, and comparable values were found in the low VE group and the high VE group.

When soybean oil or safflower oil eliminated of VE was used as a dietary fat,
VE content in the liver decreased in the low VE group and increased in the high VE group (Figs. 2 and 3). It is well known that VE requirements increase as the polyunsaturated fatty acid content in the diet also increase (16,17). A similar phenomenon was observed in this study. When hepatic VE content was compared among the low VE groups with different dietary fat sources, the value (M±SEM, μg/g tissue) was 0.556±0.055 with safflower oil, 0.71±0.060 with soybean oil, and 0.844±0.039 with lard. Corresponding to the changes of VE levels in the liver, lipid peroxide levels in the liver increased in the low VE groups, and decreased in the high VE groups (Figs. 2 and 3). 8-OHdG levels in hepatic DNA of the low VE groups tended to be higher than those from the basal VE group and high VE groups, and the tendency was clearly observed in the groups fed on the safflower oil. However, no significant difference in DNA levels of 8-OHdG among the groups was observed when either soybean oil or safflower oil was used as the dietary fat.

DISCUSSION

In this study, we fed rats either a low VE diet, a basal VE diet, or a high VE diet for 7 weeks and investigated how changes in VE content in the liver influence the hepatic levels of oxidative DNA damage and lipid peroxides. In addition, we also examined the influences of dietary fat with different compositions of polyunsaturated fatty acids on those changes. As an index of oxidative DNA damage, 8-OHdG levels in DNA were used. It has been shown that rats fed a low VE diet with safflower oil eliminated of VE for 6-7 weeks started to show VE deficiency (18). In this experiment, the rats fed the low VE diet for 7 weeks might not have developed a severe VE deficiency. However, it was expected that 7 weeks would be long enough for experimental purposes to change both the VE content and lipid peroxide levels in the livers of rats fed either the low VE or the high VE diet.

As expected, the marked decrease of VE content and the increase of lipid peroxide levels were observed in the livers of rats fed the low VE diets. On the other hand, the increase of VE content and the decrease of lipid peroxide levels were observed in livers of rats fed the high VE diets. In this way, the decrease of VE content and the increase of lipid peroxide levels correlated well. The increase of lipid peroxide levels of the liver in the low VE diet groups suggested the production of oxygen radicals which could damage DNA. However, no significant differences in 8-OHdG levels in DNA were detected among the different dietary groups.

It has been shown that the increase of polyunsaturated fatty acid content in the diet enhanced the requirement of VE (16,17). In this experiment, the linoleic acid content in the various dietary fats were 9% in lard, 50% in soybean oil, and 77% in safflower oil. From that giving the largest VE content in the liver to the smallest, if the low VE groups were to be listed according to their source of fat, they would be: lard > soybean oil > safflower oil. Although the changes of VE content in liver did not significantly influence the 8-OHdG levels in DNA, a tendency toward the increase of 8-OHdG in DNA was observed in the low VE diet group whose dietary
fat was safflower oil, but not lard.

In this study, the increase of lipid peroxides due to the decrease of VE content did not accompany the increase of 8-OHdG levels in DNA. This might be explained as follows. First, if DNA were oxidatively damaged, they might have been repaired immediately. In this experiment, we analyzed 8-OHdG level in the whole DNA, which were composed entirely of nucleus DNA. It is well known that nucleus DNA is efficiently repaired, and 8-OHdG levels in nucleus DNA are markedly lower than those in mitochondrial DNA (19). The presence of 8-OHdG-repairing enzymes has been demonstrated in mammalian cells (20). Furthermore, Kasai et al. (8) have reported the quick repair of 8-OHdG in hepatic DNA of mice who received total-body X-ray irradiation. It is also possible that nucleus DNA may hardly be damaged by oxygen radicals due to the presence of histone.

Second, DNA might be well protected against oxygen radicals by antioxidant enzymes and antioxidants even in low VE levels. In fact, one of the authors of this paper has reported that glutathione levels and glutathione-related enzymes (glutathione reductase and glutathione peroxidase) activities in VE-deficient rats were higher than those in VE-supplemented rats (18). The experimental conditions were quite similar to those of the present study. Thus, it is speculated that under the low VE conditions due to a low VE diet, other antioxidants and antioxidant systems may be protecting DNA from oxidative damage as a compensatory mechanism. It should be pointed out that unlike the administration of the low VE diet, the treatment of chemical carcinogens, which increased 8-OHdG levels (4), enhanced lipid peroxide levels and decreased glutathione-related enzymes activities as well (9).

Accumulating evidence indicates that the increase of 8-OHdG levels in DNA is detected when peroxisome proliferation is induced (4, 7, 9). It has also been shown that fats with a high content of polyunsaturated fatty acids induce peroxisome proliferation, and that an increasing amount of fat in the diet enhances \( \beta \)-oxidation and peroxisome proliferation (21, 22). Therefore, it may be possible that feeding rats a low VE diet consisting of a high amount of fat with a high content of polyunsaturated fatty acids might induce a significant increase in 8-OHdG levels in hepatic DNA. The report of Summerfield and Tappel (23) is interesting in this regard. They have measured hepatic DNA damage by DNA template activity, and observed the occurrence of DNA damage in rats fed a VE-deficient diet with 10% stripped corn oil for 6–14 months. In addition, they have shown that the DNA damage was enhanced when the content of dietary fat in the diet was increased from 10 to 26%.

This work was financially supported in part by the Environmental Agency in Japan. We are grateful to Dr. H. Kasai for advice and for providing the OHdG standard.

\[ J. \text{ Nutr. Sci. Vitaminol.} \]
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