The Prevention of Oxy Radical-Mediated Lung Tumorigenesis in Mice by Vitamin E

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Summary This work was carried out to estimate the preventive effect of vitamin E on oxy radical-enhanced lung tumorigenesis in ddY mice. We have reported that oxy radicals could be an important factor contributing to the promotive effect of glycerol on 4-nitroquinoline 1-oxide (4NQO)-induced lung tumorigenesis (1). The glycerol-promoted lung tumorigenesis of mice treated with 4NQO was reduced in mice feeding on excessive vitamin E in this study. The levels of nuclear thiobarbituric acid reactive substances (TBARS) and oxidative damage of DNA estimated as DNA single strand breaks (DNA-SSB) were significantly higher in the lungs of mice treated with 4NQO + glycerol than in those treated with 4NQO at 4 weeks after 4NQO administration. This increase was suppressed by the feeding of excessive vitamin E for 4 weeks after 4NQO injection. At 23 weeks after 4NQO injection, the feeding of excessive vitamin E for 4 and 23 weeks after 4NQO injection could cancel the promotive effect of glycerol on lung tumorigenesis. Additionally, the α -tocopherol level in serum was related with the degree of lung tumorigenesis at 23 weeks after 4NQO injection. These findings suggest that vitamin E can act as a useful agent to protect mice from oxy radical-promoted lung tumorigenesis.

Key Words vitamin E, oxy radicals, lung tumorigenesis, mice

INTRODUCTION

Oxy radical and oxidative stress are believed to play an important role in carcinogenesis (2). Serious oxidative damage on nuclei may lead to oxidative damage of DNA such as DNA-SSB and the enhancement of carcinogenesis (3–5). Many epidemiological and experimental studies show that some antioxidants can reduce cancer risk in some organs (6). With respect to the inhibitory effect of vitamin E, the results of epidemiology studies are

contradictory at present (7). However, vitamin E is considered to act as a more effective antioxidant than other antioxidants like β -carotene at relatively higher oxygen partial pressure (8). Since lung tissue is usually exposed to many oxidants and has a higher oxygen partial pressure than other tissues, vitamin E may be a useful antioxidant to protect lung tissues from oxidative stress.

Glycerol has been known to cause morphological changes in the bronchiolar non-ciliated (Clara) cell which is a possible progenitor cell of peripheral carcinoma in the lung and it promotes the development of lung tumors in ddY mice treated with 4NQO (9, 10). In addition, our previous findings suggested that the rise of oxy radical formation and oxidative stress in the lungs was a key event to enhance 4NQO-induced lung tumorigenesis in ddY mice with glycerol (1, 11). Therefore, this study was undertaken to evaluate the preventive effect of vitamin E on the glycerol-related elevation of pulmonary oxidative stress and lung tumorigenesis in ddY mice treated with 4NQO.

MATERIAL & METHODS

Animals & Treatment. Six-week-old male, specific pathogen-free (SPF), ddY and A/J strain mice were maintained under conventional clean-rack systems at 22°C and 55% humidity and also on a 12 diurnal system. The mice were fed a control CE-2 diet and special CE-2 diet containing excessive vitamin E (Japan Clea, Tokyo, Japan). A five percent glycerol solution or sterilized water was given *ad libitum*. The mice were given a 4NQO solution at a dose of 10 mg/kg body weight by a subcutaneous injection on the first experimental day (12).

Experimental design. Figure 1 shows this experimental design. The animals were divided into six groups (groups 1 to 6). Groups 3 to 6 were given 4NQO on the first experimental day. Groups 2 and 4 to 6 received glycerol for 4 weeks after the 4NQO injection. Groups 5 and 6 were fed a special CE-2 diet for 4 and 23 weeks after the 4NQO injection, respectively. At 4 weeks after the 4NQO injection, each mouse in groups 3 to 5 was killed to determine the levels of DNA-SSB, nuclear TBARS and α -tocopherol in the lungs. At 23 weeks after 4NQO injection, all mice were killed by exsanguination from the abdominal artery under pentobarbital anesthesia.

Tumor assay. The lungs were fixed by intracheal instillation of 10% buffered formalin. Tumors on the lung surface were scored under a dissecting microscope. All tumors observed in this experiment were adenoma.

Nuclei preparation & Biochemical assay. Pulmonary nuclei were isolated by differential centrifugation (12). Lipid peroxidation of nuclei was estimated as the content of TBARS by the method of Uchiyama and Mihara (13), using malondialdehyde as the standard. The level of α -tocopherol in nuclei was analyzed by high performance liquid chromatography with fluorometric detection (14). To measure DNA-SSB, the alkali unwinding assay of Morris and Shertzer (15) was used.

Data Analysis. Data were analyzed, where appropriate, by one-way analysis

Fig. 1. Experimental design.

of variance followed by Duncan's multiple-range test or chi-square test. A p value of 0.05 or less was considered to be significant.

RESULTS

Table 1 shows the results of the effect of vitamin E on oxidative stress toward pulmonary nuclei. Nuclear oxidative stress (TBARS level) increased with a significant difference by glycerol treatment, and the increased level of TBARS returned to a level similar to that in the 4NQO-treated group. The α -tocopherol level in pulmonary nuclei decreased slightly by glycerol treatment, and vitamin E treatment caused a significant increase in the level as compared to the 4NQO— and 4NQO + glycerol-treated groups. Lung DNA-SSB was significantly increased from 28.5% in the 4NQO-treated group to 35.7% in the 4NQO + glycerol-treated group. However, the increase of DNA-SSB caused by glycerol

Table 1. Nuclear TBARS, α -tocopherol levels and oxidative damages of DNA in the lungs of ddY mice at 4 weeks after 4NQO injection.

Article	Treatment group		
	4NQO	4NQO + Glycerol	4NQO + Glycerol + Vitamin E
TBARS (nmole/g lung)	10.90 $\pm$ 0.38	15.54 $\pm$ 1.29 ^a	10.71 $\pm$ 1.09 ^b
α -tocopherol (μ g/g lung)	0.38 $\pm$ 0.08	0.32 $\pm$ 0.03	1.40 $\pm$ 0.16 ^{a,b}
DNA unwinding (% unwound DNA)	28.5 $\pm$ 1.0	35.7 $\pm$ 3.0 ^a	20.1 $\pm$ 1.6 ^{a,b}

All mice were given the 4NQO-mixture by subcutaneous injection on the first experimental day. The period of glycerol and/or vitamin E treatment was 4 weeks after 4NQO injection as mentioned above. Values are expressed as the mean $\pm$ S.E. from 5 mice.

^a Significant difference from 4NQO. ^b Significant difference from 4NQO + Glycerol.

Table 2. Effect of feeding vitamin E on glycerol-enhanced lung tumorigenesis in ddY mice treated with 4NQO.

Group	No. of mice	No. of mice with tumors	% of mice with tumors	No. of total tumors	No. of tumors per mouse (mean $\pm$ S.E.)
Control (group 1)	8	0	0	0	0
Glycerol (group 2)	8	0	0	0	0
4NQO (group 3)	10	5	50	7	0.77 $\pm$ 0.26
4NQO + Glycerol (group 4)	11	9	82	21	1.91 $\pm$ 0.39 ^a
4NQO + Glycerol + Vitamin E (4 weeks) (group 5)	11	5	45	6	0.55 $\pm$ 0.21 ^b
4NQO + Glycerol + Vitamin E (23 weeks) (group 6)	9	2	22 ^b	2	0.22 $\pm$ 0.15 ^b

^a Significant difference from 4NQO. ^b Significant difference from 4NQO + Glycerol.

treatment completely disappeared through vitamin E feeding, and the level in the vitamin E-treated group was 20.1%.

Table 2 also shows the prevention of the glycerol-enhanced lung tumorigenesis

Table 3. The difference of serum vitamin E level among 6 groups.

Assay article	group 1	group 2	group 3	group 4	group 5	group 6
Serum vitamin E (μ g/ml)	1.76 $\pm$ 0.13	1.76 $\pm$ 0.12	1.70 $\pm$ 0.15	1.57 $\pm$ 0.20	2.18 $\pm$ 0.14 ^a	4.95 $\pm$ 0.59 ^b

^a Significant difference from groups 1 to 4. ^b Significant difference from groups 1 to 5.

by vitamin E in mice treated with 4NQO. Feeding on glycerol in drinking water increased the lung tumor incidence by 64% as well as lung tumor multiplicity by 72% as compared to the 4NQO-treated group. The feeding on excessive vitamin E for 4 and 23 weeks after 4NQO injection to mice treated with 4NQO + glycerol caused a 45% and 73% reduction in lung tumor incidence, and lung tumor multiplicity decrease, to 71% and 88% respectively. Additionally, as shown in table 3, the difference in the serum vitamin E level among the groups coincided with the degree of lung tumorigenesis.

DISCUSSION

Our present study demonstrates that vitamin E protects the lungs against oxy radical-promoted tumorigenesis due to antioxidative and other modifying effects of this vitamin.

Oxidative damage of DNA caused by oxy radicals and pro-oxidant states is likely to be a key factor in carcinogenesis (3–5). We have reported that oxy radicals may have a crucial role in the glycerol-related enhancement of lung tumorigenesis in mice (1, 11). The elevated levels of DNA-SSB and nuclear TBARS with glycerol returned to the levels below those in the 4NQO-treated group by the feeding of excessive vitamin E. Additionally, oxy radical-enhanced lung tumorigenesis in mice treated with 4NQO was suppressed by feeding of excessive vitamin E. These findings suggest that vitamin E can be useful antioxidant to protect nuclei from oxidative stress and oxy radical-enhanced lung tumorigenesis can be inhibited through the preventive effect of vitamin E on nuclear oxidative stress.

The inhibitory effect of vitamin E on the glycerol-related enhancement of lung tumorigenesis, was stronger in the group fed vitamin E for 23 weeks than in that fed vitamin E for 4 weeks. These findings indicate that some physiological functions other than antioxidative function with vitamin E may be effective to suppress the promotion of the development of lung tumors from 4 weeks to 23 weeks after 4NQO injection. Since the inhibitory effect of vitamin E on the activation of protein kinase C (PKC), which is a key event in the promotive stage of carcinogenesis (16), has been reported (17), this inhibition can partly account for the suppression of lung tumorigenesis by vitamin E in mice. However, this

mechanism requires further study.

The frequency of adenocarcinoma in histological types of lung cancer, is higher in Japan than in western countries (18). In addition, the increased trend of adenocarcinoma may become a serious problem in the near future. The risk factors contributing to lung adenocarcinoma in humans are still unclear, but the present findings suggest that oxy radicals play a considerable role in enhancing lung adenocarcinoma. Since vitamin E can effectively suppress oxy radical-enhanced lung tumorigenesis in mice, vitamin E may act as a useful agent in the prevention of human lung adenocarcinoma.

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