VITAMIN E AND CARCINOGENESIS

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Abstract: The role of vitamin E in carcinogenesis is reviewed. Epidemiologically, an inverse correlation between serum vitamin E levels and cancer frequency has been shown in lung and gastrointestinal (except colon) cancers, but the inverse correlation is not conclusive in cancers of the uterine cervix, colon, and breast. Clinically, vitamin E is effective in inhibiting large intestinal neoplasms. Experimentally, vitamin E inhibits skin, oral, liver, and lung carcinogenesis, however, vitamin E enhances mammary and colon carcinogenesis. The inhibitory effect of vitamin E on chemically induced carcinogenesis in some cancers may be due to the antioxidative action of vitamin E that prevents the formation of carcinogenic products and protects target cells from DNA damage. In contrast, the carcinogenic effect of vitamin E has been demonstrated in the lung, liver, forestomach, and soft tissues, but the mechanism of this carcinogenesis is yet to be elucidated. (J Toxicol Pathol 7: 179~190, 1994)

Key words: Antioxidants, Vitamin E, Carcinogens, Carcinogenicity tests, Free radical

Introduction

The role of vitamin E as an antioxidative agent, preventing peroxidation of unsaturated fatty acids by scavenging lipophilic radicals within membranes, is widely recognized^{1,2}. Vitamin E also possesses an immunomodulatory property which is similarly attributed to its antioxidative action^{3,4}. Epidemiological and case control studies in humans revealed that vitamin E prevented certain types of cancers. As a result, vitamin E has been selected as an agent for cancer chemoprevention trials that are being planned or are already underway⁵⁻⁷. However, the mechanism of the prevention of some cancers by vitamin E has

not been analysed.

Vitamin E was concluded to be a safe antioxidative food additive^{8,9} because it was neither mutagenic in *Salmonella* assay nor tumorigenic in carcinogenicity tests. However, the demonstration of the carcinogenicity of antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene, caffeic acid, catechol and sesamol¹⁰⁻¹⁵ and the publication of reports that vitamin E participated in carcinogenesis as a carcinogen¹⁶⁻²⁰ have resulted in a reevaluation of the safety of vitamin E as a food additive.

In this paper, we review the role of vitamin E in human and experimental carcinogenesis.

Modifying Effects of Vitamin E in Carcinogenesis

1. Epidemiological studies

Vitamin E is widely distributed in foods, but it is difficult to estimate the amount of consump-

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tion on the basis of dietary contents. Therefore, most of the epidemiological reports on this nutrient are based on the level of vitamin E in serum samples, while only a few are based on intake levels of vitamin E from food.

Studies on the correlation between serum vitamin E level and cancer frequency, cancer risk and cancer mortality revealed inverse, positive or negative correlation. Paired comparisons between patients and controls showed significantly low vitamin E levels in the sera of male patients with lung cancer²¹, female patients with uterine cervical dysplasia or cancer²², patients with alimentary tract cancer^{23,24}, patients with chronic atrophic gastritis²⁵, patients with cancers not related to smoking²⁴, and patients with melanoma²⁶. Serum vitamin E levels in breast cancer patients were lower than those in control patients, and this was explained to be consequence of diseases in general rather than a feature of cancer *per se*²⁷.

In contrast, significantly higher levels of vitamin E were noted in the serum of breast cancer patients than in that of controls^{28,29}. One explanation for this positive relationship is that high levels of vitamin E could enhance tumor cell proliferation by eliminating the oxygen radicals that exert a toxic effect on tumor cells. This was not likely, however, because vitamin E was distributed not in the neoplastic tissue but mainly in the accompanying adipose tissue as demonstrated by the analysis of alpha- and gamma- tocopherols in neoplastic and non-neoplastic breast tissues from the same patients³⁰.

The serum vitamin E level showed no correlation with the frequency of colon, rectum, pancreas, skin, breast, prostate, bladder, or uterine cervical cancer³¹⁻³⁶.

The correlation between serum vitamin E level and cancer risk has been examined. Low serum levels of vitamin E caused the risk of cancer to increase in the stomach³⁷, prostate³⁷, and colorectum³⁸. While, a high serum vitamin E level was protective for lung cancer³⁹.

The serum vitamin E level correlated inversely to the mortality of colon cancer patients⁴⁰, while it did not correlate to the mortality of stomach or large intestinal (except colon) cancer patients⁴¹.

An inverse correlation was observed between

intake level of vitamin E and frequency of lung cancers among nonsmokers^{42,43}. Moreover, a high intake level of vitamin E correlated to the reduction of risk in uterine cervical, pharyngeal, and oral cancer^{44,45}, but it was not correlated to the reduction of risk in laryngeal cancer⁴⁶.

2. Clinical studies

Various clinical trials have been performed to investigate the efficacy of vitamin E as a chemopreventive agent⁶⁻⁷. In a systemic screening program of commonly used prescription drugs, vitamin E was found to be protective for cancers of all sites⁴⁷. In patients with familial adenomatous polyposis of the colon, the progression of lesion from polyp to adenoma was inhibited by the supplementation of vitamin E, vitamin C and grain fiber^{48,49}. After the complete removal of colorectal adenoma, vitamin E supplementation was effective in reducing abnormalities in cell kinetics, an indicator of the preneoplastic condition, in the colon⁵⁰. Vitamin E supplementation was ineffective, however, in the regression of benign breast neoplasms⁵¹.

3. Experimental Studies

There are many reports on the modifying effects of vitamin E on chemical carcinogenesis (Table 1). All of these studies were conducted with sythetic vitamin E, except three which used natural vitamin E^{17,52,53}.

Skin cancer

When 40 μ mol (17.2 mg) of vitamin E was applied topically to mice before initiation with 3.6 μ mol of 7, 12-dimethylbenz(a)anthracene (DMBA), the frequency of skin papillomas did not decrease⁵³. However, when 1 mg of vitamin E was applied to mice before initiation with 2.56 μ g of DMBA and promotion with 10 μ g of 12-O-tetradecanoyl phorbol-13-acetate, the frequency of papillomas decreased⁵⁴. Therefore, although vitamin E was ineffective in inhibiting the tumorigenesis of the promotion component of DMBA, it was effective in inhibiting the tumorigenesis of the promotion component of 12-O-tetradecanoyl phorbol-13-acetate which stimulated the production of superoxide anion radicals and lipid hydroperoxides. The application of 1 mg of vita-

Table 1. Modifying Effects of Vitamin E in Carcinogenesis

Tissues	Species	Dose (Application)	Carcinogens	Effects	References
skin	mouse	17.2 mg (p.o., single)	DMBA (3.6 μ M), selenium	no effect	53
skin	mouse	17.2 mg (p.o., single)	DMBA (3.6 μ M), GSH	no effect	53
skin	mouse	17.2 mg (p.o., 2/wk)	DMBA (0.1 μ M), selenium	enhance	53
skin	mouse	17.2 mg (p.o., 2/wk)	DMBA (0.1 μ M), GSH	enhance	53
skin	mouse	17.2 mg (p.o., 2/wk)	DMBA (0.1 μ M), TPA	inhibit	53
skin	mouse	17.2 mg (p.o., 2/wk)	DMBA (0.1 μ M), TPA, selenium	inhibit	53
skin	mouse	17.2 mg (p.o., 2/wk)	DMBA (0.1 μ M), TPA, GSH	inhibit	53
skin	mouse	17.2 mg (p.o., 2/wk)	DMBA (0.1 μ M), TPA, mez, selenium	inhibit	53
skin	mouse	17.2 mg (p.o., 2/wk)	DMBA (0.1 μ M), TKA, mez, GSH	inhibit	53
skin	mouse	1 mg (p.o., single)	DMBA (2.56 μ M)	inhibit	54
skin	mouse	25 mg (p.o., 3/wk)	UV	inhibit	55
oral	hamster	10 mg (paint, 2/wk)	DMBA (19.5 mM)	inhibit	57
oral	hamster	10 mg (p.o., 2/wk)	DMBA (3.9 mM)	inhibit	58
oral	hamster	250 μ g (inj., 2/wk $\times$ 4)	DMBA-tumor	regress	59
oral	hamster	200 μ g (p.o., 7/wk)	DMBA-tumor, β -carotene	regress	60
liver	rat	3.6, 7.2, 1.5 g/kg diet	DEN (200 mg/kg B. wt.) + hepatectomy	inhibit	63
liver	rat	0.7 mg (p.o., 20 kw)	AAF (200 mg/kg diet)	inhibit	69
liver, forestomach	mouse	50 g/kg diet	DEN (100 mg/l)	no effect	17
liver	rat	5 g/kg diet	DEN (10 mg/kg B. wt.)	inhibit	64
liver, pancreas	hamster	10 g/kg diet	DONDPA (20 mg/kg diet)	inhibit	18
pancreas	rat	0.6 g/kg diet	azaserine	no effect	72
pancreas	rat	0.6 g/kg diet	azaserine, selenium	inhibit	72
mammary	rat	1 g/kg diet	DMBA (12 mg)	no effect	76
mammary	rat	1 g/kg diet	DMBA (10 mg)	no effect	73
mammary	rat	1 g/kg diet	DMBA (10 mg), selenium	inhibit	73
mammary	rat	5, 10 g/kg diet	DMBA (7.5 mg), selenium	inhibit	74, 75
mammary	rat	225 IU/100kal diet	MNU (25 mg/kg diet), vitamin A	no effect	77
mammary	rat	1.4 mg/kg diet	MNU (30 mg)	no effect	78
mammary	rat	1.4 mg/kg diet	MNU (30 mg)	enhance	78
mammary	rat	1.4 mg/kg diet	DMBA (50 mg)	no effect	78
mammary	rat	1.4 mg/kg diet	DMBA (50 mg)	inhibit	78
lung	mouse	50 g/kg diet	DEN (100 mg/l)	inhibit	17
forestomach	mouse	50 g/kg diet	DON (100 mg/l)	no effect	17
forestomach	mouse	10 g/kg diet	DMBA (46.8 mM)	no effect	76
forestomach	rat	10 g/kg diet	BHA (10 g/kg diet)	inhibit	81
alimentary tract	mouse	0.6 g/kg diet	DMH (10 mg/kg B. wt.)	inhibit	82
alimentary tract	mouse	40 g/kg diet	DHBNA (0.05% d.w.)	no effect	85
prostate	rat	10 g/kg diet	DMAB (50 mg/kg B. wt.)	no effect	52
CNS, kidney	rat	0.6 g/kg diet	ENA (7.5 mg/kg B.wt.)	no effect	86

Abbreviations used are; CNS: central nervous system; p.o.: per os; paint: painting; inj.: injection; DMBA: 7, 12-dimethylbenz(a)anthracene; GSH: glutathione-S-transferase; TPB: 12-O-tetradecanoylphorbol-13-acetate; mez: mezerein; UV: ultraviolet light; DEN: diethylnitrosamine; AAF: acetylaminofluorene; DONDPA: 2, 2'-dioxiso-N-nitrosodipropylamine; MNU: N-methyl-N-nitrosourea; BHA: butylated hydroxyanisole; DMH: 1, 2-dimethylhydrazine; BHBNA: N-butyl-N-(4-hydroxybutyl)nitrosamine; DMAB: 3, 2'-dimethyl-4-aminobiphenyl; ENU: ethylnitrosourea;

min E with 8.5 nmol of 12-O-tetradecanoyl phorbol-13 acetate two weeks after the application of 0.1 μ mol of DMBA did not reduce the frequency of papilloma, while the frequency of papilloma was reduced when the same dose of vitamin E was applied with 8.5 nmol of mezerein 20 weeks after the initiation with 0.1 mol of DMBA⁵³. Mezerein

was a tumor promoter and stimulated activity of ornithine decarboxylase in the target cells. In these experiments, vitamin E stimulated the activity of glutathione peroxidase and inhibited that of ornithine decarboxylase in the target cells⁵³. Therefore, the anticarcinogenic activity of vitamin E might be operated through its ability to alter the

enzymatic activities of target cells, and the activity might be linked to the glutathione-mediated protective system.

In ultraviolet-irradiated skin carcinogenesis, topical application of 25 mg of vitamin E to mice throughout the experiment reduced the incidence of skin cancer from 81% to 42%⁵⁵. Since the ultraviolet irradiation to mice stimulated the activity of epidermal ornithine decarboxylase⁵⁶, this inhibition of skin cancer by vitamin E might operate through the reduction of the activity of this enzyme.

Oral cancer

When 10 mg of vitamin E and a 0.5% (w/v) of DMBA were applied to the buccal pouch of Syrian golden hamsters alternately for 16 weeks, the latency of epidermoid carcinoma was prolonged and the average size and number of the tumors were reduced⁵⁷. When the concentration of DMBA solution was reduced to 0.1% (w/v) the 10 mg of vitamin E completely prevented the development of tumors^{57,58}. One possible mechanism of this prevention is that vitamin E blocked the oxidation of DMBA to its ultimate carcinogen, the diol epoxide. When 250 μ mol (107.7 mg) of vitamin E was injected into the tumor-bearing buccal pouch twice weekly for 4 weeks, the average size of the tumors decreased⁵⁹. Regression of the established tumors was also demonstrated by oral administration of vitamin E at a dose of 400 μ mol (172.3 mg) together with *beta*-carotene^{60,61}. These findings indicated that vitamin E stimulated the immunoreactive Langerhans cells of the buccal pouch and thereby facilitated the invasion of cytotoxic macrophages and T lymphocytes.

When 70 μ mol (30.1 mg) or 300 μ mol (129.2 mg) of vitamin E was introduced into tumor cells of oral squamous carcinoma lines by liposomes *in vitro*, 36% of the cells were killed⁶¹. However, the same doses of vitamin E introduced into normal cells did not exhibit any cytotoxicity⁶¹. The selective cytotoxic effects of vitamin E were also observed in two human oral carcinoma cell lines, when 70 μ mol (30.1 mg) of vitamin E was added to the culture media⁶².

Liver cancer

The effects of vitamin E on the development

of enzyme-altered foci in rat liver were investigated. The induction of *gamma*-glutamyltranspeptidase-positive foci was inhibited in rats fed a diet containing 0.36, 0.72 and 1.5% (3.6, 7.2 and 15 g/kg diet) vitamin E given for 6 weeks after initiation by 200 mg DEN per kg body weight plus partial hepatectomy⁶³. In another report, newborn rats were given 10 mg DEN per kg body weight and fed a diet containing 500 ppm (50 mg/kg diet) vitamin E. After 3 months, the volume of glutathione S-transferase-positive foci had significantly decreased, but after 11 months, the number and size of the foci were not different from those of rats without the treatment of vitamin E⁶⁴. In hamsters fed a diet containing 1% (10 g/kg diet) vitamin E for 40 weeks after the administration of 2,2'-dioxo-N-nitrosodipropylamine, vitamin E reduced the final frequency of glutathione S-transferase-positive foci¹⁸.

The level of hepatic glutathione-S-transferase increased when rats were given 0.025% (250 mg/kg diet) vitamin E for one month⁶⁵. And the activity of glutathione-S-transferase was induced in benzo(a)pyrene-initiated primary hepatocytes when 25 μ mol (10.8 mg/l) of vitamin E⁶⁶ was added into their culture media. Whereas, it is not conclusive whether or not the dietary vitamin E may suppress the hepatocarcinogenicity through glutathione-mediated pathway. One report showed that glutathione was effective to prevent the aflatoxin B₁-initiated rat hepatocarcinogenesis⁶⁷, while another showed no effect of glutathione for the DEN-initiated rat hepatocarcinogenesis⁶⁸. When rats were given 0.7 mg of vitamin E per day simultaneously with 2-acetylaminofluorene for 20 weeks, the concentrations of serum *gamma*-glutamyltranspeptidase and hepatocellular UDP-glucuronyl transferase were reduced⁶⁹.

A decrease of hepatic vitamin E caused the accumulation of lipid peroxides in hepatocytes. When rats were fed a diet which contained ethanol with the concentration of 7% (v/w), high levels of ethane, which is a measure of lipid peroxidation, were observed in hepatocytes. The concentration of ethane was reduced by the intraperitoneal injection of vitamin E (400 mg/kg body weight)⁷⁰. [4-Chloro-6-(2,3-xylidino) 2-pyrimidyl-thio] acetic acid could induce liver cancers in rats whose liver

contained significantly low concentration of vitamin E⁷¹.

Pancreatic cancer

When Syrian golden hamsters were fed a diet containing 1% vitamin E (10 g/kg diet) for 40 weeks after the administration of 2,2'-dioxo-N-nitrosodipropylamine, the frequency of ductal hyperplasia was reduced¹⁸. In azaserine-induced carcinogenesis in rats, the supplementation of vitamin E (600 mg/kg diet) for 69 weeks after initiation did not interfere in the growth of acinar neoplasia, while simultaneous supplementation of vitamin E and selenium (2.5 mg/kg diet) reduced the incidence of atypical nodule, adenoma and carcinoma⁷².

Mammary cancer

The effect of vitamin E and/or selenium supplementation on DMBA-induced mammary carcinogenesis was investigated in rats⁷³⁻⁷⁵. When both vitamin E (1,000 mg/kg diet) and selenium (2.5 mg/kg diet) were treated from 2 weeks before initiation by DMBA (10 mg) throughout the experiment, the mammary tumor incidence was reduced, although supplementation of vitamin E or selenium alone did not reduce the tumor incidences⁷³. The concentration of vitamin E in the supplemented diet (1,090 mg/kg diet) was 12.1 times higher than that in the control diet (90 mg/kg diet). The minimal amount of vitamin E which was effective to reduce about 50% of DMBA (7.5 mg)-induced the mammary carcinomas was around 500 mg/kg diet with the supplement of selenium (1 mg/kg diet)^{74,75}. It was speculated that the suppression of lipid peroxidation by vitamin E along with the regulation of glutathione peroxidase by selenium was needed for the inhibition of mammary carcinogenesis. Whereas, the effects of vitamin E on the immunomodulation, mitogen-induced blastogenesis and natural killer cytotoxicity were not conclusive⁷⁵.

The treatment of vitamin E with the concentration of 0.01% of diet (100 mg/kg diet) was ineffective to inhibit the mammary tumor production by DMBA⁷⁶, nor the treatment of vitamin E with the concentration of 10 times higher (225 IU 100 Kcal) than that of the control diet was effective for the inhibition of N-methyl-N-nitrosourea-induced rat mammary carcinogenesis⁷⁷.

The effects of *alpha*-tocopherol and tocotrienol were investigated for DMBA and N-methyl-N-nitrosourea induced mammary carcinogenesis in rats⁷⁸. Administrations of *alpha*-tocopherol {3.349 μ mol (1.44 mg)/kg diet}, the concentration of which was 28.9 times higher than that in the control diet, reduced neither the incidence nor the latency of mammary carcinomas induced by either DMBA or N-methyl-N-nitrosourea. Administration of tocotrienol at the same dose of *alpha*-tocopherol prolonged the latency of DMBA-induced mammary tumors, while the administration of tocotrienol increased tumor multiplicity of N-methyl-N-nitrosourea-induced mammary tumors. Therefore, it was concluded that neither vitamin E analog had a major impact on mammary tumor development after tumor induction with either DMBA or N-methyl-N-nitrosourea.

Studies on vitamin E deficiency in N-methyl-N-nitrosourea-induced mammary carcinogenesis^{79,80}, meanwhile, revealed a shortened latency and increased tumor multiplicities in rats consuming a diet deficient in both vitamin E and selenium, although no alteration in tumorigenicity was observed by the deficiency of either vitamin E or selenium alone.

Cancers in other sites

Rats fed a diet containing 1% butylated hydroxyanisole per kg diet for 52 weeks developed hyperplasia at the prefundic and mid regions of the forestomach⁸¹. The incidence of hyperplasia at the prefundic region was lower in rats fed a diet containing 1% butylated hydroxyanisole plus 1% vitamin E (10 g/kg diet) than that in rats fed a diet containing 1% (w/w) butylated hydroxyanisole alone⁸¹. When mice were fed a vitamin E supplemented diet (10 g/kg diet) and a control diet alternately up to 41 weeks this treatment showed no correlation to the incidence of forestomach tumors induced by DMBA⁷⁶. In 1,2-dimethylhydrazine-initiated carcinogenesis in mice, the administration of vitamin E either suppressed⁸² or enhanced⁸³ tumorigenicity in the lower intestinal tract. The influence of dietary vitamin E deficiency to 1,2-dimethylhydrazine-induced intestinal tumors was investigated in rats⁸⁴. When rats were fed a diet containing low level of vitamin

E (less than 0.5 mg/kg diet) for 30–34 weeks, the incidence of intestinal tumors was reduced⁸⁴. When mice were fed a diet containing 5% of vitamin E (50 g/kg diet) for 2 years, the incidence of lung tumor initiated by DEN was reduced from 82.0% to 48.0% but that of the forestomach tumor was not reduced¹⁷.

When rats were given a diet containing 1% vitamin E (10 g/kg diet) for 40 weeks, there was no effect with regard to the induction of urinary bladder cancer initiated by N-butyl-N-(4-hydroxy-butyl) nitrosamine⁸⁵, and when they were fed a diet containing 1.5% vitamin E for 31 weeks, there was no effect with regard to the induction of prostate cancer initiated by 3,2'-dimethyl-4-aminobiphenyl⁵².

In transplacental carcinogenesis, vitamin E was ineffective on the development of tumors in the nervous system and kidney by ethylnitrosourea⁸⁶. In this experiment, the dose of vitamin E was 600 mg per kg diet, and the duration of feeding was 18 months.

4. Possible mechanisms

Vitamin E could inhibit some forms of chemically induced carcinogenesis through its ability to alter the enzymatic activities of target cells, as noted in the skin^{53,–55}.

Injections of vitamin E immediately after the irradiation of ⁶⁰Co gamma-rays (8.0 Gy) to mice enhanced the 30-day survival⁸⁷. This was explained that the injection of vitamin E might enhance immune response or recovery of bone marrow of the host.

The antioxidative action of vitamin E could affect the inhibition of certain types of carcinogenesis. Vitamin E inhibited the formation of nitrosamines, the major suspects for stomach cancer, by reducing the oxidation of nitric dioxide, nitric oxide and nitrite contained in foods in the stomach^{88,89} and the formation of nitrosamines from atmospheric nitric dioxide in the skin⁹⁰. Epidemiologically, the average ratios of lipid peroxide/vitamin E in the sera of cancer patients were significantly higher than those of normal controls⁹¹. Vitamin E could also reduce the level of serum lipid peroxides in tumor-bearing mice⁹¹. Consequently, the frequency to subject target cells

to carcinogenic compounds was reduced.

Administration of vitamin E immediately after irradiation of ⁶⁰Co gamma-rays (1.0 Gy) to mice was effective to reduce the frequency of micronuclei in bone marrow cells⁹². This protective effect of vitamin E was explained by the ability of vitamin E to scavenge oxidizing free radicals. Actually, vitamin E protected liposomal membranes from lipid peroxidation induced by an X-irradiation (100 Gy)⁹³. Vitamin E inhibited the transformation of C3H10T1/2 cells induced by X-irradiation, benz(a)pyrene and tryptophan pyrolysate⁹⁴. Vitamin E also inhibited the sister chromatid exchanges of Chinese hamster ovary cells induced by the liberated free radicals from phagocytes^{95,96}. Vitamin E protected the cells of lymphoblastoid lines and primary lymphocyte cultures from bleomycin-induced chromosomal breakage which was used as an indicator of genetic instability⁹⁷. Thus, vitamin E reduced the concentration of free radicals, protected the chromosomes from the radicals and thereby might contribute to prevent the target cells from malignant transformation.

Salmonella mutagenesis assay was used to study the influence of vitamin E on the mutagenic activities of carcinogens. Vitamin E did not affect the frequency of base-substitution mutations caused by N-methyl-N'-nitro-N-nitrosoguanidine⁹⁸ or aflatoxin B₁⁹⁹, nor the frequency of frameshift mutation caused by N-acetoxy-2-acetylaminofluorene⁹⁸. Therefore, vitamin E does not seem to directly affect the repair of damaged DNA.

Direct carcinogenic potential of vitamin E

In acute and subchronic toxicity studies in Fischer 344 rats, oral administration of vitamin E at the dose of 2 g/kg body weight daily for 13 weeks significantly reduced body weight and survival and alveolar hyperplasia in the lung¹⁶. In a chronic toxicity study in CD rats, the oral administration of vitamin E at the dose of 2 g/kg body weight daily for 104 weeks did not exert any influence on growth rate, survival or tumor incidence¹⁰⁰. However, vitamin E families may be a contributive carcinogen in various organs when

Table 2. Carcinogenic Potential of Vitamin E

Vitamin E	Dose	Tissues	Species	Carcinogenicity	References
d, l- α -tocopheryl acetate	2 g/kg B. wt.	lung	rat	+	16
natural vitamin E ¹⁾	50 g/kg diet	liver	mouse	+	17
natural vitamin E	50 g/kg diet	lung	mouse	—	17
natural vitamin E	50 g/kg diet	stomach	mouse	—	17
d, l- α -tocopheryl acetate	10 g/kg diet	stomach	hamster	+	18
natural vitamin E	20 mg	subcutaneous	mouse	—	19
natural vitamin E+soya oil	20 mg+0.1 ml	subcutaneous	mouse	+	19
d- α -tocopherol	16 mg	subcutaneous	mouse	—	20
d- α -tocopherol+soya oil	16 mg+0.1 ml	subcutaneous	mouse	+	20
d, l- α -tocopheryl acetate	40 mg	subcutaneous	rat	+	19
d, l- α -tocopheryl acetate+soya oil	40 mg+0.2 ml	subcutaneous	rat	+	19
d, l- α -tocopheryl acetate+soya oil	20 mg+0.1 ml	subcutaneous	mouse	+	19

1): a mixture of natural tocopherols

it was given chronically and at sufficiently high amounts in animals (Table 2).

1. Oral administration experiments

Moore *et al.* reported the induction of squamous cells hyperplasia and papilloma by the vitamin E compound *d, l-alpha*-tocopheryl acetate in the forestomach of hamsters¹⁸. In this experiment, the *d, l-alpha*-tocopheryl acetate was mixed into a control diet at a concentration of 1% (10 g/kg diet). Nitta *et al.* reported that a 26% spontaneous liver tumor frequency was enhanced to 72% by the administration of natural vitamin E, that is, a mixture of natural tocopherols in C57BL/6N×C3H/He F1 mice¹⁷. The concentration of this natural vitamin E in a diet was 5% (50 g/kg diet), a dose about 700 times higher than that of vitamin E in the control diet.

2. Injection experiments

The carcinogenicity of vitamin E was demonstrated by subcutaneous injection experiments. The synthetic vitamin E compound, *d, l-alpha*-tocopheryl acetate induced soft tissue tumors, fibrosarcoma and malignant fibrous histiocytoma in one strain of rats and two strains of mice, when the agent was injected at doses of 40 mg for rats and 20 mg for mice subcutaneously once a week for 12 months¹⁹. A naturally occurring vitamin E, that is, a mixture of natural tocopherols, did not induce tumors by the same procedure¹⁹, although it

induced soft tissue tumors when injected subcutaneously together with vegetable oils (0.2 ml for rats and 0.1 ml for mice) which were not carcinogenic by themselves¹⁹. Similarly, a natural *alpha*-tocopherol at the dose of 16 mg per injection induced fibrosarcoma in a strain of mice when injected subcutaneously together with soya oil (0.1 ml) for 10 months²⁰. Neither the *alpha*-tocopherol nor the soya oil itself was effective for the induction of any tumors.

3. Possible mechanisms

It is helpful to define vitamin E as either a tumor initiator, promoter, complete carcinogen, or progressive agent when discussing the mechanism of its carcinogenicity. Nitta *et al.* reported the increase in spontaneous liver tumor frequency by the administration of vitamin E in a strain of mice genetically susceptible to liver carcinogens¹⁷. The genes affecting susceptibility to murine hepatocarcinogenesis controlled the progression (tumor size) not the frequency of carcinogen-induced liver tumors¹⁰¹. Therefore, this effect of vitamin E could be placed under the category of tumor initiator.

Niwa *et al.* examined the expression of the *c-myc* gene in murine hepatocellular carcinoma and soft tissue tumors induced by a vitamin E¹⁰². An elevated expression and amplification of the gene was noted in the soft tissue tumors but not in the hepatocellular carcinomas^{17,102,103}. Since gene

amplification occurred after DNA damage¹⁰³, it follows that repeated injections of vitamin E caused DNA damage and triggered the amplification. This amplification might have contributed to the promotion or progression of malignancy in the soft tissue tumors.

Summary

Many epidemiological and clinical studies have been conducted on the role of vitamin E in human cancers. Although the beneficial effects in many cases have not been conclusive, vitamin E exerted an inhibitory effect on cancer of the stomach^{24,37}, colon^{40,48,49}, lung^{21,39,42,43}, and uterine cervix⁴⁴. These cancers have been demonstrated to require multiple gene-mutations for their development¹⁰⁴⁻¹⁰⁸.

Many experimental studies have also been conducted on the role of vitamin E in chemical carcinogenesis. The inhibitory effect of vitamin E has been shown on skin, alimentary tract, liver, pancreas, mammary, lung, and prostate carcinogenesis. The involvement of the antioxidative action of vitamin E for the inhibition of carcinogenesis was strongly suggested in the skin⁹⁰, stomach^{88,89}, mammary gland⁷³⁻⁷⁵, and some cell lines⁹⁴⁻⁹⁷. In spite of this inhibitory effect of vitamin E on chemical carcinogenesis, the direct carcinogenic potential of vitamin E has been demonstrated in the forestomach¹⁸, liver¹⁷, and soft tissues^{19,20}. However, the ultimate carcinogenic structure of the vitamin E in this direct carcinogenesis remains to be elucidated.

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