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The influence of a moderately increased dose of vitamin E on 20-methylcholanthrene induced tumorigenesis in mice

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The influence of a moderately increased dose of vitamin E on tumorigenesis of 20-methylcholanthrene-induced tumours in mice was investigated. A tendency to enhancement was observed. In the experimental groups of mice the average latent period of tumours was markedly shorter than in the control groups, although the difference was not statistically significant.

Key words: neoplasms, experimental; methylcholanthrene; vitamin E; mice

Introduction

The effect of vitamin E on tumorigenesis was experimentally studied by varous authors. The results obtained were, however, highly inconsistent. Some reported an inhibitory effect of high doses of vitamin E, others observed no effect, or even an enhancing effect. The results of epidemiological studies also were controversial, which calls for further investigation of the effects of various doses of vitamin E on tumorigenesis.

The present study was undertaken to determine if excess vitamin E has an inhibitory or a stimulatory effect on 20-methylcholanthrene induced tumorigenesis in mice. Since other authors usually used very high doses of vitamin E, we decided to investigate the effect of only moderately increased doses of vitamin E.

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Materials and methods

Experiment 1

Forty young female mice weighing on average 22 g were injected subcutaneously in the right hind-leg 1 mg 20-methylcholanthrene (Sigma) in 0.1 ml olive oil and then divided into the control group (Group 1) and the experimental group (Group 2). The mice were housed 5 per cage. The control group was fed a pelleted experimental diet consisting of natural ingredients, without addition of vitamins, according to the formula shown in Table 1. Vitamins were given in drinking water. The solution of vitamins was prepared as shown in Table 2. Each mouse received 4 g of pellets and 4 ml of vitamin solution daily. The experimental group was fed the same pellets, but the solution of vitamins they received contained 240 mg vitamin E/l instead of 60 mg/l. Thus, Group 2 received 4 times as much vitamin E as Group 1.

The mice were inspected for a palpable tumour one month after injection of the carcinogen, and then once weekly. The mice which developed tumours were marked with fuchsin

Table 1. Composition of the experimental diet without vitamin supplements.

Wheat meal	40,00 %
Barley	2.00 %
Soyabean meal	13.00 %
Fish meal	15.00 %
Salt	1.00 %
Limestone	0.75 %
Sugar	2.00 %
Magnesite	0.25 %
KJ	3.92 mg/kg
$ZnSO_4 \times 7\,H_2O$	1187.33 mg/kg
MnO_2	166.58 mg/kg
$CoSO_4 \times 7\,H_2O$	1.43 mg/kg
$FeSO_4 \times 7\,H_2O$	188.60 mg/kg
$CuSO_4 \times 7\,H_2O$	549.45 mg/kg

Table 2. Composition of vitamin solution.

Vitamin	Amount/l drinking water
Vitamin A (retinol)	7000 IU
Vitamin D_3	300 IU
α-tocopherol	60 mg
Vitamin K_1 (Konakion Roche)	1.5 mg
Thiamine dichloride	4.0 mg
Riboflavin	5.0 mg
Vitamin B_6	6.0 mg
Nicotinic acid	10.0 mg
Calcium D(+) pantothenate	12.0 mg
Vitamin B_{12}	5.0 μg

or methylen blue solution. The date of the appearance of the initial tumour was recorded. The size of the tumour was recorded on first palpation, and then weekly until death.

The experiment was terminated when the last animal with a tumour died. The results were analysed with respect to the incidence of tumours, the length of the latent period and the length of survival with tumour.

Experiment 2

Experiment 2 was en exact repetition of Experiment 1. It was carried out to confirm the result of the first experiment.

Results

Experiment 1

In Group 1, tumours occurred in 16 mice; 2 mice died intercurrently and 2 survived without tumour. In Group 2, which received 4 times as much vitamin E as Group 1, tumours occurred in all 20 mice. Group 2 showed a slightly higher incidence of tumours, yet the difference between the groups was not significant. The influence of increased dose of vitamin E on the duration of latent period is shown in Table 3.

Table 3. Latent periods of tumours induced by 20-methylcholanthrene in the control group and in the group receiving high-dose vitamin E.

Group of mice	Latent periods of tumours in days
Group 1 (controls)	77, 84, 91, 91, 98, 98, 105, 112, 112, 119 119, 126, 175, 179, 182, 231
Group 2 (high-dose vitamin E)	77, 77, 77, 77, 77, 77, 84, 84, 91, 91, 91, 98, 98, 105, 105, 119, 119, 119, 156, 217
The average latent period:	Group 1: 124.93 days Group 2: 101.95 days.

The average latent period in Group 1 was 124.93 days, and in Group 2, receiving increased dose of vitamin E, only 101.95 days.

It is evident that a moderately increased dosage of vitamin E used in this experiment markedly shortened the latent period of tumours, i.e. it markedly enhanced tumorigenesis induced by 20-methylcholanthrene. The difference in the length of the latent periods between Group 1 and Group 2 is, however, not statistically significant ($p > 0.05$).

The average survival time with tumour was 35 days in Group 1 and 38.1 days in Group 2. The difference between both groups was not significant.

Experiment 2

In Group 1, 17 mice developed tumours; 1 mouse died intercurrently and 2 survived without tumour. In Group 2, which received 4 times as much vitamin E as Group 1, tumours occurred in 18 mice; 1 mouse died intercurrently and 1 survived without tumour. The influence of increased doses of vitamin E on the duration of latent period is shown in Table 4.

The average latent period in Group 1 was 107.94 days, and in Group 2 receiving increased doses of vitamin E only 89.05 days.

Table 4. Latent periods of tumours induced by 20-methylcholanthrene in the control group and in the group receiving high-dose vitamin E.

Group of mice	Latent periods of tumours in days
Group 1 (controls)	65, 72, 79, 79, 86, 93, 99, 99, 106, 113 113, 127, 127, 134, 141, 147, 155
Group 2 (high-dose vitamin E)	65, 65, 72, 72, 72, 72, 72, 86, 86, 93, 99, 99, 99, 106, 106, 106, 113, 120
The average latent period:	Group 1: 107.94 days Group 2: 89.05 days.

In the second experiment a moderately increased dosage of vitamin E markedly shortened the latent period of tumours, i.e. it markedly enhanced tumorigenesis induced by 20-methylcholanthrene. Again, the difference in the length of the latent periods between Group 1 and Group 2 was not statistically significant.

The average survival time with tumour was 40.62 days in Group 1 and 34.5 days in Group 2. The difference between the groups is not signifficant.

Discussion

In our study an enhancement of tumorigenesis in mice given a moderately increased amount of vitamin E was observed. The degree of enhancement was, admittedly, not statistically signifficant, but it was observed in two independent experiments, suggesting that the observed difference in the duration of latent periods between control and experimental mice was not coincidental. At any rate, our findings do not support the view that excess vitamin E has an inhibitory effect on tumorigenesis, but, on the contrary agree with the experimental results of several authors who observed a similar tendency to enhancement of tumorigenesis, or even a statistically signifficant enhancement due to high doses of vitamin E.

Temple and El-Khatib[1] studied the effect of vitamin E on the development of colon tumours in mice treated with 1.2-dimethylhydrazine. Compared with mice fed the control diet, those given vitamin E had a higher colon tumour incidence. This effect, which was stronger in females, was due to an increased incidence of adenomas.

Toth and Patil[2] also observed an enhancing effect of vitamin E on murine intestinal tumorigenesis induced by 1.2-dimethylhydrazine dihydrochloride. Vitamin E acetate (dl-α-tocopheryl acetate) at a 4% dose level enhanced tumour induction in the duodenum, cecum, colon, rectum and anus. The increased incidence of tumours was statistically significant.

Glauert et al.[3] studied the effect of dietary vitamin E on the development of altered hepatic foci and hepatic tumours induced by peroxisome proliferator ciprofibrate. They found that the incidence of hepatic tumours and the number and volume of altered hepatic foci were increased in rats fed larger amounts of vitamin E. The authors concluded that increasing dietary vitamin E enhances ciprofibrate-induced hepatocarcinogenesis.

Rockwood Telford[4] observed an enhancing effect of vitamin E (α-tocopherol) on tumorigenesis in mice injected with dibenzanthracene. She studied the effect of hypo- and hypervitaminosis E on the growth of lung tumours in mice and noted that the incidence of lung tumours was greatest in the hypervitaminosis E groups and so was the average number of lung tumours per animal. The hypovitaminosis group, in contrast, had the smallest number of tumours per animal.

Okishio[5] noted that supplementing basal diet with vitamin E markedly increased the incidence of tar carcinoma in rabbits.

Reddy and Tanaka[6] studied the interactions of selenium deficiency, vitamin E, polyunsaturated fat, and saturated fat on azoxymethane-induced colon carcinogenesis in male rats. They observed that the multiplicity of colon adenocarcinomas was increased by excess vitamin E in the diet.

Devor et al.,[7] who studied carcinogenicity of fecapentaene-12-diacetate on skin painting in mice, found an increased incidence of tumours, yet the difference observed was not statistically significant, when DAFP-12 was co-administered with vitamin E.

Some authors noticed no effect of high doses

of vitamin E on tumorigenesis in mice or rats treated with 3, 4, 5, 10-dibenzpyrene or 7, 12-dimethylbenzanthracene.[8, 9, 10]

In contrast to these reports, several authors interpreted their experimental data as supporting the hypothesis that high doses of vitamin E protect against carcinogenesis. The conclusions of some of these studies, e.g. those by Haber and Wisler,[11] Cook and MacNamara,[12] Kurek and Corvin,[13] however, are not very convincing: they compared the effect of diets rich in vitamin E with a diet deficient in vitamin E, or a diet containing relatively small amounts of this vitamin. In our opinion, the results obtained indicate that vitamin E deficiency increases the susceptibility of animals to tumour formation and do not suggest that high doses can protect against it.

Our view is supported by several studies[9, 14, 15] demonstrating that vitamin E deficiency increases the incidence of tumours.

Only a small number of experimental studies support the view that high doses of vitamin E have a protective effect on tumorigenesis.[16, 17, 18, 19, 20]

Some of these studies, however, were performed under special conditions. So Harman[16] supplemented the diet of rats with a very large amount of polyunsaturated fat (20% by weight of corn oil). Shamberger and Rudolph[17] applied vitamin E topically concomitantly with the tumour-promoter, croton oil. In chemoprevention of cancer, however, only oral consumption of a potentially anticarcinogenic substance is to be considered.

In our opinion the results of epidemiological studies, which are highly conflicting, do not indicate that high dose vitamin E could be used as a chemopreventive agent against cancer. The finding that subjects with serum levels of vitamin E in the lowest quintile are at a higher risk of developing cancer than persons with levels in the highest quintile[21, 22, 23] does not justify the conclusion that excess doses of vitamin E are beneficial, as another interpretation of these observations can also be that vitamin E deficiency or relative (marginal) deficiency have an adverse effect.

One should take care to avoid vitamin E deficiency. The best way to supply the organism with an adequate amount of vitamin E (and of other vitamins) is undoubtedly to eat a balanced, mixed diet containing sufficient amounts of vegetables and fruits.

Although the intake of supplements of high doses of vitamin E for chemoprevention of cancer does not seem justified, normal (physiological) amounts of vitamin E in a balanced diet certainly play a role in the protection against cancer as confirmed by the adverse effect of vitamin E deficiency.

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