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CABBAGE AND VITAMIN E: THEIR EFFECT ON COLON TUMOR FORMATION IN MICE

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SUMMARY

The effects of cabbage and vitamin E on colon carcinogenesis were investigated in Swiss mice treated with 1,2-dimethylhydrazine. Throughout the experiment the mice were fed a laboratory chow diet (46 mg vitamin E per kg) or chow containing 13 g cabbage per 100 g or 180 mg vitamin E per kg. Starting after 31 days of diet treatment the mice received 7 weekly s.c. injections of DMH. They were sacrificed 17 weeks after the first dose of DMH. While diet did not significantly alter colon tumor response, some trends were observed. Female mice given cabbage had a higher incidence (percent of mice with a tumor) and multiplicity (tumors per tumor bearing mouse) of colon tumors. Males were little affected by cabbage apart from a lower incidence of adenocarcinomas. Compared with mice fed the control diet those given vitamin E had a higher colon tumor incidence. This effect, which was stronger in females, was due to an increased incidence of adenomas. Vitamin E had little apparent affect on tumor multiplicity apart from a reduction in adenocarcinomas in females and adenomas in males. The data do not support the view that cabbage and vitamin E are protective against colon cancer.

INTRODUCTION

Several case-control studies have reported that cruciferous vegetables, paticularly cabbage, are eaten less frequently by patients with cancer of

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the colon [5,7,12]. It was therefore of interest to determine whether this effect could be reproduced in an experimental model of colon carcinogenesis.

Whether vitamin E can prevent experimental cancer is still a matter of controversy [3,4,6,8,17,23]. One interpretation for these inconsistent results is the dose of vitamin used. It is possible that vitamin E supplementation is effective at relatively low levels (200—600 mg per kg diet) but is ineffective when much larger doses are given. For instance, 500 mg vitamin E per kg diet protected mice against transplanted sarcoma cells while a 10-fold higher level was ineffective [9]. When mice were treated with DMH, fewer colon tumors were seen with 600 mg vitamin E per kg diet than with 10 mg per kg [2]. Similarly, in DMH treated rats vitamin E deficiency caused an accelerated appearance of colon tumors [19]. Conversely, 40 g vitamin E per kg diet increased colon tumor response in mice [21]. We therefore decided to examine the yield of colon tumors in mice fed diets adequate in or modestly supplemented with vitamin E.

MATERIALS AND METHODS

Mice and treatment

Swiss mice were used from a colony maintained in our animal house. At an age of 5—7 weeks they were placed on the experimental diets (mean weight, g, S.D.: males = 29.5 4.9; females = 27.6 5.5). The diets were fed ad libitum until the experiment was terminated. Mice were housed in a temperature-controlled room with a 12-h light-dark cycle.

After 31 days on the experimental diets the mice were given the first of 7 weekly s.c. injections of DMH (Sigma Chemical Co., St. Louis, MO). This was dissolved in 1 mM EDTA and neutralized with saturated sodium bicarbonate. The first dose was 23 mg DMH · diHCl/kg body wt. followed by doses of 31, 42 and then 4 doses of 56 mg/kg. The use of a gradually rising dose is based on the observation that mice develop tolerance to DMH and thus allows the number of injections to be reduced while minimizing toxic effects [20].

Diets

Diet formulation is shown in Table 1. Fresh food was given every 1—2 days.

Tumor assessment

Seventeen weeks after the first DMH injection, mice were sacrificed and internal organs grossly examined. The colon (including the cecum) was opened and carefully examined. Suspected tumors were removed and placed in neutral buffered formalin. They were confirmed after staining (H & E) [11,15].

TABLE 1

COMPOSITION OF DIETS

Ingredients	Diet, composition by weight[a]		
	Control	Cabbage	Vitamin E
Chow (%)[b]	100	87	100
Cabbage (%)[c]	—	13	—
Vitamin E (mg/kg)	46[d]	41	180[e]

[a] Composition is expressed on a dry weight basis, except cabbage which is weight before homogenization. In each case water was added to the diet (900 ml/kg chow).

[b] Rodent laboratory chow meal (Ralston Purina Co., St. Louis, MO). The diet consists mainly of cereal and vegetable foods and contains 23.4% protein, 16% neutral detergent fiber, 5.5% fat and an adequate content of all nutrients. Physiological energy is 3300 Kcal/kg.

[c] Bought locally and homogenized in tap water using a blender.

[d] Chow contains 65 i.u. vitamin E/kg. We have assumed 1 mg = 1.4 i.u.

[e] The supplemental vitamin E (D-α-tocopherol acetate, Sigma) was dissolved in a small volume of ethanol.

The above procedures were carried out by observers who were unaware as to which dietary group the samples and mice had come from. Data were analyzed by two-tailed Student's *t*-test and by chi-square.

RESULTS

DMH toxicity and body weight

Six male mice on the vitamin E diet died (33% of the group). This occurred following the first injection. Other deaths during the period of injections were insignificant (2/83) but most groups manifested symptoms of mild toxicity, particularly weight loss of about 3—12%. Diet did not affect weight gain or final body weight (Table 2).

Tumor data

The great majority of tumors were found in the mid- or distal-colon. Additionally, four were at the ileo-cecal junction. Diet had no apparent effect on the location of colon tumors. Two female, cabbage fed mice which had colon tumors, also had liver tumors.

Comparison of tumor data of mice fed diets supplemented with cabbage or vitamin E indicated no significant differences in comparison with the respective control groups. Nevertheless, certain trends are apparent. Cabbage supplemented female mice had a higher incidence (percent of mice with a tumor) and multiplicity (tumors per tumor bearing mouse) of colon tumors

TABLE 2

EFFECT OF DIETARY CABBAGE AND VITAMIN E ON THE INCIDENCE AND TYPE OF COLON TUMORS IN MICE TREATED WITH DMH

Mice were sacrificed 17 weeks after the first dose of DMH.

Diet	Sex	No. of mice	Weight[a]	% of mice with colon tumors (tumor incidence)			Colon tumors/tumor bearing mouse (tumor multiplicity)[a]		
				Total	Adenoma	Adeno-carcinoma	Total	Adenoma	Adenocarcinoma
Control	F	14	37.4 ± 3.5	42.9	42.9	35.7	3.17 ± 1.60	2.17 ± 1.17	1.00 ± 0.63
Cabbage	F	16	35.5 ± 3.5	81.3[b]	62.5	68.7	4.92 ± 4.41	3.15 ± 3.62	1.77 ± 1.42
Vitamin E	F	15	38.1 ± 3.4	80.0	73.3	33.3	2.67 ± 1.92	2.25 ± 2.13	0.42 ± 0.51
Control	M	17	39.8 ± 2.6	70.6	47.0	64.7	3.08 ± 1.73	1.92 ± 1.51	1.17 ± 0.58
Cabbage	M	16	39.3 ± 3.9	56.3	37.5	37.5	2.89 ± 2.37	1.78 ± 2.05	1.11 ± 0.93
Vitamin E	M	11	38.3 ± 3.5	81.8	63.6	63.6	2.78 ± 1.56	1.44 ± 1.23	1.33 ± 1.00

[a] Values are mean ± S.D.

[b] Two female colon tumor bearing mice also had a liver tumor.

(Table 2). Cabbage feeding had little effect on males apart from causing an apparent fall in the incidence of adenocarcinomas.

Mice, particularly females, fed supplementary vitamin E had a higher colon tumor incidence than those fed the control diet (Table 2). This was due to an increased incidence of adenomas rather than of adenocarcinomas. Tumor multiplicity was little affected by vitamin E except that there was a fall in adenocarcinomas in females and of adenomas in males.

DISCUSSION

The results presented here indicate that cabbage provides no protection against DMH induced colon tumors in mice. Indeed, in females there was actually a higher incidence and multiplicity of tumors when cabbage was given (not significant). The level of cabbage (13 g whole cabbage per 100 g diet, equivalent to 135 g per 2500 Kcals) was chosen so as to be comparable with the human situation. Our results therefore seem at variance with several case-control studies which have indicated that cabbage or related cruciferous vegetables may be protective against colon cancer [5,7,12].

Srisangnam et al. [18] also studied the effect of cabbage in DMH treated mice. In their model, tumors did not occur in the colon but were mainly in the spermatic cord followed by the liver and kidney. As in the experiment described here a low level of supplemental cabbage (10 or 20% dehydrated cabbage) caused an increase in tumor yield, albeit non-significant. However, a large cabbage intake (40% of diet) was protective.

Other evidence, apart from case-control studies, led us to expect that cabbage would be protective against experimental colon cancer. Wattenberg and others [1,13,16,22] have shown that cabbage and related cruciferous vegetables induce xenobiotic-metabolizing enzymes in rodent liver and intestine (e.g. benzpyrene hydroxylase and aryl hydrocarbon hydroxylase). This is partly or wholly accounted for by the presence of various indole compounds [10,13]. In another study it was demonstrated that feeding of these chemicals prior to challenge with polycyclic aromatic hydrocarbon carcinogens inhibits subsequent neoplasia of mouse forestomach and rat mammary gland [24].

There are several possible explanations for our failure to observe any protective effect of cabbage against colon carcinogenesis. Firstly, our control diet was Rodent Chow. This contains alfalfa, which has similar properties to cruciferous vegetables as an inducer of xenobiotic-metabolizing enzymes [1,16,22]. The cabbage may therefore have been of no further benefit. Secondly, the enzymes induced by cabbage may not be relevant to DMH induced colon carcinogenesis. Thirdly, the level of cabbage may have been too low.

We were also surprised by the trend (not significant) for supplemental vitamin E to enhance colon carcinogenesis. This occurred particularly in females and was due to a rise in the incidence of adenomas. However, tumor

multiplicity was lower in females for adenocarcinomas and in males for adenomas.

The level of supplementation used was modest (46 vs. 180 mg per kg diet, equivalent to 35 vs 136 mg/2500 Kcal as compared to a typical human intake of 8 mg/2500 Kcal). Thus the data does not support our hypothesis that vitamin E in that dose range is anti-carcinogenic. Similar observations were recently reported by Reddy and Tanaka [14]. They observed that supplemented vitamin E (50 vs. 750 mg per kg diet) did not affect the incidence of colon tumors in DMH treated rats but tended to increase tumor multiplicity. Possibly vitamin E is protective when the 'low' vitamin E diet is deficient or marginal, rather than nutritionally adequate as in our control diet. Such a possibility is consistent with other studies on DMH induced colon tumors in rodents. Thus vitamin E supplementation was protective in mice using a control diet containing only 10 mg per kg [2] while in rats it delayed tumor appearance as compared to a deficient diet (under 5 mg per kg) [19]. This should be further investigated.

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