Selenium and Cabbage and Colon Carcinogenesis in Mice [1]

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ABSTRACT—The influence of dietary selenium and cabbage on the formation of colon tumors in female Swiss mice treated with 1,2-dimethylhydrazine [(DMH) CAS: 540-73-8] was reported. Mice received a control diet (laboratory chow), the control diet plus selenium in the drinking water (1 mg/liter), or the control diet with added cabbage (12.8 g/100 g diet). They also received 8 weekly sc injections of DMH. The experiment was divided into two time periods: *a*) from 5 weeks before the first injection until 3 days after the last one (initiation period), and *b*) the subsequent 19.5 weeks until sacrifice of the mice (promotion period). Selenium had a strong protective effect when given during the initiation period; adenomas were reduced to a much greater extent than adenocarcinomas. The only effect of selenium supplementation in the promotion period was a small decrease in adenomas. Cabbage apparently had two opposing actions. It increased tumor incidence, particularly adenocarcinomas, if given in the initiation period, but it reduced adenoma formation considerably when given in the promotion period.—JNCI 1987; 79:1131-1134.

A finding that emerged from several case-control studies is that colon cancer patients have a low intake of cabbage and other cruciferous vegetables (*1–3*). Experiments on rodent liver and intestine indicate that these foods are effective by inducing xenobiotic-metabolizing enzymes such as benzpyrene hydroxylase and aryl hydrocarbon hydroxylase (*4–7*).

On the basis of these findings, Temple and El-Khatib (*8*) tested whether cabbage provides protection against DMH-induced colon carcinogenesis in mice. Although differences were not significant, cabbage tended to increase colon tumor formation in females though not in males. There are at least two possible explanations, apart from chance, for these surprising results. First, the stock diet was Purina Rodent Chow. This contains alfalfa, which resembles cruciferous vegetables as an inducer of xenobiotic-metabolizing enzymes (*5–7*). Thus the cabbage may have been without additional effect. Second, the enzymes induced by cabbage might actually enhance the carcinogenicity of DMH.

Several lines of evidence strongly indicate that selenium is also protective against cancer. Thus epidemiologic studies have revealed an inverse relationship between selenium intake and several major cancers (including of the colon) (*9*). Furthermore, cancer patients often have a history of a low-serum selenium level (*9*). Of 37 animal studies recently reviewed, two-thirds reported that supplementary selenium caused a reduction in tumor incidence of at least 35% (*9*). The rat colon is one of the tumor models for which this has been well established (*10–15*). With carcinogen-induced mammary tumors in rats and mice, it appears that selenium is protective at several stages of carcinogenesis but particularly during early promotion (*16*). However, its stage of effectiveness for colon carcinogenesis is unknown.

The present study was undertaken to investigate the effects of cabbage and selenium on colon tumor formation in DMH-treated mice. To identify the stages at which they are effective, we fed these dietary components during either the initiation or promotion periods.

MATERIALS AND METHODS

Mice and treatment.—Female swiss (ICR) mice were used from a colony maintained in the university animal facilities. They were housed in a temperature-controlled room with a 12-hour light-dark cycle. At an age of 5–7 weeks they were placed on the experimental diets (mean weight, g, ± SD: 21.8±2.2). After being fed these diets for 5 weeks, the mice were given 8 weekly sc injections of DMH (CAS: 540-73-8; Sigma Chemical Co., St. Louis, Mo.). This was dissolved in 1 m*M* EDTA and neutralized with saturated sodium bicarbonate. The first dose was 17 mg DMH·diHCl/kg body weight; each successive dose was increased by 21% (total dose: 291 mg/kg). Use of a gradually rising dose is based on the finding that mice develop tolerance to DMH; thus the number of injections is cut while still minimizing toxic effects (*17*).

The following dietary treatments were used: *a*) Control diet: 98.1% Wayne Rodent Blox meal (Continental Grain Co., Chicago, IL) and 1.9% corn oil. Wayne meal consists mainly of cereal and vegetable foods (but no alfalfa) and contains (dry weight basis) 24% protein, 3.6% crude fiber, 4.1% fat, 0.15 mg selenium/kg, and an adequate concentration of all nutrients. Metabolizable energy is 2,970 kcal/kg.

b) Cabbage diet: 12.8% cabbage, 85.5 Wayne Rodent Blox meal, and 1.6% corn oil. The cabbage was purchased locally and homogenized in tap water by means of a blender.

Both diets were prepared by mixing the ingredients with water (total of 1 liter/kg chow), and the food was

ABBREVIATIONS USED: BOP = *N*-nitrosobis(2-oxopropyl)amine; DMH = 1,2-dimethylhydrazine; PAH = polycyclic aromatic hydrocarbon.

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given in this form. Fresh food was given every 1-2 days (usually daily) from a stock prepared roughly every 5 days and stored at 5°C.

c) Selenium: sodium selenite in drinking water, providing 1 mg selenium/liter.

In each case food and water were provided ad libitum. Where indicated, dietary treatments were changed 3 days after the last injection.

Tumor assessment.—Mice were sacrificed 27 weeks after the first DMH injection. After inspecting internal organs, 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 (hematoxylin and eosin) (*18, 19*). The above procedures were done by observers who were unaware as to which dietary group the samples and mice belonged. Data were analyzed by two-tailed Student's *t*-test and by Fisher's exact probability test.

RESULTS

Diet intake.—Diet intake was measured in the week before the first injection and 4 weeks after the final injection. Mice on the control diet ate 4.9 g/mouse per day (dry weight basis). Intake of cabbage diet was 5.3 g/mouse per day comprising 0.68 g whole cabbage and 4.6 g other components (dry weight). Selenium-supplemented mice consumed approximately 4.2 ml/mouse per day (4.2 μg selenium). This increased their daily selenium intake from 0.7 μg (provided by the control diet) to 4.9 μg.

Body weight.—Diet had no apparent effect on body weight gain during the experiment. Weights when sacrificed are shown in tables 1 and 2. At that time the non-DMH-treated controls were heavier than the other mice ($P<.001$). This averaged 12.2 g in DMH-treated mice and 19.1 g in non-DMH-treated mice.

Tumor data.—Tumors were almost entirely confined to the distal half of the colon, generally 1-5 cm from the anus, with a smaller number at the anus. Additionally, 1 was in the cecum and 1 was in the uterus. Diet had no apparent effect on tumor size or location. Tumors were not detected in the control group receiving no DMH (table 2).

When selenium was given during the initiation period, tumor incidence fell by half ($P<.025$) and tumor multiplicity (tumors per tumor-bearing mouse) fell by 36% (table 1). The decrease in adenomas (76% for incidence, $P = .001$; 46% for multiplicity) was much greater than for adenocarcinomas. On the other hand, the only effect of selenium supplementation during the promotion period was a small and nonsignificant decrease in the incidence and multiplicity of adenomas. Selenium showed only a small protective effect if given for the whole experiment.

When cabbage was fed during the initiation period, there was a modest increase in tumor incidence, particularly adenocarcinomas (table 2). However, feeding it in the promotion period resulted in a drop in adenoma formation: Incidence was down 30%, and multiplicity was down 50% ($P<.05$).

DISCUSSION

These results provide the first demonstration of the stage of effectiveness of selenium against colon tumor formation. When mice were given selenium supplementation at a relatively modest level (1 mg/liter drinking water) during the initiation period (i.e., before and during DMH treatment), there was a substantial drop in the incidence and multiplicity of tumors. Adenoma, rather than adenocarcinoma, was the tumor type mainly affected. Selenium supplementation during the promotion period (i.e., starting only after the last DMH injection) gave merely a little protection against adenomas. Curiously, when selenium was given throughout the experiment, only a little protective effect was seen.

With carcinogen-induced mammary tumors in rats and mice, however, although the evidence is not altogether clear, selenium appears to act at several stages with its major action being during early promotion (*16*).

Originally it was predicted that cabbage would protect mice against DMH-induced colon cancer. This was based on case-control studies of colon cancer patients (*1-3*). The presumed mechanism is that cabbage and related vegetables contain various indole compounds that induce xenobiotic-metabolizing enzymes and thereby

TABLE 1.—*Effect of dietary selenium on the incidence and type of colon tumors in mice treated with DMH*

Dietary treatment[a]		No. of mice	Weight,[b] g	Percent of mice with colon tumors—tumor incidence[c]			Colon tumors/tumor-bearing mouse—tumor multiplicity[b,c]		
Initiation	Promotion			Total	Adenoma	Adenocarcinoma	Total	Adenoma	Adenocarcinoma
Control	Control	40	35.2±6.1	65.0	57.5	32.5	2.69±3.00	1.85±1.93	0.85±1.29
Selenium	Control	22	34.0±4.4	31.8[d]	13.6[e]	22.7	1.71±1.50	1.00±1.53	0.71±0.49
Control	Selenium	24	33.3±4.7	62.5	50.0	33.3	2.27±2.79	1.47±1.73	0.80±1.26
Selenium	Selenium	21	34.5±4.5	57.1	42.9	38.1	2.58±1.98	1.42±1.51	1.17±1.11

[a] Mice received the control diet or the control diet plus selenium. The indicated dietary treatments were given from 5 wk before the first DMH injection until 3 days after the last one (initiation) or for the following 19.5 wk until sacrifice of the mice (promotion period).
[b] Values are means ± SD.
[c] For statistical analysis of tumor data, comparisons were made only with the unsupplemented control group.
[d] Significantly different by Fisher's exact probability test, $P<.025$.
[e] Significantly different by Fisher's exact probability test, $P=.001$.

TABLE 2.—*Effect of dietary cabbage on the incidence and type of colon tumors in mice treated with DMH*[a]

Dietary treatment		No. of mice	Weight, g	Percent of mice with colon tumors—tumor incidence			Colon tumors/tumor bearing mouse—tumor multiplicity		
Initiation	Promotion			Total	Adenoma	Adenocarcinoma	Total	Adenoma	Adenocarcinoma
Control	Control	40	35.2±6.1	65.0	57.5	32.5	2.69±3.00	1.85±1.93	0.85±1.29
Cabbage	Control	25	32.7±3.9	80.0	68.0	48.0	3.15±2.60	2.00±1.59	1.15±1.23
Control	Cabbage	20	33.8±3.4	60.0	40.0	35.0	1.67±0.98	0.92±0.79[b]	0.75±0.75
Cabbage	Cabbage	22	33.3±4.1	54.5	45.5	40.9	3.42±2.35	2.00±1.48	1.42±1.08
Control[c]	Control	7	41.3±5.3[d]	0	0	0	—	—	—

[a] Mice received the control diet alone or with cabbage. Other details are as in table 1, footnotes *a*, *b*, and *c*.
[b] Significantly different by Student's *t*-test, $P<.05$.
[c] Non-DMH-treated control group.
[d] Significantly different from other mice combined by Student's *t*-test, $P<.001$.

direct carcinogens toward a detoxification pathway (*4–7, 20*).

In this study we used a level of cabbage comparable to that in human diets (12.8 g/100 g diet, equivalent to 118 g/2,500 kcal). It appears that cabbage has two distinct effects. Feeding it in the initiation period modestly increased tumor incidence, particularly adenocarcinomas. When fed during the promotion period, incidence and multiplicity of adenomas fell. This antipromotion action may reflect the reportedly strong cation-exchange capacity of cabbage fiber (*21*).

The apparent enhancement of carcinogenesis after feeding cabbage during the initiation period resembles other experiments on DMH-treated animals. Temple and El-Khatib (*8*) observed that feeding cabbage to mice throughout the experiment tended to increase colon tumor formation. Pence et al. (*22*) recently studied indole-3-carbinol, an inducer of xenobiotic metabolizing enzymes and one of the compounds thought to explain the supposed anticarcinogenic action of cabbage. Feeding it increased adenocarcinoma formation in the rat intestine (mainly colon). Similarly, Srisangnam et al. (*23*) observed that cabbage tended to increase tumor yield in mice. However, in their experiment the tumors were not in the colon but mainly in the spermatic cord followed by the liver and kidney. Cabbage has also been reported to increase the incidence of pancreatic carcinoma in hamsters treated with BOP (*24*).

In contrast to the above findings when the carcinogen is a PAH, a protective effect has been observed. Thus indole-3-carbinol and related compounds protected rats against mammary tumors induced by 7,12-dimethylbenz[*a*]anthracene and tumors of the forestomach induced by benzo[*a*]pyrene (*25*).

How are these seemingly contradictory results to be explained? It is likely that cabbage and its active ingredients consistently induce xenobiotic metabolizing enzymes. In some cases, such as DMH and BOP, this enhances the production of the ultimate carcinogen, whereas with PAH the reverse is true. Since cabbage and other cruciferous vegetables are apparently protective against human colon cancer, this indicates that the carcinogen responsible has a metabolism (and a chemical structure?) resembling PAH rather than DMH or BOP. This indication suggests a probable avenue of further research.

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