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Factors Influencing the Anticarcinogenic Efficacy of Selenium in Dimethylbenz[a]anthracene-induced Mammary Tumorigenesis in Rats[1]

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ABSTRACT

The present study was designed to investigate the effect of selenium supplementation on dimethylbenz[a]anthracene-induced mammary tumorigenesis in female Sprague-Dawley rats fed either a 5 or 25% corn oil diet, denoted as low fat (LF) or high fat (HF), respectively. Selenium supplementation of LF and HF diets were begun at 21 days of age. In Experiment 1, rats (50 days of age) were given 5 mg of dimethylbenz[a]-anthracene p.o. and were supplemented with 0.1 (adequate level), 0.5, 1.5, and 2.5 ppm of selenium (as sodium selenite) in the diet. The total number of tumors found were as follows (30 rats/group): 26, 23, 19, and 10, respectively, in the LF group; and 65, 66, 41, and 21, respectively, in the HF group. In Experiment 2, rats (50 days of age) were given 10 mg of dimethylbenz[a]anthracene, and selenium was added to the diets at 0.1, 2.5, and 5.0 ppm. Tumor yields were found to be 71, 32, and 15, respectively, in the LF group and 135, 85, and 46, respectively, in the HF group. There was also a trend towards a longer latency period of tumor appearance with selenium supplementation. In conclusion, high dietary selenium levels are able to protect against mammary tumorigenesis, but rats on a HF diet still develop more tumors than those on a LF diet at comparable selenium supplementation.

INTRODUCTION

One aspect of current research in selenium is focused on its ability to inhibit tumor development in laboratory animals. There are many reports in the literature documenting that selenium supplementation above nutritional requirement protects against neoplasia induced by carcinogens (5, 8, 9, 17, 19, 25–28) or viruses (20, 22, 23). The experimental models that have been examined include tumors of the mammary tissue, colon, liver, and skin. Selenium is also known to limit the growth of transplantable Ehrlich ascites tumor cells (6). In most cases, selenium is added to the diet or the drinking water; parenteral administration, although less commonly practiced, is just as effective as the p.o. route.

Very little is known about the relationship between the host nutritional status and the anticarcinogenic efficacy of selenium. Of particular interest is the fat intake of the animals, since diets rich in fat are known to enhance mammary tumor development in rodents (3, 10, 11, 18). We have reported previously (12, 14) that augmentation of mammary tumorigenesis due to selenium deprivation depends on the fat intake of the animals. The present study was designed to investigate the optimal level of selenium supplementation on the inhibition of DMBA[2]-induced mammary carcinogenesis in rats that were given different doses of carcinogen and fed either a high-fat or a low-fat diet.

MATERIALS AND METHODS

Animals and Diets. Female Sprague-Dawley rats were purchased from Charles River Breeding Laboratories, Wilmington, Mass. They were fed a synthetic Torula yeast-based regimen containing either 5 or 25% corn oil from weaning (21 days of age) until termination of the experiment. Food and water were available *ad libitum*. The composition of these diets has been reported previously (14), with the exception that $ZnCl_2$ was added in the present experiments to provide 12 mg of Zinc per kg of diet. The diets were formulated such that the intake of all nutrients would be the same except for fat and dextrose, assuming that rats would consume an equal number of calories. The basal diets were deficient in selenium and contained less than 0.02 ppm of selenium as determined by fluorometric analysis (1). Selenium, in the form of sodium selenite, was supplemented to each of the basal diets at different levels as indicated in the tables. Animals were weighed weekly to monitor their growth.

Mammary Tumor Induction. Mammary tumors were induced by i.g. administration of 5 (Experiment 1) or 10 mg (Experiment 2) of DMBA (Sigma Chemical Co., St. Louis, Mo.) at 50 days of age. The animals were fasted 12 hr before and after administration of DMBA, which was dissolved in corn oil. The method of DMBA administration was described in detail previously (11). Rats were palpated once a week, tumors were identified by their location, and the size was determined by measurement with a vernier caliper. Experiments were terminated 22 weeks after carcinogen administration. At autopsy, tumors were excised, fixed in Bouin's reagent, and sectioned for histology.

Hormone Assays. Serum prolactin, estrone, and estradiol were determined by radioimmunoassays as described previously (13, 15). Animals were sacrificed at proestrus; the stages of the estrus cycle were ascertained by the vaginal smear technique.

RESULTS

Table 1 shows the effect of various levels of selenium supplementation on the mammary tumor incidence of rats that were fed either a 5 or 25% corn oil diet and given 5 mg of DMBA (Experiment 1). At 0.1 ppm of selenium, which is considered to meet the nutritional requirement of rats (control level), the incidence was 40 and 70%, respectively, in the 5 and 25% fat groups. No inhibition of tumorigenesis was detected at 0.5 ppm of selenium. Supplementation with 1.5 and 2.5 ppm of selenium reduced the incidence to 29 and 24%, respectively, in the low-fat group, and 55 and 33%, respec-

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[2] The abbreviations used are: DMBA, 7,12-dimethylbenz[a]anthracene; i.g., intragastric(ally).

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tively, in the high-fat group. It should be pointed out that, at the 2.5-ppm selenium level, a tumor incidence of 24% in the low-fat group was not statistically different from the control value of 40%, possibly due to small sample size. However, the decrease in tumor incidence from 70 to 33% in the high-fat group was highly significant ($p < 0.01$).

Table 2 shows the initial and final body weights, total tumor yield, latency period of tumor appearance, and rate of tumor growth in the various groups of Experiment 1. Supplementation of selenium, even at 2.5 ppm, did not influence the growth rate of the rats (results not shown). The initial and final body weights were very similar in all 8 groups. Over 90% of the tumors obtained in each group were adenocarcinomas, suggesting that selenium had no effect on the proliferation of malignant *versus* benign lesions.

Compared to the 0.1-ppm selenium intake level (control), supplementation at 2.5 ppm reduced the total number of tumors from 26 to 10 in rats on the low-fat diet and from 65 to 21 in rats fed the high-fat diet. This decrease was obscured when results were expressed as number of tumors per tumor-bearing rat. Despite an apparent reduction in the average number of tumors found per rat, the differences were not statistically significant. Such analysis suggests that animals might have different sensitivity to selenium. There was also a trend towards a longer latency period of tumor appearance in proportion to selenium intake. However, the delay was significant only in the high-fat 2.5-ppm selenium-supplemented group when compared to its control ($p < 0.05$).

Selenium did not seem to affect the growth rate of the tumors once they became palpable, as measured by the increase in tumor diameter during the initial month of development. From our experience with this tumor model, we found that about 40 to 50% of the tumors stopped growing or even regressed 6 to 8 weeks after they became palpable. We therefore divided the tumors into 2 categories at the time of sacrifice: those with continued growth and those that stopped growing or regressed Analysis of the data showed that selenium supplementation had no effect on the distribution of these 2 classes of tumors.

In Experiment 2, rats were given 10 mg of DMBA, and selenium was added to the diet at 0.1, 2.5, and 5.0 ppm. The results are shown in Tables 3 and 4. At these different levels of selenium, tumor incidence (Table 3) was found to be 70, 45, and 23%, respectively, in the low-fat group and 100, 77, and 55%, respectively, in the high-fat group. The decrease in either group from the control value was statistically significant. It is worth noting that, with this higher dose of DMBA, the enhanced incidence due to dietary fat was detected at all levels of selenium supplementation ($p < 0.02$).

As can be seen in Table 4, supplementation of selenium at 5 ppm resulted in a 10% decrease in the growth rate of rats compared to those given 0.1 ppm. This was most likely due to reduced diet consumption in the former group. Periodic measurement of food intake confirmed this assumption. Supplementation of selenium at the 5-ppm level reduced the total tumor yield from the control level of 71 to 15 in the low-fat group and from 135 to 46 in the high-fat group. As in the previous experiment, the reduction was obscured when results were expressed as number of tumors per tumor-bearing rat (differences not statistically significant). High selenium supple-

Table 1

Effect of selenium supplementation on DMBA-induced mammary tumorigenesis in rats fed either a 5 or 25% fat diet

Rats were given 5 mg of DMBA i.g. (Experiment 1).

Selenium supplement (ppm)	Tumor incidence		χ^2 analysis
	5% Fat	25% Fat	
0.1	12/30 (40.0%)	21/30 (70.0%)	$p < 0.02$
0.5	11/30 (36.7%)	20/29 (68.9%)	$p < 0.02$
1.5	9/31 (29.0%)	16/29 (55.2%)	$p < 0.05$
2.5	7/29 (24.1%)[a]	10/30 (33.3%)[b]	NS[c]

[a] Not significantly different from control 0.1-ppm level value.
[b] Significantly different from control 0.1-ppm level value ($p < 0.01$).
[c] NS, not significant.

Table 3

Effect of selenium supplementation on DMBA-induced mammmary tumorigenesis in rats fed either a 5 or 25% fat diet

Rats were given 10 mg of DMBA i.g. (Experiment 2).

Selenium supplement (ppm)	Tumor incidence		χ^2 analysis
	5% Fat	25% Fat	
0.1	21/30 (70.0%)	30/30 (100%)	$p < 0.02$
2.5	13/29 (44.8%)[a]	23/30 (76.7%)[b]	$p < 0.02$
5.0	7/30 (23.3%)[c]	16/29 (55.2%)[c]	$p < 0.02$

[a] Significantly different from control 0.1-ppm level value ($p < 0.05$).
[b] Significantly different from control 0.1-ppm level value ($p < 0.01$).
[c] Significantly different from control 0.1-ppm level value ($p < 0.001$).

Table 2

Effect of selenium supplementation on body weight, tumor yield, and rate of tumor growth in rats fed either a 5 or 25% fat diet

Rats were given 5 mg of DMBA i.g. (Experiment 1).

Dietary group (% fat)	Selenium supplement (ppm)	Initial body wt (g)	Final body wt[a] (g)	Total no. of tumors	No. of tumors/tumor-bearing rat	Av. latency period[b] (days)	Rate of tumor growth[c] (mm/month)
5	0.1	152 ± 2[d]	290 ± 6[d]	26	2.2 ± 0.3[d]	92 ± 7[d]	14 ± 3[d]
	0.5	150 ± 2	292 ± 6	23	2.1 ± 0.3	89 ± 6	12 ± 3
	1.5	151 ± 3	289 ± 6	19	2.1 ± 0.3	97 ± 7	12 ± 3
	2.5	150 ± 3	288 ± 7	10	1.4 ± 0.2	109 ± 8	10 ± 2
25	0.1	153 ± 2	294 ± 6	65	3.1 ± 0.4	85 ± 6	13 ± 3
	0.5	155 ± 3	290 ± 6	66	3.3 ± 0.5	86 ± 6	15 ± 3
	1.5	151 ± 2	290 ± 7	41	2.6 ± 0.4	92 ± 7	14 ± 3
	2.5	151 ± 3	288 ± 7	21[e]	2.1 ± 0.3	106 ± 7[f]	11 ± 3

[a] Final body weight refers to carcass weight, *i.e.*, body weight minus tumor weight.
[b] Time between DMBA administration and the appearance of the first palpable tumor.
[c] Linear regression used to calculate the increase in diameter for the initial month of growth.
[d] Mean ± S.E.
[e] Significantly different from the control 0.1-ppm level value ($p < 0.01$).
[f] Significantly different from the control 0.1-ppm level value ($p < 0.05$).

ment also resulted in a consistent prolongation of the latency period.

In order to determine the contribution of reduced food intake to the inhibitory response observed at 5 ppm of selenium, another experiment was initiated in which the 0.1-ppm selenium-supplemented rats were pair fed with those that were given 5 ppm of selenium. Rats were treated with 10 mg of DMBA i.g. The results are shown in Table 5. Rats in Group 1 were fed *ad libitum* either a 5 or 25% fat diet containing 0.1 ppm of selenium. At 22 weeks after carcinogen administration, 68 and 96% of the animals, respectively, developed tumors. When the animals were subjected to the same dietary regimens but were pair fed (approximately 10% decrease in food intake) with those that were given 5 ppm of selenium, there was a slight decrease in tumorigenesis in both the low-fat and high-fat groups to 56 and 80%, respectively (Group 3). This was compared to an incidence of 25 and 48%, respectively, in the *ad libitum*-fed selenium-supplemented low-fat and high-fat rats (Group 2). The relative contribution of reduced food intake and selenium to inhibition of tumorigenesis was perhaps better assessed by comparing the total tumor yield per group. In the low-fat rats, food restriction alone reduced the number of tumors formed from 51 to 39 (76%), whereas selenium decreased this further to 13 (25%). In the high-fat rats, food restriction alone decreased the tumor yield from 103 to 78 (76%), and selenium lowered this to 31 (30%).

Since the development of DMBA-induced mammary tumors is very much dependent on the influence of circulating estrogens, progesterone, and prolactin, the concentrations of estrone, estradiol, and prolactin in the serum were determined in non-tumor-bearing rats that were fed a 25% fat diet, supplemented with either 0.1 or 2.5 ppm of selenium. Rats (25 rats/group) were maintained on these diets for 8 weeks before sacrifice. The estrus cycle was monitored by daily vaginal smear for 3 cycles. Cytology was analyzed according to the method of Bukovsky *et al.* (2), scored on a scale of 1 to 10 based on the number of different cell types observed. There was no difference in the estrus cycling regardless of selenium intake. Serum hormone levels (determined at proestrus) in the respective 0.1- and 2.5-ppm selenium-supplemented groups were as follows: prolactin (ng/ml), 296 ± 38 (S.E.) and 334 ± 47; estrone (pg/ml), 142 ± 20 and 120 ± 17; estradiol (pg/ml), 100 ± 15 and 122 ± 21. Thus, selenium supplementation did not result in any significant alterations in blood prolactin and estrogen levels.

DISCUSSION

The present study confirms previous findings by other investigators that selenium supplementation above dietary requirement inhibits tumorigenesis. A review of this subject by Griffin (7) appeared recently. In addition, our observations lead to the conclusion that the optimal level of selenium for manifestation of this protective effect depends on the dose of the carcinogen and the nutritional status of the animal, specifically in relation to fat intake. Higher selenium supplementation is necessary to neutralize the insult produced by a larger dose of DMBA, a finding reminiscent of a report by Wortzman *et al.* (29) which showed that the ability of selenium to defend against 2-acetylaminofluorene-induced hepatic DNA damage is readily over-

Table 4

Effect of selenium supplementation on body weight, tumor yield, and rate of tumor growth in rats fed either a 5 or 25% fat diet

Rats were given 10 mg of DMBA i.g. (Experiment 2).

Dietary group (% fat)	Selenium supplement (ppm)	Initial body wt (g)	Final body wt[a] (g)	Total no. of tumors	No. of tumors/tumor-bearing rat	Av. latency period[b] (days)	Rate of tumor growth[c] (mm/month)
5	0.1	154 ± 3[d]	294 ± 7[d]	71	3.4 ± 0.5[d]	71 ± 6[d]	11 ± 2[d]
	2.5	155 ± 3	290 ± 7	32[e]	2.5 ± 0.3	81 ± 6	9 ± 2
	5.0	139 ± 3[e]	259 ± 6[e]	15[e]	2.1 ± 0.3	95 ± 7[f]	9 ± 2
25	0.1	156 ± 2	289 ± 6	135	4.5 ± 0.6	65 ± 5	13 ± 3
	2.5	155 ± 3	290 ± 7	85[e]	3.7 ± 0.5	73 ± 6	11 ± 2
	5.0	142 ± 3[e]	255 ± 6[e]	46[e]	2.9 ± 0.4	88 ± 7[g]	10 ± 2

[a] Final body weight refers to carcass weight, *i.e.*, body weight minus tumor weight.
[b] Time between DMBA administration and the appearance of the first palpable tumor.
[c] Linear regression used to calculate the increase in diameter for the initial month of growth.
[d] Mean ± S.E.
[e] Significantly different from the control 0.1-ppm level value ($p < 0.01$).
[f] Significantly different from the control 0.1-ppm level value ($p < 0.05$).
[g] Significantly different from the control 0.1-ppm level value ($p < 0.02$).

Table 5

Determination of the influence of reduced food intake on the anticarcinogenic effect of selenium

Rats were fed 10 mg of DMBA i.g. There were 25 rats/group.

Group	Dietary fat (%)	Mode of feeding	Selenium supplement (ppm)	Final body wt (g)	Tumor incidence (%)	Total no. of tumors
1	5	*Ad libitum*	0.1	297 ± 6[a]	68	51
	25	*Ad libitum*	0.1	298 ± 7	96	103
2	5	*Ad libitum*	5.0	262 ± 6	25[b]	13[b]
	25	*Ad libitum*	5.0	260 ± 6	48[b]	31[b]
3	5	Pair fed with Group 2	0.1	260 ± 7	56	39
	25	Pair fed with Group 2	0.1	265 ± 7	80	78[c]

[a] Mean ± S.E.
[b] Significantly different from control value in Group 1 ($p < 0.01$).
[c] Significantly different from control value in Group 1 ($p < 0.02$).

come by increasing the dose of the carcinogen. However, we found that selenium was unable to counteract completely the enhancing effect of dietary fat in mammary carcinogenesis, since rats fed a high-fat diet still developed more tumors than those fed a low-fat diet at comparable selenium intake level.

Little information is available on the mode of action of selenium as an anticarcinogenic agent. Reports from the laboratory of Daoud and Griffin (4) and Marshall *et al.* (19) showed that selenium impedes activation and accelerates detoxification of 2-acetylaminofluorene. Other mechanisms that have been discussed by Schrauzer (21) include the glutathione peroxidase antioxidant activity, the immune system, and the repair of chromosomal damage. However, he cautioned that none of these selenium-related biochemical functions has been convincingly established in its anticarcinogenic action.

The most common route of selenium administration is in the food or drinking water. Rats consume at least twice as much water as food on a weight basis. A concentration of 4 ppm of selenium in the drinking water would be equivalent to 8 ppm in the diet. Jacobs (16) reported that 4 ppm of selenium in the drinking water did not influence the body weight or the survival of male Sprague-Dawley rats treated with dimethylhydrazine. In our experiment, 5 ppm of selenium in the diet caused a decrease in growth of female Sprague-Dawley rats. Such an observation is in agreement with the finding by Schroeder and Mitchener (24) that 2 ppm of selenium (as selenite) in the drinking water suppressed growth and led to early mortality in male, though less in female, Long-Evans rats. Thus, caution should be exercised in using high levels of selenium in experimental research.

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