Selenium as an Anticarcinogen

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The role that selenium may play as an anticarcinogen in the chemoprevention of murine mammary carcinogenesis is discussed. Consideration is given to the inhibitory activity of selenium against both the initiation and promotion stages of 7,12-dimethylbenz[a]anthracene- and 1-methyl-1-nitrosourea-induced mammary carcinogenesis in the rat. The data presented provide evidence that selenium can block both the metabolic sequence that initiates neoplastic transformation and the series of events that follow exposure of the organism to a carcinogenic agent and that promote the development of a malignancy.

Historically the major interest in selenium has been as a toxicant (Smith et al., 1937) and carcinogen (Nelson et al., 1943). Only in the past 25 years has attention shifted to the role that selenium plays in physiological processes as an essential component of the diets of men and animals (Schwartz and Foltz, 1957). The potential use of selenium as an anticarcinogen has received attention only in the past decade (Griffin, 1982). At this time, a significant effort is being directed to the elucidation of the activity of selenium as a chemopreventive agent. Chemoprevention refers to the intake or use of chemical agents to interrrupt either the metabolic sequence that initiates neoplastic transformation or the series of events that follow exposure of an organism to a carcinogenic agent and that promote the development of a malignancy. In this symposial presentation evidence is presented that selenium acts as a chemopreventive agent against mammary carcinogenesis.

Mammary carcinogenesis is considered a multistage process; however, most investigations have focused on the two general phases of initiation and promotion. Whereas the initiation stage of mammary carcinogenesis is considered to be short in its duration, promotion is believed to be a protracted process. In most studies of chemically induced rat mammary carcinogenesis, either 7,12-dimethylbenz[a]anthracene (DMBA) or 1-methyl-1-nitrosourea (MNU) has been used as the carcinogen. These two compounds can be employed with great utility in investigating the effects of selenium on initiation and promotion. DMBA requires activation by the mixed function oxygenase system (Sims et al., 1974), whereas MNU is a direct alkylating agent (Krüger et al., 1970). This difference permits the delineation of the effects of selenium on carcinogen activation from those that may be exerted on other aspects of the process of initiation. On the other hand, the process of promotion is thought to run essentially the same course independent of the carcinogen used to induce mammary gland neoplasms. Nonetheless, MNU does induce tumors that have biological characteristics closer to those of human breast cancers than does DMBA (McCormick et al., 1981).

Overview of the Inhibition of Experimental Breast Cancer by Selenium. *Studies in Rats.* Feeding selenium *prior* to and for a *short period after* administration of 7,12-dimethylbenz[a]anthracene has been reported to reduce cancer incidence and the number of cancers per animal and to prolong cancer latency (Thompson and Tagliaferro, 1980; Thompson et al., 1981a,b, 1982; Ip, 1981a; Welsch et al., 1981). The effective doses of selenium have ranged from 2 to 5 ppm in the diet and from 2 to 4 ppm in the drinking water. Inhibitory activity has been reported against doses of 5, 7.5, 10, 15, and 20 mg of DMBA/rat and in animals on 5, 20, and 25% (w/w) fat diets. Initiation of selenium treatment *subsequent* to carcinogen administration has been reported to reduce cancer incidence and the number of cancers induced and to prolong cancer latency in animals given either DMBA or MNU (Ip, 1981a; Welsch et al., 1981; Thompson and Becci, 1980; Thompson et al., 1984). Doses of either DMBA ranging from 5 to 20 mg or MNU, 50 mg/kg of body weight, were given in those studies, and the effect was observed in animals on low-fat (5% w/w) and high-fat (20 or 25% w/w) diets. It appears that a concentration of 5 ppm of selenium as sodium selenite in the diet or 2 ppm or more of selenium in the drinking water are necessary to exert a significant inhibitory response. The greatest inhibition of mammary tumorigenesis has been observed when selenium feeding is initiated prior to carcinogen treatment and is continued for the duration of the experiment (Thompson and Tagliaferro, 1980; Ip, 1980, 1981a,b; Ip and Sinha, 1981a,b). Selenium must be provided continuously to sustain the anticancer activity (Ip, 1981a; Welsch et al., 1981), an observation consistent with the fact that it is a "nonaccumulating" trace element. Combination treatments such as ovarietomy plus selenium or retinyl acetate plus selenium have been shown to have an additive protective effect, (Ip, 1981a; Thompson et al., 1981a,b; Ip and Ip, 1981). Selenium supplementation reportedly does not affect serum concentration of estrogen, progesterone, or prolactin (Ip, 1981b), nor does it modify either the ratio of hormone responsive to nonresponsive tumors induced or the frequency of induction of benign or malignant mammary gland neoplasms (Welsch et al., 1981). It has been reported that 2.5 ppm of selenium as sodium selenite inhibits development of hyperplastic al-

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Table I. Effect of Selenium on the Initiation and Promotion Stages of Chemically Induced Mammary Carcinogenesis

level of Se supplementation,[a] ppm	period of Se supplementation,[b] weeks	no. of rats	inhibition of cancer occurrence,[c] %	comments
unsupplemented	−2 to +16	20	control	laboratory chow diet (0.2 ppm of Se);
5.0	−2 to +2	20	50.5	20 mg of DMBA/rat; terminated 16
5.0	−2 to +16	20	67.5	weeks postcarcinogen (Thompson and Tagliaferro, 1980)
unsupplemented	−2 to +1	17	control	torula yeast diet (0.05 ppm of Se), 5%
0.15	−2 to +1	19	20.2	fat; 15 mg of DMBA/rat; terminated
1.05	−2 to +1	19	28.1	17 weeks postcarcinogen (Thompson
2.06	−2 to +1	16	48.3	et al., 1982)
unsupplemented	+1 to +18	25	control	laboratory chow diet (0.2 ppm of Se);
4.0	+1 to +18	25	24.3	50 mg of MNU/kg of body weight; terminated 18 weeks postcarcinogen (Thompson et al., 1981a,b)
unsupplemented	+1 to +14	25	control	laboratory chow diet (0.5 ppm of Se); 50 mg of MNU/kg of body weight; terminated 14 weeks postcarcinogen (Thompson and Becci, 1980)
unsupplemented	+1 to +19	20	control	torula yeast diet (0.1 ppm of Se), 20%
4.0	+1 to +19	20	23.8	fat; 50 mg of MNU/kg of body weight;
5.0	+1 to +19	20	35.7	terminated 19 weeks postcarcinogen
6.0	+1 to +19	20	17.4	(Thompson et al., 1984)
5.0[d]	+1 to +19	20	4.3	
6.0[d]	+1 to +19	20	21.4	

[a] Selenium supplied as sodium selenite unless otherwise noted. [b] Time zero represents the point at which carcinogen was administered. [c] The mean number of cancers per rat induced in the respective control group minus the comparable value induced in the treatment group divided by the mean number of cancers per rat induced in the control group expressed as a percent. [d] Selenium supplied as selenomethionine.

veolar nodules that are induced when rats are given 5 mg of DMBA at 50 days of age (Ip and Sinha, 1981b).

In comparison to animals on an adequate (0.1 ppm) selenium diet, selenium deficiency has been reported to enhance DMBA-induced mammary carcinogenesis (Ip and Sinha, 1981b). The enhancement was greatest in animals receiving a high polyunsaturated fat diet. Levels of mammary gland glutathione peroxidase were reduced and peroxide levels elevated in deficient animals. Thus it has been suggested that the antioxidant properties of selenium are involved in its protection against tumorigenesis. On the other hand, a lack of correlation between the anticarcinogenic efficacy of supplemental levels of selenium and their ability to suppress lipid peroxidation in mammary tissue has also been reported (Ip and Sinha, 1981a). These data have been used as evidence that the inhibitory action of supplemental selenium is not mediated by its antioxidant function in lipid metabolism. However, selenium has been reported to be more effective at a given dose of DMBA in animals consuming high levels of dietary fat (Ip, 1981b).

Studies in Mice. Selenium has been reported to reduce the incidence of mammary tumors induced by mammary tumor virus in C_3H/St female mice (Schrauzer and Ischmael, 1974; Schrauzer et al., 1976, 1978). Supplementation of the animal's drinking water with 1 ppm of selenium as sodium selenite reduced the incidence of mammary tumors from 90 to 10%. Supplementation of diets with 1 ppm of selenium as high-selenium yeast also reduced tumor incidence from 77% to 27% (Schrauzer et al., 1980). It has also been demonstrated that continuous selenium treatment was needed to sustain the effect and that initiation of selenium supplementation 13.8 months into the study resulted in a significant reduction (77% to 46%) in tumor incidence. Supplemental selenium (6 ppm of selenium as selenium dioxide) has also been reported to inhibit DMBA-induced mammary tumorigenesis in C57BL × DBA/28 F_1, $C_3H/StWe$, and Balb/c female mice (Welsch et al., 1981; Medina and Shepherd, 1980, 1981). In these studies, selenium inhibited both chemically and virally induced mammary tumors as well as DMBA-induced ductal hyperplasias and virus-induced alveolar hyperplasias. Selenium did not, however, alter the growth of established mammary tumors. It was suggested that preneoplastic lesions were the most sensitive to selenium-mediated inhibition (Medina and Shepherd, 1980, 1981; Medina and Osborn, 1981). Further, it was reported that there are preneoplastic mammary gland outgrowths that are sensitive and others that are nonresponsive to selenium-mediated inhibition.

Effects of Selenium on Process of Initiation. The interest of our laboratory in the effect(s) of selenium on the process of carcinogenesis stems from a study, the preliminary results of which we reported in 1980 (Thompson and Tagliaferro, 1980). In that investigation rats were fed a laboratory chow diet containing either no supplemental selenium or 5 ppm of selenium as sodium selenite. Animals were fed these diets from 35 to 64 days of age. At 50 days of age, the rats received a 20-mg dose of DMBA. At 64 days of age, i.e., 14 days after DMBA was given, one group of rats receiving selenium was switched to the control diet. The study was terminated 16 weeks after DMBA was given. The percent inhibition of tumor occurrence reported in that study is summarized in Table I. A reduction in cancer incidence and the number of cancers per rat and the lengthening of the tumor free time were affected by either short- or long-term selenium treatment, providing evidence that selenium had an effect on the initiation as well as the promotion stage of mammary carcinogenesis. In order to further evaluate the effect(s) of selenium on initiation, a second experiment was initiated (Thompson et al., 1982). In that study, a torula yeast diet supplemented with sodium selenite to contain either no selenium or 0.15, 1.05, or 2.06 ppm of selenium was fed from 28 to 64 days of age. Thereafter, the rats were fed a chow diet containing 0.2 ppm of selenium. At 50 days of age each rat received either 7.5 or 15 mg of DMBA. The study was terminated 17 weeks after DMBA was given. The most significant inhibitory effect of selenium was noted at the high dose of carcinogen (Table I). Analysis

Table II. Effect of Dietary Selenium on Aryl Hydrocarbon Hydroxylase Activity (AHH)

dietary selenium,[a] ppm	AHH,[b] nmol of OH-BP (mg of protein)$^{-1}$ h^{-1}
	Liver[c]
0.1	11.1 ± 1.4
5.0	12.5 ± 4.2
	Mammary Gland[c]
0.1	2.8 ± 0.2
5.0	3.4 ± 0.5

[a] Selenium was fed as sodium selenite in AIN-76 diet from 35 to 50 days of age. [b] AHH activity was assayed in liver and isolated mammary epithelial cells 24 h following the intragastric instillation of 20 mg of DMBA. NIH guidelines for the use of chemical carcinogens were followed. [c] Each value is the mean ± SE of nine animals; differences in mean values were not statistically significant.

of the data indicated a dose-dependent reduction in tumor prevalence and prolongation of the latency of tumor appearance with increasing levels of dietary selenium.

Subsequently, a series of studies was undertaken in an attempt to establish the mechanism(s) by which selenium blocks initial stages of DMBA-induced mammary gland carcinogenesis. One set of experiments was based on the work of Rasco et al. (1977). They reported that in cultured human lymphocytes selenium inhibited the activity but not the induction of aryl hydrocarbon hydroxylase (AHH), a key regulatory enzyme in determining the mutagenicity of metabolites of polycyclic aromatic hydrocarbons such as DMBA. We examined the effect of feeding diets containing either 0.1 or 5.0 ppm of selenite on the AHH activity of supernatants of rat liver and mammary gland 24 h subsequent to the induction of AHH by intragastric administration of 20 mg of DMBA (Table II). Methods were essentially identical with those outlined by Greiner et al. (1980). In agreement with the work of other investigator (Gairola and Chow, 1982), no significant differences in AHH activities were noted between treatment groups receiving either adequate or supplemental dietary selenium, although AHH was consistently higher in the tissues from animals receiving supplemental selenium. Since Martin et al. (1981) have reported that feeding supplemental dietary selenium does alter the mutagenicity of hepatic microsomal metabolites of DMBA, a study was initiated in which the effect of feeding diet containing either 0.1, 2.5, or 5.0 ppm of selenite from 28 to 50 days of age on carcinogen binding to DNA was evaluated. At 50 days of age [^{3}H]DMBA (70 Ci/mmol), 1 mCi/rat in 20 mg of carrier DMBA, was given via gastric intubation. Mammary epithelial cells were isolated, DNA was extracted via alcohol precipitation, and its specific activity was determined. The resulting data, presented in Table III, provide evidence that selenium did alter either some aspect of DMBA metabolism or some other factor that affects the susceptibility of mammary gland DNA to active metabolites of DMBA. The ultimate consequence of this (these) effect(s) as indicated by our carcinogenesis data is a lower yield of tumors.

Effects of Selenium on Promotion. The investigation of the role of selenium as a chemopreventive agent against tumor promotion has yielded considerable evidence that it can act as an antipromoter. In our initial study of this question (Thompson and Becci, 1980), selenium treatment was begun 7 days after female Sprague-Dawley rats received 50 mg of MNU/kg of body weight administered intravenously. Selenium, as sodium selenite, was incorporated into a chow diet at the concentration of 5 ppm,

Table III. Effect of Dietary Selenium on Incorporation of [^{3}H]DMBA into Mammary Epithelial Cell DNA

dietary selenium,[a] ppm	time of sacrifice after DMBA,[b] h	sp act.,[c,d] dpm/mg of DNA × 10^{-3}
0.1	6	2.2 ± 0.2
2.5	6	2.0 ± 0.3
5.0	6	4.4 ± 1.8
0.1	24	4.6 ± 0.3
2.5	24	4.0 ± 0.6
5.0	24	2.5 ± 0.1

[a] Selenium was fed as sodium selenite in AIN-76 diet from 35 to 50 days of age. [b] DMBA was given at 50 days of age. Each animal received 1 mCi of [^{3}H]DMBA (70 Ci/mmol) in 20 mg of carrier DMBA via gastric intubation. NIH guidelines for the use of chemical carcinogens were followed. [c] Each value is the mean ± SE of five animals. [d] Mammary epithelial cells were isolated following collagenase digestion and DNA of the cells was recovered via ethanol precipitation.

Table IV. Effect of Dietary Selenium on [^{3}H]Thymidine Incorporation into Mammary Epithelial Cell DNA from Carcinogen-Treated Rats

	sp act. of mammary gland DNA,[a,b] dpm/mg of DNA, for dietary selenium, ppm,[c] of	
age, days	0.1	5.0
42	27 ± 3	23 ± 6
50	26 ± 3	20 ± 10
57	35 ± 8	30 ± 5
78	54 ± 11	28 ± 10
85	119 ± 23	30 ± 10
90	71 ± 25	27 ± 24
97	50 ± 5	60 ± 11

[a] At 50 days of age animals were given a sc injection of 50 mg of MNU/kg of body weight. NIH guidelines for the use of chemical carcinogens were followed. [b] Two hours prior to sacrifice rats were given an ip injection of 50 μCi of [^{3}H]thymidine 0.72 Ci/mmol)/100 g of body weight. Mammary epithelial cells were isolated following collagenase digestion and DNA was recovered via ethanol precipitation. Each value is the mean ± SE of five rats. [c] Selenium was fed as sodium selenite in AIN-76 diet.

and the study was terminated 14 weeks after MNU was given. Selenium supplementation inhibited carcinogenesis significantly (Table I), reducing the rate of tumor occurrence and the average number of cancers per rat. In two follow-up studies, graded levels of selenium were fed during the promotion stages of DMBA- and MNU-induced mammary carcinogenesis (Thompson et al., 1984). The data resulting from that study, as partially summarized in Table I, suggest that increasing the level of selenium as sodium selenite above 5 ppm in the diet provided no additional protective effect against chemically induced mammary carcinogenesis. On an equimolar basis selenite appeared more protective and less toxic than selenomethionine. This observation is consistent with the fact that seleno amino acids are both incorporated into tissue proteins and degraded to an inorganic form of selenium in which state it is ultimately eliminated from the body. As a result of the sequestering of organic selenium in tissue proteins, it is expected that less selenium from selenomethionine would cycle into the inorganic selenium pool than when equivalent amounts of selenite are fed. The carcinogenesis data therefore provide important evidence that an inorganic form of selenium is involved in its chemoprevention of carcinogenesis.

Data presented in Table IV parallel the findings of other investigators and suggest that the antipromotional effect

of selenium occurs early in the neoplastic process and is transient. The data indicate that selenium suppresses the rate of thymidine incorporation into mammary gland DNA after carcinogen treatment. It appears that selenium exerts an antiproliferative effect that is progressively lost with advancing stages of the carcinogenic process.

Summary. An important question in the field of chemoprevention is whether the ingestion of nutritionally safe amounts of selenium can alter the course of carcinogenesis in man. Emerging evidence does suggest that selenium can inhibit the induction of cancer in experimental systems; however, the significance of this effect to the human disease process remains a matter of speculation. Of practical concern is whether differences in the amount of selenium ingested in areas of the world designated high vs. low with respect to environmental selenium are sufficient to account for a protective effect against cancer. Our data indicate that researchers investigating this problem should pay particular attention to the sources of ingested selenium since the form of selenium consumed as well as the amount may be crucial in determining the role that this essential trace element plays in cancer prevention. Furthermore, it seems likely that the interaction of selenium with other dietary factors such as vitamins A and E and dietary fat may be equally important determinants of its effect on the neoplastic process.

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