Enhancement of Mammary Tumorigenesis by Dietary Selenium Deficiency in Rats with a High Polyunsaturated Fat Intake\(^1\)

Clement Ip and Dilip K. Sinha

Department of Breast Surgery and Breast Cancer Research Unit, Roswell Park Memorial Institute, Buffalo, New York 14263

ABSTRACT

The effect of selenium depletion on mammary tumorigenesis following dimethylbenz[a]anthracene administration was examined in female Sprague-Dawley rats that were fed different levels and types of fats. Four basal diets deficient in selenium were used: (a) 1% corn oil; (b) 5% corn oil; (c) 25% corn oil; and (d) a high saturated fat diet containing 1% corn oil and 24% hydrogenated coconut oil. The comparable selenium-adequate diets were obtained by adding 0.1 ppm of selenium to each of the basal diets. In animals that received an adequate supplement of selenium, an increase in fat intake was accompanied by an increased tumor incidence when corn oil was used in the diets. A high saturated fat ration, on the other hand, was much less effective in this respect. Only in those rats that were maintained on a high polyunsaturated fat diet (25% corn oil) did selenium depletion result in a further increase in tumor incidence and tumor yield. Such an augmentation was not observed in animals given either a 1 or a 5% corn oil ration or a diet rich in saturated fat. Regardless of selenium status, almost all of the tumors formed were adenocarcinomas. An enhancement of tumorigenesis as a result of selenium deficiency in rats fed the 1% corn oil regimen was detected provided a high dose of dimethylbenz[a]anthracene was used, suggesting that alterations in dimethylbenz[a]anthracene metabolism might be involved under this condition. The antioxidant property of selenium is discussed as a possible mechanism by which selenium protects against tumorigenesis, especially in animals with a high polyunsaturated fat intake.

INTRODUCTION

Nutritional modification of carcinogenesis has enjoyed a revival of interest in the past decade because of the growing awareness that dietary deficiencies, excesses, and imbalances can play an important role in the etiology of neoplastic diseases. Although there are many reports in the literature demonstrating that selenium supplements at levels well above the dietary requirement are able to produce an inhibitory response on carcinogenesis in experimental animals (for summary, refer to Refs. 13 and 27), information is lacking on the effect of selenium deficiency on the development of neoplasia. Studies on selenium insufficiency can be easily performed in the laboratory using synthetic diets that contain Torula yeast as the protein source and a mineral mix which has no selenium salt.

Several investigators (2, 5, 12) have pointed out the strong positive association between the per capita consumption of fat and the age-adjusted mortality rate from breast cancer. Diets rich in fat are also known to enhance the incidence of chemically induced mammary tumors in rodents (6, 7, 16). In view of the well-established ability of selenium to protect against endogenous lipid peroxidation (9, 10, 14), the objective of the present study was to determine whether selenium deficiency had any differential effect on the susceptibility to DMBA\(^2\)-induced mammary tumorigenesis in rats fed different levels and types of fats.

MATERIALS AND METHODS

Female Sprague-Dawley rats (Charles River Breeding Labs., Wilmington, Mass.) were fed one of 8 synthetic diets (4 different types of diets with and without selenium supplement) from weaning until termination of the experiment. They were housed in stainless steel cages with water and food available ad libitum. The 4 basal diets were designated as follows: (a) 1% corn oil; (b) 5% corn oil; (c) 25% corn oil; and (d) a high saturated fat diet containing 1% corn oil and 24% hydrogenated coconut oil. The reason for adding a small amount of corn oil in the last described diet (d) is because a ration consisting of hydrogenated coconut oil as the only source of lipid is deficient in essential fatty acids.

Compositions of these diets are shown in Table 1. They were formulated so 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 (23). It should be pointed out that tocopherol-stripped corn oil was used in all diets. The basal diets were deficient in selenium and contained less than 0.02 ppm of selenium as determined by the fluorometric technique (1). The other 4 selenium-adequate diets were obtained by adding 0.1 ppm of selenium (23), in the form of sodium selenite, to each of the basal diets.

Mammary tumors were induced by intragastric administration of DMBA (Sigma Chemical Co., St. Louis, Mo.) at 50 days of age (16). The dosage of DMBA is denoted in Tables 2 to 4. Tumor palpation was carried out as described previously (17). Animals were sacrificed 22 weeks after DMBA treatment. At autopsy, rats were dissected and examined for nonpalpable tumors. All tumors were excised, fixed in Bouin’s reagent, and sectioned for histology.

RESULTS

Results of the effect of selenium deficiency on the incidence of DMBA-induced mammary tumors in rats fed diets containing different levels of polyunsaturated fat or a high saturated fat diet are shown in Table 2. Although tumor incidence increased in proportion to the amount of corn oil in the diet, a deficiency of selenium seemed to have minimal effect on tumor development in animals maintained on either a 1 or 5% fat ration (Group 1 versus Group 2 and Group 3 versus Group 4). Only

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\(^2\) The abbreviation used is: DMBA, 7,12-dimethylbenz[a]anthracene.
in those rats that were fed a high polyunsaturated fat diet (25% corn oil) did selenium depletion result in a marked enhancement of mammary tumorigenesis. Based on the 2 × 2 contingency method of \( \chi^2 \) analysis, the difference in tumor incidence between Groups 5 and 6 is statistically significant (\( p < 0.01 \)). Moreover, the shortest latency period of tumor appearance was observed in rats that were given a 25% corn oil diet that was deficient in selenium. In other words, the effect of selenium deficiency on mammary carcinogenesis was magnified in the presence of a high intake of polyunsaturated fat. Results in Table 2 also indicate that a diet rich in hydrogenated coconut oil was much less effective in promoting mammary carcinogenesis. Furthermore, dietary selenium deficiency failed to exert any adverse influence on the oncogenic process in rats fed the saturated fat diet (Group 7 versus Group 8).

The yield and histology of the tumors obtained from these 8 groups of animals are shown in Table 3. Selenium depletion led to only a slight increase in the number of tumors/tumor-bearing rat in the low-fat and saturated-fat groups, but the effect was more pronounced in those rats fed a high polyunsaturated fat diet. Regardless of selenium status, almost all of the tumors formed were adenocarcinomas, indicating that selenium deficiency had a negligible effect on the proliferation of malignant versus benign lesions.

Periodic measurements of food intake showed that rats fed either the 1 or 5% fat diet consumed more than rats fed the 25% fat diet with the result that all animals had very similar caloric intake (about 54 kcal/day). There was no significant variation in body weights of rats fed these different levels and types of fats. Selenium deficiency did not seem to affect the growth of the animals. Final body weights of the various dietary groups were as follows: Group 1, 283 ± 5 g; Group 2, 285 ± 5 g; Group 3, 282 ± 6 g; Group 4, 288 ± 6 g; Group 5, 289 ± 7 g; Group 6, 292 ± 4 g; Group 7, 282 ± 4 g; and Group 8, 288 ± 5 g.

In an attempt to determine if selenium deficiency could influence the dose-dependent mammary tumorigenesis by DMBA, rats on the 1% corn oil diet were treated with 5, 10, or 15 mg of DMBA at 50 days of age. Results are shown in Table 4. It can be seen that selenium depletion did not have any significant effect on DMBA carcinogenesis at lower doses (5 or 10 mg). Tumor incidence and tumor yield were increased by selenium deficiency only when a higher dose (15 mg) of DMBA was used (\( p < 0.02; \chi^2 \) analysis), suggesting that alterations in DMBA metabolism might be involved.

**DISCUSSION**

The most notable finding in the present study is the observation that rats on a high polyunsaturated fat ration were more sensitive to selenium deficiency in manifesting a further enhancement of DMBA-induced mammary tumorigenesis. Both tumor incidence and the number of tumors/tumor-bearing rat were elevated in rats fed a 25% corn oil diet that was deficient in selenium as compared to those maintained on a similar regimen but supplemented with an amount of selenium that met the nutritional requirement. Such an augmentation was not detected in animals given either a low fat diet or a diet rich in saturated fat. In our experiments, the 5% fat diet was used as a control to approximate the fat content of laboratory chow; thus the 1% and 25% fat diets represent a marginal deficiency and an excess, respectively.

Burk et al. (4) have shown that tissue selenium is rapidly

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Compositions of synthetic diets</th>
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<tbody>
<tr>
<td></td>
<td>1% corn oil (% by wt)</td>
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<tr>
<td>Stripped corn oil</td>
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<td>Caloric density (kcal/g)</td>
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<table>
<thead>
<tr>
<th>Table 2</th>
<th>Effect of selenium deficiency on incidence of palpable mammary tumors in rats fed different levels and types of fats</th>
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<tbody>
<tr>
<td>Fat in diet (%)</td>
<td>No. of rats with palpable tumors at following times (wk) after DMBA administration</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------------------------</td>
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<tr>
<td>Group</td>
<td>Corn oil</td>
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<tr>
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<tr>
<td>7</td>
<td>1</td>
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<td>8</td>
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</tbody>
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\( ^a \) No new animal developed palpable tumor beyond 19 weeks after DMBA.

\( ^b \) Time between DMBA administration and the appearance of the first palpable tumor.
adequate intake of vitamin E, did not affect the growth of the yeast diet. Chronic selenium deficiency, in the presence of an depleted within 2 to 4 weeks from weanling rats fed a Torula tissues of rats that were deficient in selenium and/or vitamin E function.

Increased in vitro peroxidation, as determined by the thio-barbituric acid method, has been demonstrated in several tissues of rats that were deficient in selenium and/or vitamin E (9, 10). The concerted action of vitamin E and selenium in protecting against the endogenous accumulation of lipid hydroperoxides has been reviewed in detail by Hoekstra (14). Lability to peroxidation depends on the intake of polyunsaturated fatty acids (18) with the consequence that uncontrolled peroxidation may result in structural and functional damages to cellular components (3, 8, 24). Malondialdehyde is formed as an end product of lipid peroxidation (31). This compound has been shown to react with proteins and nucleic acids (19, 26). Thus, lipid peroxidation, although a normal occurrence in animal tissues, can lead to a host of deleterious effects in cells if it remains unchecked. Moreover, malondialdehyde has been reported to be a carcinogetic initiator on mouse skin (28) and is found to be mutagenic in the Ames test (22, 29). Our observation that rats fed a high polyunsaturated fat diet are more sensitive to selenium depletion than those on a low fat diet or a high saturated fat diet suggests that lipid peroxidation may, either via malondialdehyde or in some unidentified manner, be involved in the carcinogetic process.

Reports from Daoud and Griffin (11) and Marshall et al. (20) indicated that supplementation of 4 ppm of selenium in the drinking water of rats impeded activation and accelerated detoxification of the carcinogen 2-acetylaminofluorene. The data shown in Table 4 suggest that selenium deficiency may also influence the metabolism of DMBA. Lending support to this conclusion is the finding by Rasco et al. (25) that selenium in the culture medium inhibited aryl hydrocarbon hydroxylase activity of human lymphocytes. Another avenue worthy of exploration is the host defense-immune mechanism. Dogs fed a diet high in polyunsaturated fatty acids but deficient in vitamin E and selenium have been found to develop a severely depressed lymphoproliferative response to mitogen (30). Dietary supplementation with selenium at levels above nutritional requirement increases the number of IgM-producing cells and the synthesis of IgM antibody (32). Whether immunological dysfunction affects the development of neoplasia under the dual influences of selenium deficiency and a high fat intake remains to be elucidated. Given the diverse approaches that may be implicated in unraveling the relationship between selenium, fat, and cancer, the tumor-diet system used in the present study seems to provide a suitable model for investigation into the potential use of selenium supplement in the chemoprevention of cancer in the human population due to insufficient selenium intake in many areas of the world, especially in those countries with a high fat consumption.

ACKNOWLEDGMENTS

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