Effect of an Inorganic and Organic Form of Dietary Selenium on the Promotional Stage of Mammary Carcinogenesis in the Rat

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ABSTRACT

The relative effectiveness of either sodium selenite or selenomethionine in the inhibition of mammary carcinogenesis was studied in virgin female Sprague-Dawley rats. In one experiment, rats were given 50 mg of 1-methyl-1-nitrosourea per kg of body weight s.c. at 50 days of age. Beginning 7 days post-1-methyl-1-nitrosourea, they were assigned to a basal diet containing 0.1 ppm of selenium or basal diet supplemented to contain either 4, 5, or 6 ppm of selenium as sodium selenite or 5 or 6 ppm of selenium as selenomethionine. Selenium treatment was continued until termination of the study 135 days after 1-methyl-1-nitrosourea treatment. Sodium selenite, at the 5-ppm level, was the most effective chemopreventive agent. The highest level of selenomethionine (6 ppm) caused grossly apparent liver damage. No liver damage was noted in sodium selenite-treated rats. In a second experiment, rats were given 5 mg of 7,12-dimethylbenz(a)anthracene at 50 days of age. Beginning 7 days post-7,12-dimethylbenz(a)anthracene treatment, rats were assigned randomly to the control group or to one of two selenium treatment groups receiving either 3.4 ppm of selenium as sodium selenite or 3.4 ppm as selenomethionine in their drinking water. Selenium supplementation was continued throughout the study until its termination at 111 days postcarcinogen. Sodium selenite significantly reduced cancer incidence and the average number of cancers per rat. Treatment with selenomethionine was less effective and caused severe liver damage. Although both sodium selenite and selenomethionine can inhibit some aspect of the postinitiation stage(s) of mammary carcinogenesis, selenium provided as sodium selenite was the more effective and less toxic of the two chemicals. Increasing the dose of sodium selenite above 5 ppm did not enhance the inhibitory activity of selenium.

INTRODUCTION

Experimental evidence continues to accumulate supporting the hypothesis that selenium can inhibit one or more stages of the carcinogenic process in several target tissues, including the mammary gland (7, 13, 16, 20, 25, 28). The anticancer effect(s) of selenium has been found to be exerted against chemically induced cancers in the rat and mouse and against virally induced lesions in the mouse (7, 13, 20, 25). In certain mouse tumor systems, selenium has been reported to exert its greatest protective effect on early stages of the disease process, and continuous selenium treatment has been observed to be necessary to sustain anticancer activity (13, 14). At the levels of dietary selenium reported to inhibit mammary carcinogenesis, no alterations in the circulating levels of estrogen and progesterone have been observed, and inhibition of mammary carcinogenesis by selenium has been reported in the presence or absence of the ovaries (7). These data taken collectively have resulted in considerable interest in the potential of selenium as an anticarcinogen.

Very little work has been conducted to determine the effect(s) of feeding organic forms of selenium on chemically induced neoplasia. That which has been reported has been done primarily with high-selenium yeast in which the forms in which selenium exists are not defined completely (20). It was therefore decided to determine the anticarcinogenic efficacy of selenomethionine in the MNU- and DMBA-induced mammary carcinogenesis systems. This choice was made since selenomethionine has been reported to be one of the predominant organic forms of selenium in cereals, vegetables, and grains and, hence, is a form of selenium that is ingested commonly (10, 31). Furthermore, it has been shown that the selenium in selenite and selenomethionine is absorbed to a similar degree and enters the same metabolic pool in the body (24). In addition, despite the fact that the form of selenium at the active site of glutathione peroxidase appears to be 1-selenocysteine, sodium selenite and selenomethionine have about equal potency for maintaining and restoring glutathione peroxidase in rats, and equal selenium retention in the body results from feeding either compound (2, 15, 19). It was anticipated that this study would also provide mechanistic insights into the mode of action of selenium as an antipromoter, since the initial steps in the metabolism of the 2 compounds differ.

MATERIALS AND METHODS

All experiments were conducted with virgin female Sprague-Dawley rats obtained from Taconic Farms, Inc., Germantown, NY. Animals were fed a torula yeast diet, the composition of which is shown in Table 1. A 20% fat diet was selected because such high fat diets have been reported to promote mammary carcinogenesis (3). Given that the antipromotional activity of selenium was being investigated, diet conditions were chosen to amplify the promotion phase of carcinogenesis. The diet was also formulated to contain 0.5% dl-methionine, the most limiting amino acid in torula yeast (5). It is important to note that this is far below the 2.0% level of dietary methionine known to be toxic to the rat (6, 17). We also felt it to be extremely important to provide sulfur-containing amino acids sufficiently above requirement levels so that the selenomethionine would be available for metabolic utilization as selenium and would not be used to satisfy the requirement for methionine (22). Furthermore, increasing the concentration of dietary methionine has been reported to reduce selenium toxicity (11). dl-Selenomethionine was used since it has been reported to be as equally well utilized as sodium selenite for regeneration of glutathione peroxidase activity in the rat (29).

Experiment 1. The purpose of this study was to confirm and extend...
previous observations of the effect of selenium in the experimental mammary carcinogenesis system induced with 50 mg of MNU/kg of body weight (25, 27). The study differed from those reported previously in that tumor appearance was accelerated by feeding of a 20% (w/w) fat diet. Although selenium has been reported to be effective in the DMBA-induced mammary tumor system promoted by high dietary fat (8), this has not been shown for the MNU induction system. Furthermore, very little selenium dose-response work has been done to date. Diminished anticarcinogenic effectiveness with 4 ppm of selenium in the MNU system has been reported (27), and no work has been done above 5 ppm of selenium. We therefore examined the dose range from 4 to 6 ppm of selenium, the upper limit being necessitated by the induction of selenium toxicity above this level (9). Furthermore, we sought to examine the effect of selenium supplied as selenomethionine incorporated to provide the same final concentration of selenium provided by sodium selenite, i.e., 5 or 6 ppm of selenium. Between 35 and 57 days of age, animals were provided with distilled water and a lab chow diet (Purina 5001) which contained 0.2 ppm of selenium. At 50 days of age, 120 rats received a s.c. injection of 50 mg of MNU/kg of body weight (26). The MNU was dissolved in acidic saline (pH 4.0) immediately prior to injection. Seven days after MNU treatment, animals were randomized into 1 of 6 groups and received the torula yeast diet containing 0.1 ppm of selenium, to which was added either no additional selenium or sufficient selenium to make diets of 4, 5, or 6 ppm of sodium selenite or 5 or 6 ppm of selenium as selenomethionine. Concentrations of selenium in these diets were determined fluorometrically (18). Food intakes of individually housed, 0.9% NaCl solution (saline)-injected animals assigned to each treatment were determined during the first 8 weeks of the study and were used to estimate the amount of selenium ingested per day. Animals were maintained under these conditions for the remainder of the study until its termination 135 days after carcinogen treatment.

### RESULTS

**Effect of selenium administered in the diet on MNU-induced mammary carcinogenesis**

All rats were given MNU (50 mg/kg of body weight) as described in "Materials and Methods." Twenty rats were assigned to each treatment group. All rats survived until the study was terminated 135 days after treatment with MNU.

<table>
<thead>
<tr>
<th>Selenium (ppm)</th>
<th>Source of selenium</th>
<th>Estimated selenium intake (µg)</th>
<th>Final cancer incidence (%)</th>
<th>Mean no. of cancers/rat</th>
<th>Mean no. of cancers/cancer-bearing rat</th>
<th>Median cancer-free time (days)</th>
<th>Body wt (g)</th>
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<tr>
<td>Basal</td>
<td>Selenite</td>
<td>2</td>
<td>90</td>
<td>4.2^d</td>
<td>4.5^d</td>
<td>61^d</td>
<td>259 ± 4.3^d</td>
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<tr>
<td>4.0</td>
<td>Selenite</td>
<td>40</td>
<td>90</td>
<td>3.2^d</td>
<td>3.5^d</td>
<td>61^d</td>
<td>247 ± 4.4^d</td>
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<tr>
<td>5.0</td>
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<td>47</td>
<td>95</td>
<td>2.9^d</td>
<td>2.1^d</td>
<td>71^d</td>
<td>241 ± 2.6^d</td>
</tr>
<tr>
<td>6.0</td>
<td>Selenite</td>
<td>51</td>
<td>95</td>
<td>4.3^d</td>
<td>4.7^d</td>
<td>71^d</td>
<td>249 ± 5.3^d</td>
</tr>
<tr>
<td>5.0</td>
<td>Selenomethionine</td>
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<td>95</td>
<td>4.8^d</td>
<td>5.1^d</td>
<td>66^d</td>
<td>252 ± 3.1^d</td>
</tr>
<tr>
<td>6.0</td>
<td>Selenomethionine</td>
<td>63</td>
<td>80</td>
<td>3.3^d</td>
<td>4.1^d</td>
<td>72^d</td>
<td>241 ± 5.1^d</td>
</tr>
</tbody>
</table>

* Concentration of selenium in the diet. The basal diet was supplemented with sodium selenium to contain 0.1 ppm of selenium as confirmed by analysis.

* Selenite was supplied as the sodium salt.

* Group mean body weights at the time the study was terminated.

* Statistically different from r; p < 0.05.

* Mean ± S.E.

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**Table 1**

**Composition of the torula yeast diet**

The diet used in both Experiments 1 and 2 was as described in "Materials and Methods."

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>% composition</th>
</tr>
</thead>
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<td>Corn starch</td>
<td>20</td>
</tr>
<tr>
<td>Cereal</td>
<td>20</td>
</tr>
<tr>
<td>Torula yeast</td>
<td>30</td>
</tr>
<tr>
<td>Fiber</td>
<td>5</td>
</tr>
<tr>
<td>Corn oil</td>
<td>20</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>1.0</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>3.5</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.5</td>
</tr>
</tbody>
</table>

* USF XIX. Obtained from the Lakes States Division, St. Regis, Rhinelander, WI.

* Obtained from Sulkafolk, Brown Co., Berlin, NH.

* Mazola corn oil obtained from Best Foods Co., Englewood, NJ.

* American Institute of Nutrition formulations obtained from Teklad, Madison, WI.

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**Table 2**

**Effect of selenium administered in the diet on MNU-induced mammary carcinogenesis**

All rats were given MNU (50 mg/kg of body weight) as described in "Materials and Methods." Twenty rats were assigned to each treatment group. All rats survived until the study was terminated 135 days after treatment with MNU.

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**Experiment 2.** This experiment was conducted in order to evaluate the results of Experiment 1 in the different but analogous mammary tumor system induced with DMBA. Given the strong promotional activity of dietary fat in this system, a low dose of DMBA was used in order to increase the sensitivity of the assay system for detection of differences in tumor induction.

Between the ages of 35 and 111 days, animals were provided ad libitum with distilled water and an adequate selenium diet, the composition of which is shown in Table 1. At 50 days of age and following an 18-hr fast, rats received 5 mg of DMBA p.o. The carcinogen was dissolved in sesame oil an delivered in a volume of 1 ml. Seven days after carcinogen treatment, animals were randomized into 1 of 3 groups of 21 rats each. At this time, selenium treatment via the drinking water was initiated. Animals either continued to receive distilled water or were given water containing 3.4 ppm of selenium supplied as either sodium selenite or selenomethionine, a level of selenium reported to be effective in other mammary tumor systems (28). The selenium solutions were prepared and given to the rats fresh every other day. Selenium concentrations were determined fluorometrically (18). Water intakes of individually housed, solvent-intubated rats assigned to each treatment were determined during the first 8 weeks of the experiment and were used to estimate the amount of selenium ingested per day. Animals were maintained under these conditions for the remainder of the study.

In both experiments, rats were weighed each week and palpated for the detection of mammary tumors twice weekly. At necropsy, the skin of each rat was transilluminated, and all grossly observable tumors were removed and processed for histopathological evaluation according to the criteria of Young and Hallowes (30). Only tumors confirmed to be mammary carcinomas are reported.

Statistical analyses of the data were performed as follows. The tumor counts were transformed using the square root transformation as recommended by Snedecor and Cochran (21). The transformed counts were then tested for response to selenium dose by regression analysis. Differences in tumor incidence were tested using the x-square test without the overly conservative continuity correction (1). The groups were tested for a hypothesized delay in time to tumor appearance with increased levels of dietary selenium by the nonparametric test of Mantel (12).
selenium per day. The reason for this is unclear. Median cancer-free time was marginally extended at both 5 and 6 ppm of selenium. Selenomethionine also suppressed cancer occurrence, but it appeared that 6 ppm organic selenium was not as effective as was 4 ppm selenium. At necropsy, hard nodular cirrhosis was observed in 50% of the animals receiving the highest level of selenomethionine. Livers were shrunken in size. Histologically, extensive necrosis, hemorrhage, and fibrosis were observed. Comparable effects were not noted in animals on the other dietary treatments. The rate of body weight gain was decreased similarly to the same extent among the various selenium-treated groups. This implies that a difference exists in the marginal toxicity of selenite and selenomethionine, as indicated by reduced weight gain and the gross liver damage observed in the animals fed 6 ppm of selenium as selenomethionine.

In Table 3, data is presented which summarizes the effects of selenium administered in the drinking water on the promotion stage of DMBA-induced mammary carcinogenesis. Selenium provided as either selenite or selenomethionine significantly reduced cancer induction in comparison to animals receiving no supplemental selenium. However, as reflected by the comparison of estimated dose of selenium ingested from each diet group, selenite was more protective than was selenomethionine, both in terms of reducing the number and in incidence of mammary carcinomas. The protective effect of selenium against promotion was, however, actually eliminated by increasing the dose of DMBA to 15 mg in animals that were fed the high-fat diet. Hard nodular cirrhosis was also noted in 35% of the animals receiving the selenomethionine in the drinking water, whereas the livers of the selenite-treated group were within normal limits. Body weights were depressed similarly in both groups in comparison to the control.

**DISCUSSION**

This investigation provides evidence that dietary selenium in the form of sodium selenite provides greater inhibition of mammary carcinogenesis than does an equivalent amount of selenium in the form of selenomethionine. Similar differences between selenomethionine and sodium selenite have been observed with transplantable tumors (16). As has been reported by Sunde et al. (22), selenium provided from selenomethionine can either be incorporated into tissue protein in place of methionine or catalyzed into the selenite pool for subsequent utilization or elimination. The extent to which the former pathway is favored is, in part, determined by the adequacy of dietary methionine. As noted above, this experiment was designed to minimize the diversion of selenomethionine to satisfy the requirement for methionine and, hence, permit a better comparison of the protective activity of selenium from 2 defined sources.

It has been reported previously that feeding the levels of dietary selenium in the dose range used in this study results in a 10 to 15% reduction in the rate of body weight gain. This observation was again confirmed. However, it has been reported that this degree of growth retardation cannot account for the protective activity of selenium (7, 23, 25). The unanticipated finding of hard nodular cirrhosis at the highest dose of selenomethionine fed in diet or drinking water illustrates the toxicity of the compound and the narrow dose range over which the transition from marginal to overt toxicity occurs. This observation supports the need for additional research to clarify those aspects of selenium toxicity that are characteristic of selenomethionine *per se* (9).

Although the fact that selenium can act as an anticarcinogen is well established, little progress has been made toward elucidating the mechanism(s) by which selenium inhibits the neoplastic process. The results of this investigation suggest that no advantage is gained from the use of selenomethionine rather than selenite for cancer prophylaxis. The observation of selenomethionine toxicity accompanied by a diminished protective effect against mammary carcinogenesis raises an important question. Why is the same amount of selenium essentially nontoxic and highly protective when supplied as selenite but more toxic and less protective when supplied as selenomethionine? One answer could be that selenite is more effectively detoxified than is selenomethionine. This possibility suggests that the chemopreventive activity of selenium may occur, at least in part, as a consequence of its effective detoxification.

**ACKNOWLEDGMENTS**

We wish to thank Anne M. Ronan for her technical assistance and Karen Savard for her assistance in the preparation of this manuscript.

**REFERENCES**


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*H. J. Thompson, unpublished observation.*
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