Effect of Short-Term Feeding of Sodium Selenite on 7,12-Dimethylbenz(a)anthracene-induced Mammary Carcinogenesis in the Rat

Henry J. Thompson, L. David Meeker, Peter J. Becci, and Stephen Kokoska

ABSTRACT

The inhibitory activity of short-term feeding of one of four concentrations of dietary selenium against the induction of mammary gland carcinomas by 7,12-dimethylbenz(a)anthracene (DMBA) was studied in female Sprague-Dawley rats. When 28 days old, the animals were placed on a Torula yeast diet formulation which contained, by analysis, either 0.05, 0.15, 1.05, or 2.06 μg of selenium, as sodium selenite, per g of diet. Mammary cancer was induced by a single p.o. administration of either 7.5 or 15.0 mg DMBA at 50 days of age. The animals were maintained on the above diets until 14 days after carcinogen treatment at which time all animals were transferred to a chow diet containing 0.21 μg of selenium per g of diet. The study was terminated 120 days after DMBA administration. The concentrations of selenium in the liver and mammary tissue measured at the time of DMBA treatment increased with increasing levels of dietary selenium (p < 0.05). At the low dose of DMBA, there was a trend toward reduction in the number of cancers with increased amounts of selenium, but the only significant difference occurred between groups fed the next to lowest and the highest level of selenium. At the high dose of DMBA, the number of observed cancers showed a strong dose effect (p < 0.05). In addition, tumor load was significantly reduced in selenium-supplemented rats (p < 0.05), and there was a significant delay (p < 0.05) in the time to appearance of the cancers of animals receiving the highest level of selenium when compared with those receiving the lowest level. The dietary concentrations of selenium shown to inhibit the early stage(s) of cancer induction in this system were both significantly lower and fed for a shorter time interval than that which was previously reported.

INTRODUCTION

The apparent anticarcinogenic activity of selenium in the form of sodium selenite has been reported and verified in a number of tumor systems (4). Several investigators have studied the effects of dietary selenium on the process of tumor initiation (5, 7, 20, 22). We first described selenium inhibition of DMBA-induced mammary carcinoma using such an approach in 1980 (20), and Ip (7) has recently reported a similar study. In both these investigations, it was suggested that pharmacological amounts of selenium (5 ppm as sodium selenite) inhibit initiation although treatment effects on the very early stages of tumor promotion could not be ruled out. This paper provides evidence that much lower levels of dietary selenium produce a dose-dependent reduction in tumor occurrence while delaying tumor appearance following DMBA administration. Possible mechanisms of action which could account for this effect include: prevention of the carcinogen from reaching or reacting with critical sites via changes in carcinogen metabolism or tissue susceptibility, facilitation of the process(es) which repairs carcinogen-induced damage, or prevention of cellular events secondary to, but essential for, neoplastic transformation.

MATERIALS AND METHODS

Virgin female Sprague-Dawley rats obtained from Taconic Farms, Germantown, N. Y., at 21 days old were used. The animals were housed in stainless steel wire mesh-bottomed cages (4 rats/cage) in a controlled environment with temperature maintained at 22 ± 1°C (S.D.) and a 12-hr light-dark cycle. From 21 to 28 days of age animals were provided with a low-selenium (0.02 μg/g selenium; vitamin E-fortified, 50 μg/g) powdered diet, the composition of which has been reported (16). A Torula yeast diet formulation was used in order to keep the basal dietary level of selenium low. At 28 days of age, the animals were randomized into one of 4 groups of 50 rats each and were fed the Torula yeast diet supplemented with one of 4 levels of selenium in the form of sodium selenite (Sigma Chemical Company, St. Louis, Mo.). The total concentrations of selenium in the treatment diets were selected to range from marginally adequate to a pharmacological level known to be well tolerated and yet below the 5 ppm already shown to be effective in reducing tumor numbers (7). The actual levels of selenium in the diets were determined fluorometrically (18) and are shown in Table 1. Food intake and body weight were quantified during this period. At 50 days of age and following an 18-hr fast, 45 of the animals in each group were randomly assigned to 2 groups of 20 and 25 rats, respectively, to receive a p.o. dose of either 15 or 7.5 mg of DMBA dissolved in 1.0 ml of sesame oil. The remaining rats in each group were killed at this time, and the concentrations of selenium in the abdominal inguinal mammary glands and in the liver were determined. Portions of each tissue were processed for histological evaluation.

Fourteen days after carcinogen treatment, all rats were changed to a Purina chow (5001) diet determined by fluorometric analysis to contain 0.21 μg selenium per g. Thereafter, the rats were weighed each week and were palpated for the detection of mammary tumors twice weekly. Because of the large number and size of tumors occurring in the low-selenium groups by 120 days after DMBA treatment, all animals were sacrificed and necropsied at that time. The skin of each rat was transilluminated, and all grossly observable tumors were removed and processed for histopathological evaluation.

Statistical analyses of the data were performed as follows: Tumor
Effect of dietary selenium on food intake, body weight, and the concentration of liver and mammary gland selenium

<table>
<thead>
<tr>
<th>Dietary selenium (µg/g)</th>
<th>Food intake (g)</th>
<th>Body wt (g)</th>
<th>Liver</th>
<th>Mammary gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>10.3 ± 1.3³</td>
<td>139 ± 3³</td>
<td>0.80 ± 0.19³</td>
<td>0.02 ± 0.01³</td>
</tr>
<tr>
<td>0.15</td>
<td>10.5 ± 1.3³</td>
<td>140 ± 3³</td>
<td>2.5 ± 0.19</td>
<td>0.06 ± 0.03</td>
</tr>
<tr>
<td>1.05</td>
<td>10.4 ± 1.3³</td>
<td>137 ± 3³</td>
<td>3.3 ± 0.09</td>
<td>0.11 ± 0.04</td>
</tr>
<tr>
<td>2.06</td>
<td>9.3 ± 1.3³</td>
<td>126 ± 3³</td>
<td>4.7 ± 0.70</td>
<td>0.20 ± 0.04</td>
</tr>
</tbody>
</table>

³ µg selenium per g diet determined fluorometrically. Selenium was provided as sodium selenite.

² Measured as the disappearance of diet from the food cup.

The increases in tissue concentrations of selenium with increasing dietary selenium were significant by regression analysis (p < 0.05).

Mean ± S.D. expressed per g tissue processed in this manner.

RESULTS

The i.g. administration of DMBA resulted in the induction of 888 tumors of which 870 were diagnosed as mammary gland carcinomas. The 18 benign lesions were evenly distributed among treatment groups. Only lesions which were cancers are reported and discussed below.

Increasing the dietary level of selenium resulted in a dose-dependent increase in liver and mammary gland selenium determined in animals killed on the day carcinogen was given (p < 0.05; Table 1). Histological evaluation of these tissues indicated that they were all within normal limits at the light microscopic level. Feeding the highest level of selenium did result in a reduction in food intake and in the rate of body weight gain (Table 1). However, following cessation of selenium treatment, body weights of animals fed the highest level of selenium recovered to control values within 14 days, and the tumor-free carcass weights of all groups were similar at the end of the study (Table 2).

Table 3 provides a summary of the tumors observed during the experiment. At the low dose of carcinogen, there is a trend toward reduction in the number of tumors with increased amounts of selenium as determined by regression analysis. However, the only significant difference occurred between the next to lowest (0.15 ppm) selenium group and the highest (2.06 ppm) selenium group (p < 0.05). The fresh tumor weights of the animals given the 3 highest levels of selenium show a reduction compared with those given the lowest level (p < 0.05). However, no significant effect of selenium treatment on time to tumor appearance was observed in the animals given the low dose of DMBA.

At the high dose of carcinogen, the number of observed tumors (square root of tumor counts) showed a strong dose effect with a significant negative slope (p < 0.05) of the regression line. The reversal in the rank ordering of the mean number of cancers (compared with the ranked doses) provides additional strong evidence of an inverse relationship between selenium dose and number of tumors induced (p < 0.05).

Animals receiving 1.05 ppm of selenium showed a marginally significant decrease in tumor counts (p = 0.06) while those receiving the high dose of 2.06 ppm of selenium showed a highly significant decrease (p < 0.05) when compared with the animals receiving the lowest dose. At this dose of carcinogen, the fresh tumor weights taken from all animals receiving the 3 highest selenium levels were reduced (p < 0.05) from those receiving the lowest level. In addition to this decrease in tumor mass due to selenium, there was a significant delay (p < 0.05) in the time to appearance of all tumors of the animals receiving the highest level of selenium when compared with those on the lowest level, as well as a significant delay in the time between latency and first tumor appearance (p < 0.05).

The analysis above refers to the animals which survived until the end of the experiment. As is typical with this system, a number of animals died shortly after receiving the carcinogen, and 2 tumor-bearing animals died during the first half of the experiment. Deaths were not associated with any particular treatment group. Those animals bearing one or more tumors at their time of death were included as tumor-bearing animals in a comparison of tumor incidence among the treatment groups. This comparison showed no significant difference in incidence among the treatment groups.

DISCUSSION

The addition of 0, 2, or 4-ppm selenium as selenium dioxide in the drinking water to laboratory chow-fed Sprague-Dawley rats for 30 days before and after the administration of 5 mg DMBA has been reported to suppress significantly mammary gland carcinogenesis, but a dose-responsive effect was not
observed (21). Those results were interpreted as evidence that selenium can inhibit the initiating phase of DMBA-induced mammary carcinogenesis, although effects on the early promoting phase were not ruled out.

The results of this investigation provide evidence that selenium inhibits the initiation and/or very early promotional stage(s) of DMBA-induced mammary carcinogenesis and that the degree of inhibition of cancer occurrence reflected by reduction in the number of cancers induced was positively correlated with the dose of selenium ingested. Our study differs from that of Welsch et al. (21) in that, in the present investigation, selenium was fed for a shorter period of time (2 weeks less) subsequent to administration of DMBA; the selenium was fed at a much lower level than selenite, not selenium dioxide; and the selenium was incorporated into a purified diet which contains a low basal amount of selenium. Welsch et al. administered selenium in the drinking water to rats and fed them a chow diet which contained variable amounts of selenium and undefined factors which affect the bioavailability of selenium. All of these factors may have contributed to the differences in the 2 studies. That a dose-dependent effect of selenium was observed was not anticipated, since Ip (6) has reported data suggesting a dose-response relationship when selenium was fed to Sprague-Dawley rats given varying doses of DMBA from weaning to the termination of the study. However, they did not specifically analyze their data to test this hypothesis. It is not clear why enhancement of mammary carcinogenesis was not noted in this study in the animals receiving the low dose of DMBA and the lowest level of selenium since, under different conditions but using the same tumor system, Ip and Sinha (9) have reported such an effect. Differences in the duration of selenium deficiency (appropriately 6 months in Ref. 9 versus 6 weeks in this study) may have been contributory to the differences in effect which were observed. In the present investigation, short-term feeding of one of 4 dietary levels of selenium resulted in proportionate increases in liver and mammary gland selenium concentrations determined at the time carcinogen was given. Only at the highest dose of selenium was food intake decreased and rate of body weight gain reduced. It is important to note that all animals were fed the same diet during the postinitiation stage of the experiment, that within 2 weeks after cessation of selenium treatment all animals were growing similarly, and that tumor-free body weights were essentially identical at the end of the study. Short-term selenium treatment at the doses administered tended to reduce the number of tumors and the total tumor mass and to prolong the time-to-appearance of all cancers. These effects complement those reported by Ip (7).

In evaluating the accumulating literature on the anticarcinogenicity of selenium, it is frequently noted that pharmacological doses of selenium reduce food intake and rate of body weight gain. No one has yet convincingly ruled out the possibility that inhibition of carcinogenesis by selenium is not a nonspecific cytotoxic effect, although the results of several in vitro studies provide evidence to the contrary (13–15). Nonetheless, this point is certainly worth underscoring in any discussion of the mechanism(s) which may be involved in selenium-mediated inhibition of the process of tumor induction. To date, at least 4 hypotheses have been advanced to explain the anticarcinogenicity of selenium. Pharmacological concentrations of selenium have been reported to influence carcinogen metabolism both in vitro and in vivo, and evidence continues to accumulate that selenium interferes with hepatic metabolism of xenobiotics (5, 12, 22). However, concern has been registered about the extrahepatic significance of such an effect. Indeed, in studies in which selenium has been reported to block hepatic metabolism of 1,2-dimethylhydrazine, increased methylation of the DNA from colon epithelial cells was reported (5). The ultimate consequence(s) of such an effect is not known. A second mechanism by which selenium may be acting as an anticarcinogen during the process of initiation is through a reduction in the number of cells exposed to carcinogen or by the suppression of cell turnover rates in target tissues (5, 22). An inhibition of cell proliferation by selenium has been reported using a variety of experimental conditions; therefore, it is conceivable that such an event would reduce tissue susceptibility to carcino-

### Table 3: Effect of dietary selenium on mammary carcinogenesis

<table>
<thead>
<tr>
<th>Dietary selenium (μg/g)</th>
<th>Carcinogen dose</th>
<th>No. of surviving rats/group</th>
<th>No. of cancers</th>
<th>Median time of appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>TNC</td>
</tr>
<tr>
<td>0.05 Low</td>
<td>25 (25)</td>
<td>20</td>
<td>80</td>
<td>108</td>
</tr>
<tr>
<td>0.15 Low</td>
<td>24 (24)</td>
<td>22</td>
<td>92</td>
<td>125</td>
</tr>
<tr>
<td>1.05 Low</td>
<td>23 (23)</td>
<td>17</td>
<td>74</td>
<td>88</td>
</tr>
<tr>
<td>2.06 Low</td>
<td>24 (24)</td>
<td>19</td>
<td>79</td>
<td>78</td>
</tr>
<tr>
<td>0.05 High</td>
<td>17 (16)</td>
<td>17</td>
<td>100</td>
<td>143</td>
</tr>
<tr>
<td>0.15 High</td>
<td>19 (18)</td>
<td>18</td>
<td>95</td>
<td>127</td>
</tr>
<tr>
<td>1.05 High</td>
<td>19 (19)</td>
<td>17</td>
<td>89</td>
<td>122</td>
</tr>
<tr>
<td>2.06 High</td>
<td>16 (16)</td>
<td>14</td>
<td>88</td>
<td>73</td>
</tr>
</tbody>
</table>

* Amount of selenium in μg/g of diet; concentration determined fluorometrically. The rats were fed these diets between 28 and 64 days of age as described in "Materials and Methods."

* The procedure for carcinogen treatment is described in "Materials and Methods." Low dose is 7.5 mg DMBA per rat; high dose is 15.0 mg DMBA per rat.

* Twenty-five rats were initially assigned to each group receiving the low dose of DMBA, and 20 rats were initially assigned to each group receiving the high dose. The numbers indicate the number of rats in each group surviving carcinogen treatment.

* TNC, total number of cancers; MNC, mean number of cancers per rat; MNCB, mean number of cancers per cancer-bearing animal; MTAAC, median time to appearance of all cancers; MTAFC, median time to appearance of the first cancer per rat.

* Numbers in parentheses, number of rats surviving until the end of the study.

* Significantly different from g (p < 0.05). Statistical significance based on analyses performed on treatment groups given the same carcinogen dose.
Selenium Inhibition of Mammary Cancer

Ogen independent of any effect of selenium on carcinogen metabolism. If this were found to be occurring in the mammary gland, it would be important to determine whether it represents a pharmacological or toxic response to selenium treatment. A third line of evidence suggests that selenium may facilitate the repair of carcinogen-induced damage in DNA. Although evidence exists to support this hypothesis (14), there is an equally convincing set of data suggesting that this mechanism cannot account for the anticarcinogenicity of selenium during the process of initiation (5, 22). Finally, although the prospect has not been adequately addressed, on a theoretical basis the scavenging of carcinogen-generated free radicals by selenium-dependent glutathione peroxidase could account, at least in part, for the anticarcinogenicity of selenium. We are aware of only the negative data of Ip and Sinha (8) in this regard.

Several aspects of the effect of selenium on mammary carcinogenesis are now being studied in our laboratories under experimental conditions designed to differentiate among physiological, pharmacological, and toxic responses. In this regard, it is relevant to point out that our laboratories have found that the growth of animals under 60 days old is very sensitive to suppression by pharmacological levels of dietary selenium; that in terms of growth, female rats appear to be less tolerant of selenium than are males; that there is a significant interanimal variance in ability to tolerate selenium treatment both in terms of growth and liver necrosis; and that the effects of selenium on food intake and body weight gain are transient. All these factors must be taken into account in the design, execution, and interpretation of selenium chemoprevention studies. Furthermore, future investigations of this question could provide new insights into the inhibitory activity of selenium if: (a) experiments are designed to include groups treated with DMBA or N-methyl-N-nitrosourea so that the role of selenium in the modulation of carcinogen metabolism can be assessed relative to its overall inhibitory effect on early stages of mammary carcinogenesis; and (b) feeding of supplemental selenium is restricted to the time prior to carcinogen treatment and diet intakes of all treatment groups are equalized to eliminate the potential effects of differences in nutrient intake on susceptibility of the target tissue. Although Torula yeast-based diet formulations will continue to be the diets of choice when experimental protocols require feeding of diets deficient in selenium, the use of casein-based formulations such as AIN 76 should be considered when large supplements of selenium are to be given. Torula yeast diets do not support rodent growth as satisfactorily as do casein-based diets, and nutrient availability from Torula yeast diets has not been widely studied. On the other hand, casein-based diets are used very extensively, particularly in studies of the relationships of diet and nutrition to cancer induction as well as in studies of mammary gland biology. Consequently, although sometimes necessary, the use of Torula yeast-formulated diets makes comparisons with other carcinogenesis-diet experiments difficult. The majority of work done in selenium chemoprevention is subject to this concern.

Finally, it has been our observation that the relationship between carcinogen dose and number of observed cancers in this mammary tumor system is nonlinear and possibly sigmoidal. This observation implies that the choice of size of the dose of carcinogen will have considerable influence upon the magnitude of anticarcinogenic activity observed for a given concentration of selenium. For example, the choice of carcinogen dose yielding a tumor response which falls at either the low-level region or the high-plateau region of the sigmoidal dose-response curve would result in little apparent anticarcinogenic activity by selenium, whereas, if the tumor response falls on the linear portion of the dose-response curve, the same amount of selenium could produce a marked inhibitory effect. It appears that the low dose of DMBA used in this experiment could correspond to the former situation and the high dose of DMBA could correspond to the latter situation. The ramifications of this are that it may be difficult to demonstrate a significant effect of selenium on the process of initiation using very low doses of carcinogen in this rat mammary tumor system unless large numbers of animals are used, considering the character of the dose-response curve and the amount of statistical variability in the tumor response data. Consequently, studies of mechanisms will be critical in evaluating the usefulness of selenium in cancer prophylaxis in humans.

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REFERENCES


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