Effects of Dietary Selenium Concentration on the Development of Enzyme-altered Liver Foci and Hepatocellular Carcinoma Induced by Diethylnitrosamine or N-Acetylaminofluorene in Rats

Robert A. LeBoeuf, Brian A. Laishes, and William G. Hoekstra

Department of Biochemistry, College of Agricultural and Life Sciences [R. A. L., W. G. H.], and McArdle Laboratory for Cancer Research [B. A. L.], University of Wisconsin-Madison, Madison, Wisconsin 53706

ABSTRACT

Three protocols were used to determine the effects of dietary selenium concentration on the development of \(\gamma\)-glutamyltranspeptidase (GGT)-positive foci and hepatocellular carcinoma induced by either diethylnitrosamine (DEN) or N-acetylaminofluorene in rats. In the first experiment, foci were induced by a carcinogenic dose of DEN (100 mg/kg body weight, p.o.) at 20–22 h after two-thirds partial hepatectomy. One wk after DEN administration, during which time 0.1 ppm (representing a control level), 3.0, or 6.0 ppm selenium as Na2SeO3 was fed for 8 or 16 wk, at which time focal analysis was conducted using quantitative stereology. The results demonstrated that 3.0 and 6.0 ppm dietary selenium, initiated 1 wk following carcinogen administration, decreased focal growth rate without affecting the number of GGT foci compared to 0.1 ppm selenium. Decreased focal growth was temporary and reversible with 6.0 ppm selenium which may be related to chronic selenium observed after 16 wk of 6.0 ppm selenium feeding.

In a second experiment involved a noncarcinogenic dose of DEN (25 mg/kg body weight, p.o.), then 0.1 or 6.0 ppm selenium feeding for 8 wk, followed by 0.05% phenobarbital (PB), a liver tumor promoter in a diet containing 0.1 ppm selenium. Analysis of GGT foci at 5 or 8 wk of PB feeding indicated that 6.0 ppm selenium caused a trend towards an increase in the number of foci/cm\(^2\) of liver and mean focal volume and a significant increase in GGT focal volume as a percentage of liver volume by 8 wk of PB feeding. Thus, high dietary selenium concentrations prior to PB enhance the tumor-promoting ability of PB.

In a third experiment, using male Fischer 344 rats (150 g), 0.1 or 6.0 ppm selenium was fed concurrently with 0.02% AAF which was fed in a cyclic regimen. After 4 cycles, where 1 cycle equaled 4 wk of AAF, followed by 1 wk of control diet (0.1 ppm selenium), 6.0 ppm selenium significantly decreased the mean focal volume and focal volume as a percentage of liver volume, while not affecting the number of foci/cm\(^2\) of liver, again indicating a selenium effect on focal growth while not affecting the number of "preneoplastic" lesions in the liver. Six ppm selenium feeding after AAF treatment had no effect on the percentage of incidence of hepatocellular carcinoma (100%) but did cause a significant decrease in the percentage of liver volume occupied by macroscopic subcapsular liver lesions compared to 0.1 ppm selenium. Three ppm selenium was without effect. The results from these studies are discussed in terms of an inhibitory effect of high selenium on cell proliferation and thus an inhibitory or delaying effect on carcinogenesis.

INTRODUCTION

Numerous studies have demonstrated anticarcinogenic effects of dietary selenium in experimental animals when administered in the food or drinking water at concentrations of 20 to 60 times the nutritional requirement. Protection by selenium has been demonstrated in chemically induced carcinogenesis in liver (6, 10, 13), colon (1, 2, 20–22), mammary gland (16, 17, 19, 31, 34, 52, 55), and skin (49). The genesis of spontaneous mammary tumors in mice (33, 47) as well as the growth of transplanted tumor cells (9, 18, 35, 53, 54) is also inhibited by high dietary and injected selenium. Several reports have suggested that the effect of selenium on chemically induced cancer may be mediated through alterations in carcinogen metabolism (4, 5, 11, 21, 23, 30). Other mechanisms of inhibition must also be present as indicated by those studies in which high dietary selenium fed after carcinogen administration caused a significant reduction in hepatic and mammary tumor incidence (10, 16, 52, 55). This interpretation is also supported by studies in which selenium exerted protective effects against tumor development and growth in nonchemically induced tumor systems (9, 18, 33, 35, 47, 53, 54).

The purpose of our experiments was to study the effect of dietary selenium concentration on early and late stages of hepatocarcinogenesis induced by DEN and AAF. Two of the experiments were designed such that any observed selenium effects would be independent of alterations in carcinogen metabolism. For our experimental end points, we quantitated GGT-positive foci to study early stages of hepatocellular carcinoma and macroscopic subcapsular protrusions to study late stages of hepatocarcinogenesis. Our results indicate that high dietary selenium can affect postinitiation stages of hepatocarcinogenesis as indicated by a decreased growth of "preneoplastic" foci. In addition, selenium can decrease or enhance the carcinogenic process, depending on the dietary concentration of selenium, the duration of selenium feeding, and the experimental system under study.

MATERIALS AND METHODS

Diet. The diet composition for the experiments involving DEN was previously described (25), with the exception that 20% casein was used instead of 15%, at the expense of glucose monohydrate, and an addi-

---

1 Research supported by the College of Agricultural and Life Sciences, University of Wisconsin-Madison, and in part by NIH Grants AM-14881, CA-24818, and CA-07175. A preliminary report of these experiments was presented at the meetings of the Federation of American Societies for Experimental Biology, Chicago, IL (29).

2 To whom requests for reprints should be addressed.

Received 8/8/83; revised 7/2/85; accepted 7/5/85.
SELENIUM EFFECTS ON LIVER FOCI AND CARCINOMA DEVELOPMENT

FACTORS AND THEIR EFFECTS ON LIVER FOCI AND CARCINOMA DEVELOPMENT

The animals in the present studies were housed in stainless steel cages. This diet met all nutritional requirements of the laboratory rat according to the National Research Council (36). Selenium supplementation as Na2SeO3 at 0.1, 3.0, and 6.0 ppm selenium was accomplished by adding a selenium:glucose monohydrate premix. The 0.1 ppm selenium-supplemented diet which represents a control concentration of selenium was analyzed fluorometrically to contain 0.13 ppm selenium (37). The diet used for the experiments involving AAF was similar to that described above for the DEN experiments with the exception that 0.2% DL-methionine and 0.4% DL-threonine were added to the diet. The experiment involving AAF was originally designed to last 50 wk post-AAF feeding, a length of time shown previously (15) to be necessary for carcinoma development using a cyclic feeding protocol. Amino acid supplementation to the diet was an attempt to minimize selenium toxicity over the 50-wk feeding period (28, 43, 56). AAF (Aldrich, Milwaukee, WI) was supplemented to give a final dietary concentration of 0.02% by adding a premix of 1% AAF in glucose monohydrate to the basal diet.

DEN: Selenium Experiment (Chart 1a). Female Sprague-Dawley rats (Harlan-Sprague Dawley, Madison, WI) weighing approximately 200 g were received and housed in hanging stainless steel wire cages at 3 rats/cage. They were fed a basal diet containing 0.1 ppm selenium for 1 wk. PH (14) was then performed on the rats followed by intragastric administration of DEN (100 mg/kg body weight; Eastman Kodak, Rochester, NY) in 0.9% saline at 20–22 h post-PH. This dose of DEN is capable of causing hepatocellular carcinoma without further treatment (44). This large dose of DEN was used in order to induce hepatic lesions which required no further exogenous modifiers for the subsequent development of hepatocellular carcinoma. In this way, the direct effect of selenium on the development of liver GGT foci could be evaluated.

RESULTS

DEN: Selenium Experiment (Chart 1a; DEN, 100 mg/kg Body Weight). Dietary selenium treatment had no effect on body or liver weight after 8 wk of selenium feeding, but 6.0 ppm selenium caused a reduction in body weight and an increase in liver weight by 16 wk which was significant compared to 0.1 ppm (Table 1).
SELENIUM EFFECTS ON LIVER FOCI AND CARCINOMA DEVELOPMENT

Survival of animals was greater than 95% in all groups. The effect of dietary selenium concentration on focal development was evaluated in 2 separate experiments after 8 wk of selenium feeding to test the reproducibility of this protocol. Individual values from each experiment are listed in Table 2, and statistics were performed on pooled replicates from both experiments. Dietary selenium concentration had no effect on the number of foci/cm³ of liver, while both 3.0 and 6.0 ppm selenium caused a significant reduction of mean focal volume and focal volume as a percentage of liver volume by an average of 45% and 50%, respectively, compared to 0.1 ppm selenium. The reproducibility between individual experiments was excellent. At 16 wk, there was a significant increase in the number of foci/cm³ of liver compared to 8 wk, and dietary selenium had no effect on the number of foci/cm³, mean focal volume, or focal volume as a percentage of liver volume. However, the difference between 0.1 and 3.0 ppm selenium at 16 wk compared to that observed at 8 wk was less than 2 GGT-positive sections per standard liver section compared to an average of 0.089 ± 0.025 (0.082 ± 0.004) for the 0.1-ppm selenium treatment group. Within the 0.1- or 3.0-ppm selenium treatment group, mean focal volume was not significantly different at 16 wk versus 8 wk. This was characterized by an equal increase in the number of foci in smaller and larger size classes distributed according to the mean volume observed at 8 wk. A significant increase in focal volume as a percentage of liver volume did occur in all treatment groups by 16 compared to 8 wk. In contrast to the 8-wk time point, after 16 wk of 6.0 ppm selenium feeding, the mean focal volume and focal volume as a percentage of liver volume returned to a similar or slightly greater value compared to 0.1 ppm selenium, indicating a temporary or reversible effect of selenium at 6.0 ppm under these experimental conditions. Unlike 0.1 and 3.0, the mean focal volume of the 6.0-ppm selenium treatment group was significantly increased at 16 wk compared to 8 wk. Histological examination of hematoxylin-eosin sections after 16 wk of selenium feeding indicated that 11 of 12 rats fed 6.0 ppm selenium had evidence of focal fatty changes and cholangioma with some bile duct proliferation indicative of selenium toxicity. Mildly fatty changes were observed in 3 of 12 livers from the 0.1-ppm selenium group and 4 of 12 livers from 3.0-ppm selenium group. Cholangioma was present in only 1 of 12 livers from 0.1 ppm selenium and was completely absent from 3.0-ppm selenium livers.

DEN:Selenium:PB Experiment (Chart 1b; DEN, 25 mg/kg, plus PB). Six-ppm selenium feeding for 8 wk prior to 5 wk of PB feeding had no significant effect on the number of foci/cm³, mean focal volume, or focal volume as a percentage of liver volume while causing a trend towards an increase compared to 0.1 ppm selenium in all variables measured (Table 3). After 8 wk of PB feeding, 6.0 ppm selenium feeding prior to PB caused a significant increase in the focal volume as a percentage of liver volume. A trend towards an increase in the number of foci/cm³ of liver and mean focal volume was again observed (Table 3). Livers of rats given no DEN plus 8 wk of PB had no GGT foci. Rats given DEN, 25 mg/kg body weight, after PH, without subsequent PB feeding, had an average of less than 2 GGT-positive transsections per standard liver section at 8 wk post-DEN regardless of dietary selenium concentration. At 14 wk post-DEN without PB, foci were clearly present but had a frequency of less than 1 transsection/cm³ of standard liver section compared to an aver-

### Table 1

<table>
<thead>
<tr>
<th>Treatment (ppm selenium)</th>
<th>Body wt (g)</th>
<th>Liver wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 wk selenium feeding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>264 ± 5</td>
<td>8.3 ± 0.4</td>
</tr>
<tr>
<td>3.0</td>
<td>259 ± 5</td>
<td>9.1 ± 0.4</td>
</tr>
<tr>
<td>6.0</td>
<td>263 ± 5</td>
<td>9.1 ± 0.4</td>
</tr>
<tr>
<td>16 wk selenium feeding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>292 ± 5</td>
<td>9.8 ± 0.4</td>
</tr>
<tr>
<td>3.0</td>
<td>286 ± 6</td>
<td>10.6 ± 0.3</td>
</tr>
<tr>
<td>6.0</td>
<td>274 ± 7?</td>
<td>11.1 ± 0.4</td>
</tr>
</tbody>
</table>

* Mean ± SE for 12 animals/treatment group.

Table 2

<table>
<thead>
<tr>
<th>Treatment (ppm selenium)</th>
<th>No. of animals/group</th>
<th>No. of foci/cm³</th>
<th>Mean focal volume (mm³)</th>
<th>Focal/liver volume (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 wk selenium feeding</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>7</td>
<td>95 ± 17</td>
<td>0.089 ± 0.025</td>
<td>0.87 ± 0.31</td>
</tr>
<tr>
<td>3.0</td>
<td>12</td>
<td>111 ± 12 (105 ± 10)</td>
<td>0.072 ± 0.011 (0.078 ± 0.011)</td>
<td>0.86 ± 0.21 (0.86 ± 0.18)</td>
</tr>
<tr>
<td>6.0</td>
<td>9</td>
<td>83 ± 11</td>
<td>0.045 ± 0.007</td>
<td>0.50 ± 0.11</td>
</tr>
<tr>
<td>16 wk selenium feeding</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>12</td>
<td>177 ± 15</td>
<td>0.082 ± 0.014</td>
<td>1.53 ± 0.36</td>
</tr>
<tr>
<td>3.0</td>
<td>12</td>
<td>179 ± 14</td>
<td>0.046 ± 0.004</td>
<td>0.90 ± 0.14</td>
</tr>
<tr>
<td>6.0</td>
<td>12</td>
<td>180 ± 11</td>
<td>0.086 ± 0.014</td>
<td>1.49 ± 0.22</td>
</tr>
</tbody>
</table>

* Mean ± SE.

^a Numbers in parentheses, mean ± SE for pooled animals from 2 separate experiments, for which individual means ± SE are also shown. Only one experiment was conducted with the 3.0-ppm selenium treatment.

^b Significantly different (P < 0.05) from means in a column not followed by Footnote c at 8 or 16 wk according to Duncan's multiple range test.

^c Significantly different (P < 0.001) from means of the same dietary treatment group at 8 versus 16 wk according to the Student t test.

^d Significantly different (P < 0.025; Student's t test) from means of the same dietary treatment group at 8 versus 16 wk.

^e Significantly different (P < 0.05; Student's t test) from means of the same dietary treatment group at 8 versus 16 wk.

CANCER RESEARCH VOL. 45 NOVEMBER 1985

5491
SELENIUM EFFECTS ON LIVER FOCI AND CARCINOMA DEVELOPMENT

Table 3
Effect of dietary selenium concentration on the development of liver GGT (+) foci when selenium is fed after DEN and prior to PB administration
(DEN:selenium:PB experiment)

<table>
<thead>
<tr>
<th>Treatment (ppm selenium)</th>
<th>No. of foci/group</th>
<th>No. of foci/cm²</th>
<th>Mean focal volume (mm³)</th>
<th>Focal/liver volume (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 wk PB feeding</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>5</td>
<td>156 ± 61*</td>
<td>0.016 ± 0.002</td>
<td>0.232 ± 0.082</td>
</tr>
<tr>
<td>6.0</td>
<td>4</td>
<td>199 ± 81</td>
<td>0.018 ± 0.005</td>
<td>0.351 ± 0.120</td>
</tr>
<tr>
<td>8 wk PB feeding</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>12</td>
<td>118 ± 29</td>
<td>0.063 ± 0.008</td>
<td>0.631 ± 0.094</td>
</tr>
<tr>
<td>6.0</td>
<td>12</td>
<td>154 ± 21</td>
<td>0.083 ± 0.011</td>
<td>1.178 ± 0.159*</td>
</tr>
</tbody>
</table>

* Mean ± SE.

Female Sprague-Dawley rats (200 g) were given DEN (25 mg/kg body weight) (p.o.) at 20 h post-PH. Rats were fed a diet containing 0.1 ppm selenium for 1 wk post-DEN and then either 0.1 or 6.0 ppm selenium for 8 wk. Rats were subsequently fed a diet containing 0.1 ppm selenium for 1 wk, followed by a diet containing 0.05% PB with 0.1 ppm selenium for 5 or 8 wk (Chart 1b), at which time focal analysis was conducted. No significant effect on number of foci per liver or mean nodule volume was observed with 6.0 ppm selenium compared to 0.1 (Table 4). Three ppm selenium was without effect. Dietary selenium concentration subsequent to AAF had no effect on body or liver weight (data not shown). Survival of rats from the cessation of AAF feeding until the termination of the experiment was 84%, 79%, and 89% for 0.1, 3.0, and 6.0 ppm selenium, respectively, therefore without treatment effect.

DISCUSSION

In our studies, enzyme-altered foci were used as a quantitative index to assess the effects of dietary selenium concentration on early stages of hepatocarcinogenesis. While unequivocal evidence is lacking that foci are precursors of carcinomas, existing data strongly suggest that foci represent relevant cells in the lineage of hepatocellular carcinomas and can provide information as to the carcinogenic potential of an experimental system (7, 8, 42, 44, 45) due to the consistent nature in which foci appear during the postinitiation stages of hepatocarcinogenesis. Because of the strong association between foci and hepatocellular carcinoma, the results from these experiments indicate that high dietary selenium can affect postinitiation stages of hepatocarcinogenesis. The concentrations of selenium used in our studies have previously been shown (by others) to alter tumor development in a number of experimental systems. In the experiments using DEN, 100 mg/kg body weight, high dietary selenium slowed the growth of foci as indicated by a smaller mean focal volume compared to 0.1 ppm selenium. Inhibition of cell proliferation as a mechanism of the anticarcinogenic effect of selenium has been previously suggested using "H-thymidine incorporation into colon (12) and colon weights (9) as indicators of proliferation in vivo. Studies in vitro have also indicated an effect of selenium on cell proliferation (29, 31, 32). Depending on the dietary selenium concentration, in the present experiments, selenium effects on mean focal volume were temporary and reversible as indicated by an initial decrease at 8 wk and subsequent return to control values by 16 wk in the 6.0-ppm selenium treatment group. The difference in the persistent effect due to less focal involvement in these livers as indicated by the decreased focal volume as a percentage of liver volume with 6.0 ppm selenium. Animals fed either 0.1 ppm selenium or 6.0 ppm selenium without AAF through 3 cycles had no foci. AAF:Selenium Experiment 2 (Chart 1, c2; High Selenium Subsequent to AAF Feeding). After 4 cycles of AAF feeding in a diet containing 0.1 ppm selenium, livers from these rats showed a number of neoplastic nodules, several exhibiting markedly atypical cellular hyperplasia in addition to a few small hepatocellular carcinomas as diagnosed histologically. Selenium at 0.1, 3.0, or 6.0 ppm was subsequently fed for 21 wk to rats which had received 0.1 ppm selenium plus AAF. Dietary selenium concentration had no effect on the percentage of animals in each group diagnosed histologically to have hepatocellular carcinoma (Table 4). Evaluation of standard liver sections and nodule cross-sections indicated the presence of hepatocellular carcinoma, cholangioma, cholangiocarcinoma, mixed cell type hepatocellular carcinoma, and neoplastic nodules. Six ppm selenium had no significant effect on the number of nodules per liver or the mean nodule volume, while causing a trend towards a decrease compared to 0.1 ppm selenium. A significant reduction of 35% in nodule volume as percentage of liver volume was observed with 6.0 ppm selenium compared to 0.1 (Table 4). Three ppm selenium was without effect. Dietary selenium concentration subsequent to AAF had no effect on body or liver weight (data not shown). Survival of rats from the cessation of AAF feeding until the termination of the experiment was 84%, 79%, and 89% for 0.1, 3.0, and 6.0 ppm selenium, respectively, therefore without treatment effect.

CANCER RESEARCH VOL. 45 NOVEMBER 1985
5492

Downloaded from cancerres.aacrjournals.org on April 14, 2017. © 1985 American Association for Cancer Research.
Table 4

Effect of dietary selenium concentration on the development of subcapsular microscopic liver lesions (nodules) and hepatocellular carcinoma following AAF administration

<table>
<thead>
<tr>
<th>Treatment (ppm selenium)</th>
<th>No. nodules</th>
<th>Mean nodular volume (cm²)</th>
<th>Nodular volume (% of liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>16/16</td>
<td>30.4 ± 2.67</td>
<td>69.1 ± 6.9</td>
</tr>
<tr>
<td>3.0</td>
<td>15/15</td>
<td>30.2 ± 4.0</td>
<td>69.0 ± 8.1</td>
</tr>
<tr>
<td>6.0</td>
<td>16/17</td>
<td>24.8 ± 1.7</td>
<td>45.3 ± 4.1**</td>
</tr>
</tbody>
</table>

*Results of the histological examination of nodules are described in “Results.”

**Individual nodule volumes were calculated from 2 perpendicular diameters measured on each nodule.

One g of liver was assumed to occupy 1 cm³ for this calculation.

Mean ± SE.

"Significantly different (P < 0.05) from 0.1 ppm selenium according to Duncan’s multiple range test.

Experimental evidence by others supporting this finding exists.

In studies in which selenium has been shown to decrease the number of animals with hepatocellular carcinoma or total numbers of carcinoma following 3'-methylaminobenzene or AAF administration, the precursor lesions may still have been present in the liver, but their growth rate slowed such that carcinoma appearance was delayed beyond the experimental end point (6, 10, 13). This interpretation is supported by an increased latency period in carcinoma appearance following concurrent feeding of high selenium and AAF (13). Similar results have been reported with dimethylhydrazine-induced colon cancer (1, 22), where initially high selenium caused a decrease in tumor incidence of the colon but at longer time points had no effect. The number of colon tumors per animal followed a similar pattern.

Similar inhibitory effects of high selenium on focal growth induced by DEN were observed when selenium was fed concurrently with AAF in the cyclic protocol. While the cyclic AAF protocol is less accurate for foci quantitation than the DEN protocol in our laboratory, the results indicate that high selenium decreased mean focal volume without affecting the number of foci in the liver similar to results obtained using DEN. In the DEN experiment, selenium effects must be independent of alterations in carcinogen metabolism. In the first AAF experiment, it is uncertain whether high selenium is directly affecting the growth of cells or altering the metabolism of AAF as has been previously described (5, 23, 30). The lack of effect of selenium on the number of foci/liver induced by AAF suggests that selenium is not affecting the efficiency at which AAF can initiate foci appear-
SELENIUM EFFECTS ON LIVER FOClS AND CARCINOMA DEVELOPMENT

ance. Alterations in AAF metabolism by selenium may, however, alter the promoting properties of AAF as indicated by a smaller mean focal volume with high selenium feeding. High selenium also prevented the increase in liver weight caused by AAF which may indicate that selenium is decreasing the toxic effects of AAF.

While 6.0 ppm selenium was fed for a total of 16 wk in the AAF:selenium study, evidence of chronic selenium as indicated by growth was not observed in contrast to the 16-wk DEN study, where both decreased body weight and histological evidence for selenium toxicity were observed. This difference could be due to the interrupted pattern of high selenium feeding, the additional 0.2% dietary methionine, or the use of male rats in the AAF study.

Additional support of an effect of high dietary selenium on preneoplastic growth is the moderate inhibitory effect of 6.0 ppm selenium on the development of nodules subsequent to AAF feeding. Selenium treatment did not, however, affect the incidence of hepatocellular carcinoma. The feeding of high selenium at relatively late stages of carcinogenesis may be less effective than feeding at early stages in terms of retarding tumor development as has been previously demonstrated (19, 34). Alternately, the cyclic protocol of AAF administration used to induce carcinoma may have used a dose of AAF in excess of that in which any selenium effects may be observed. The duration for carcinoma development in our experiments was shorter than that previously reported by approximately 20 wk using the cyclic protocol of AAF administration (15). This difference may be attributable to differences in experimental diet. It would be essential to establish a dose-response curve of AAF dose and carcinoma development to quantitate the efficiency of selenium treatment on carcinoma development.

Studies in the mammary gland have demonstrated that high selenium in the drinking water as SeO2 or selenite decreases the incidence of dimethylbenz[a]anthracene-induced ductal hyperplasias, which are believed to represent preneoplastic lesions in the mammary gland (19, 34). These studies support the proposal that high selenium can affect the development of early lesions in the carcinogenic process. It would be of great interest to apply quantitative stereological techniques to these studies in order to determine whether selenium actually decreased the incidence of hyperplasias, which in 3 dimensions may or may not give the same results as in 2 dimensions (3), or whether selenium decreased the mean volume of the hyperplasias, suggestive of decreased growth.

In contrast to an inhibitory effect on foci development, 6.0 ppm selenium, when fed after DEN but prior to the promoter PB, resulted in an enhancement of the percentage of liver occupied by foci. Whether this is a direct effect of selenium on the cells or an alteration of PB metabolism by high hepatic selenium remaining after selenium feeding is uncertain. With the absence of foci prior to PB treatment, it is impossible to assess the effects of dietary selenium concentration on foci development prior to PB. It would be plausible, however, to suggest that any selenium effects were reversible as has been previously shown when high selenium concentrations are reduced to control levels (17, 46).

In support of an enhancing effect of selenium on carcinogenesis, it was reported that 4 ppm selenium administration as Na2SeO3 in the drinking water resulted in an inhibition of large bowel cancer while enhancing small bowel cancer in rats induced by dimethylhydrazine (1). These results indicate that, in addition to the dose of selenium and the experimental protocol used, the organ system under study may also be critical as to whether selenium has an inhibitory or enhancing effect on carcinogenesis.

In addition to the chemical form of administered selenium, other factors which may modulate selenium effects on carcinogenesis are the sex, age, and strain of the animal under study as well as other dietary components such as methionine, vitamin E, and protein concentrations which affect the biological responses to high dietary selenium (28, 43, 56).

In summary, effects of selenium on focal growth may represent a "selective toxicity" to proliferating cells by virtue of the fact that they are proliferating compared to a relatively nonproliferating background and thereby decrease carcinogenesis. A separate report (27) describes studies in which selenium has been shown to decrease proliferation in both regenerating liver and in vitro with minimal deviation hepatoma cells and fibroblasts, thus supporting our hypothesis that selenium effects on carcinogenesis are at least, in part, mediated through inhibition of cell proliferation. If, however, adaptive cell proliferation occurs in response to high selenium in an organ, an enhancement of carcinogenesis is likely, resulting in the development of cells that are resistant to high doses of selenium. A selenium interaction with tumor promoters may also enhance promoting activity. In addition, our results suggest that the continued administration of selenium is necessary for the anticarcinogenic effects of selenium, because removal or repair of preneoplastic lesions did not occur with high selenium.

ACKNOWLEDGMENTS

We are grateful to Professor Henry Pitot for histopathological evaluation of tissue samples and Dr. Harold C. Campbell for assistance in the focal analysis. We would also like to thank Mark Buckley for his excellent surgical assistance and Jane Weeks and Mary Erbs for the histological preparations of samples.

REFERENCES

SELENIUM EFFECTS ON LIVER FOCI AND CARCINOMA DEVELOPMENT


Effects of Dietary Selenium Concentration on the Development of Enzyme-altered Liver Foci and Hepatocellular Carcinoma Induced by Diethylnitrosamine or N-Acetylamino-fluorene in Rats

Robert A. LeBoeuf, Brian A. Laishes and William G. Hoekstra


Updated version Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/45/11_Part_1/5489

E-mail alerts Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.