Selenium and the Acute Effects of the Carcinogens, 2-Acetylaminofluorene and Methylazoxymethanol Acetate

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ABSTRACT

Selenium inhibits the development of 2-acetylaminofluorene-induced hepatic tumors and methylazoxymethanol-induced colon tumors. It has been suggested that selenium exerts these protective effects by inhibiting metabolic activation of the carcinogen. We have studied the effects of selenium upon the acute inhibition of RNA and DNA synthesis induced by 2-acetylaminofluorene or methylazoxymethanol in intact liver, regenerating liver, and colon of weanling male Sprague-Dawley rats. Some animals received selenium in the drinking water (4 ppm) for 1 week, while others received a single injection of selenium (1 mg/kg i.p.) prior to being treated with the carcinogens. No protection against the effects of the carcinogens on RNA or DNA synthesis was noted with either treatment of selenium. Disulfiram did protect against the 2-acetylaminofluorene-induced inhibition of hepatic RNA synthesis, and pyrazole prevented the inhibition of RNA synthesis induced by methylazoxymethanol in both liver and colon. Serum selenium levels are reported. The data indicate that selenium does not influence the acute alterations induced by the carcinogens 2-acetylaminofluorene or methylazoxymethanol and suggest that the tumor-preventive effects of selenium are probably due to a mechanism other than interference with carcinogen activation and interaction with cellular macromolecules.

INTRODUCTION

Epidemiological studies have shown an inverse relationship between the dietary intake of selenium and cancer mortality (21, 23). In experimental studies, the administration of selenium in the drinking water prior to or with treatment of the animals with carcinogen has resulted in a decreased incidence of tumors in various organs (9, 14). Such effects include a decrease in the incidence of hepatic tumors induced by either 3’-methyl-4-dimethylaminoazobenzene (3) or 2-AAF,4 (11), of colon tumors induced by MAM acetate and 1,2-dimethylhydrazine (15) and azoxymethane (24), of mammary tumors induced by 7,12-dimethylbenz[a]anthracene (18, 28), and of mammary tumors occurring in mammary tumor virus-positive C3H/St mice (20). Selenium administration, however, did not decrease the number of tracheal tumors induced by 1-methyl-1-nitrosourea (25).

The mechanism of action of selenium is not clear. It has been suggested that selenium may alter the metabolic activation of carcinogens (2, 16). Rat hepatic microsomes were used to study the metabolism of 2-AAF in vitro (16). Microsomal preparations from rats pretreated with selenium produced more of the noncarcinogenic ring hydroxy metabolite than the carcinogenic N-hydroxy form. Also, selenium administered to rats decreased the in vivo metabolism of the colon carcinogen, 1,2-dimethylhydrazine (10). It is of interest to determine whether such effects of selenium could actually interfere with the biological properties of the carcinogen. Since metabolic activation of the carcinogen is required for induction of both acute and chronic effects, we studied the effects of selenium on inhibition of RNA synthesis in livers of animals that had received 2-AAF (8). Also, since selenium inhibits the carcinogenic effects of MAM, the end product of metabolized 1,2-dimethylhydrazine (5), we studied the effect of selenium on the MAM acetate-induced inhibition of nucleic acid synthesis in liver and colon (31, 32). The tumorigenic and RNA-inhibitory effects of this carcinogen are dependent on metabolism by NAD*-dependent dehydrogenases and not on microsomal metabolism (8). Preliminary findings of these studies have been published (1).

MATERIALS AND METHODS

Male Sprague-Dawley rats (CD line) were purchased from Charles River Breeding Laboratories, Brookline, Mass., and used when 4 weeks old. [methyl-3H]Thymidine, [5-3H]cytidine, and [5-3H]orotic acid were purchased from New England Nuclear, Boston, Mass.; MAM acetate was obtained from Sigma Chemical Co. St. Louis, Mo.; selenium as sodium selenite was from Alfa Products, Danvers, Mass.; disulfiram (Antabuse) and 2,3-diaminonaphthalene HCl, 99%, were from Aldrich Chemical Co. Inc., Milwaukee, Wis.; and decahydroquinoline (spectro grade) and pyrazole were from Eastman Kodak Co., Rochester, N. Y.

MAM acetate was dissolved in sterile 0.9% NaCl solution and injected i.p., 10 ml/kg (35 mg/kg); 2-AAF was dissolved in a solution of PG: DMSO (3:2) and injected i.p., 2.5 ml/kg (25 or 100 mg/kg); disulfiram was homogenized in 4% starch solution and given p.o. by intubation, 5 ml/kg (1 g/kg) into rats which had been fasted for 3 hr; pyrazole was dissolved in 0.9% NaCl solution and injected i.p., 10 ml/kg (180 or 360 mg/kg). Control animals for each of the treatments mentioned above were given only 0.9% NaCl solution, i.p., only PG:DMSO (3:2) solution, i.p., or only 4% starch solution p.o. Sodium selenite was dissolved in drinking water for the chronic studies or in 0.9% NaCl solution and injected i.p., 10 ml/kg, in the acute studies. Radioactive solutions were prepared in sterile 0.9% NaCl solution and injected i.v., 10 ml/kg.

Effects of Selenium and Disulfiram on 2-AAF-Induced Inhibition of RNA Synthesis in Regenerating Liver. Groups of rats received Purina laboratory chow and either drinking water or 4 ppm selenium in the drinking water for 1 week. On the eighth day the rats were partially hepatectomized under ether anesthesia (12) and maintained on their respective treatments. Nineteen hr later, rats within each group were given injections of either PG:DMSO solution or 2-AAF solution, 25 mg/kg i.p., and were maintained on their respective treatments.
Other groups of rats were partially hepatectomized and treated with 19 hr later, i.p., with either 0.9% NaCl solution or sodium selenite solution equivalent to 1 mg of selenium per kg body weight. One hr later, rats treated with each group received either PG:DMSO solution or 2-AAF solution, 25 mg/kg i.p.

Other untreated groups of rats were also partially hepatectomized, and 15 hr later their food was removed. Three hr later, the rats received via p.o. intubation either 4% starch solution, 5 ml/kg, or disulfiram in 4% starch solution at a dose of 0.5 or 1 g/kg. The animals were then allowed food, and 2 hr later the animals in each group received either PG:DMSO solution or 2-AAF solution, 100 mg/kg i.p. In other experiments, the rats received 2 hr after disulfiram treatment either 0.9% NaCl solution or MAM acetate, 35 mg/kg i.p.

In each of the experiments described above, 2 hr after carcinogen treatment, the rats were given \( [^{5-3H}] \)cytidine, 50 µCi/2 µmol/kg i.v., and were killed 10 min later. The livers were removed and analyzed for synthesis of RNA.

Effects of Selenium and Pyrazole on MAM Acetate-induced Inhibition of RNA Synthesis in Regenerating Liver. Groups of rats received Purina laboratory chow and either drinking water or 4 ppm selenium in the drinking water for 1 week. On the eighth day, the rats were partially hepatectomized under anesthetic ether and were maintained on their respective treatments. Nineteen hr later, rats within each group were given injections of either 0.9% NaCl solution or MAM acetate solution, 35 mg/kg i.p., and were maintained on their respective treatments.

Other untreated groups of rats were also partially hepatectomized and, 19 hr later, were given either 0.9% NaCl solution, sodium selenite solution at a dose of 1 mg selenium per kg, or pyrazole solution at a dose of 180 mg/kg i.p. One hr later, rats within each group received either 0.9% NaCl solution or MAM acetate solution at a dose of 35 mg/kg i.p.

In each of the experiments described above, 5 hr after carcinogen treatment, all the rats were given \( [^{5-3H}] \)orotic acid, 50 µCi/2 µmol/kg i.v., and were killed 10 min later. The livers were removed and analyzed for synthesis of RNA.

Effects of Selenium and Pyrazole on MAM Acetate-induced Inhibition of RNA Synthesis in Colon. Groups of rats received Purina laboratory chow and either drinking water or 4 ppm selenium in the drinking water for 1 week. Other groups of rats received either 0.9% NaCl solution, selenium selenite solution at a dose of 1 mg/kg, or pyrazole solution at a dose of 180 mg/kg i.p. On the eighth day of the drinking water experiment or 1 hr after the i.p. injections, rats within each group received either 0.9% NaCl solution or MAM acetate, 35 mg/kg i.p. Those rats that were given selenium in the drinking water continued to receive this treatment following injection of carcinogen. Three hr after treatment with carcinogen, all the rats received \( [^{5-3H}] \)cytidine, 50 µCi/2 µmol/kg i.v., and were killed 10 min later. The colons were removed, cut longitudinally, rinsed clean, and analyzed for synthesis of RNA.

Effects of Selenium and Pyrazole on MAM Acetate-induced Inhibition of DNA Synthesis in Colon and Intact Liver. Rats were placed on a diet of Purina laboratory chow and either drinking water or 4 ppm selenium in the drinking water for 1 week. Other groups of rats were given injections of 0.9% NaCl solution or solution of pyrazole, 360 mg/kg i.p. On the eighth day of the drinking water experiment or 1 hr after the i.p. injections rats in each group were given injections of either 0.9% NaCl solution or MAM acetate, 35 mg/kg i.p. Those rats that were given selenium in the drinking water continued to receive this treatment. Six hr later, all the rats received \( [^{3H}] \)thymidine, 50 µCi/2 µmol/kg i.v. After a 10-min pulse, the animals were killed by ether anesthesia and exsanguination. The livers and colons were removed, cleaned, and analyzed for DNA synthesis.

Measurement of DNA and RNA Synthesis. A 10% homogenate of liver or colon was prepared using glass-distilled water. For analysis of RNA or DNA synthesis, an equal volume of 20% TCA was added to the homogenate, and the resultant pellet was washed 3 times with 10% TCA. For analysis of RNA synthesis, the pellet was incubated overnight at 37° in 0.5 n NaOH. The digest was neutralized, the DNA and protein were precipitated, and the supernatant containing hydrolyzed RNA was analyzed for radioactivity and RNA content, using the orcinol procedure (19). For analysis of DNA synthesis, the TCA-washed pellet was heated twice at 90° with 10% TCA for 15 min. The supernatant containing hydrolyzed DNA was analyzed for radioactivity and DNA content using the diphenylamine procedure (19). Radioactive samples were added to Liquisint (National Diagnostics, Somerville, N. J.), and the activity was determined using a Packard Tri-Carb liquid scintillation spectrometer. The data were analyzed statistically using Student’s t test.

Serum Selenium Levels. Blood samples were obtained from animals in each of the treatment groups. Sera were prepared and the selenium levels were determined according to a fluorescence assay using 2,3-diaminonaphthalene (27).

RESULTS

Growth and Water Consumption. The daily weight and water intake was recorded in groups of rats receiving selenium in the drinking water. Selenium administered at a concentration of 4 ppm for 1 week to animals that were initially 4 weeks old had an effect on their growth. For example, in one set of studies, on Day 0, the control group of animals (n = 22) had an average body weight of 66.0 ± 0.94 (S.E.) g, and the average body weight of those rats that were to receive selenium (n = 23) was 66.5 ± 1.00 g. On Day 7, those rats that had consumed selenium in their drinking water, 4 ppm, had an average body weight of 81.8 ± 2.41 g, while those that had received only drinking water weighed, on Day 7, 114.7 ± 1.59 g. Thus, the selenium-treated rats showed a 23% weight gain, while the controls showed a 74% weight gain (p < 0.001).

Animals from the control groups consumed, within 24 hr, 0.36 ± 0.03 ml H₂O per g rat on experimental Day 1 and 0.31 ± 0.03 ml H₂O per g rat on Day 7 (p > 0.30), while those rats that had had selenium in the drinking water consumed, within 24 hr, 0.31 ± 0.03 ml H₂O per g rat on Day 1 and 0.17 ± 0.04 ml H₂O per g rat on Day 7 (p < 0.025). There were generally 4 to 5 weanling rats per cage.

Effect of Selenium in the Drinking Water on Carcinogen-induced Inhibition of Hepatic RNA Synthesis. Initial studies of the inhibition of nucleic acid synthesis were performed using several doses of each carcinogen. That dose of each carcinogen that produced the greatest inhibition of nucleic acid synthesis on the linear portion of the dose-response curve was used in these studies. Thus, any interference with activation of the carcinogen would have been easily discernible.

Selenium administered for 1 week in the drinking water did not decrease the inhibitory effect of 2-AAF or of MAM acetate on RNA synthesis in regenerating liver (Table 1). Selenium itself did inhibit RNA synthesis in regenerating liver about 26%, and the total hepatic content of RNA at the end of 1 week of treatment with selenium in the drinking water was 8% lower than the group that had received drinking water alone.

Acute Toxicity of Selenium. We wished to determine whether large single doses of selenium would interfere with the effects of the carcinogens. We treated partially hepatectomized rats with various doses of selenium i.p. and then treated them with carcinogen under actual experimental conditions. Rats were given 1.0 and 3.0 mg selenium per kg i.p. By 4 hr, 4 of 10 rats that had received 3.0 mg/kg died, and none of 27 rats that had received 1.0 mg/kg died.

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Studies with Single Treatments of Selenium or Disulfiram and 2-AAF in Regenerating Liver. A single injection of selenium, 1 mg/kg i.p., did not protect against the 2-AAF-induced inhibition of RNA synthesis (Table 2). As a positive control, we tested the effects of disulfiram, an inhibitor of the microsomal mixed-function oxidase system (see Ref. 5), on the acute effects of 2-AAF. The data suggest that disulfiram, 1 g/kg, did prevent the inhibitory effects of 2-AAF (Table 2); the dose of carcinogen was 4 times greater than that used in either the selenium-treated drinking water or parenteral selenium experiments.

Disulfiram by itself did inhibit RNA synthesis markedly. To establish whether RNA synthesis could, in fact, be inhibited to an extent greater than that observed using disulfiram, we tested the effects of disulfiram against the inhibition of RNA synthesis induced by MAM acetate, a carcinogen not requiring activation by the microsomal system. The data indicate (Table 2) that MAM acetate can inhibit RNA synthesis even further than that induced by disulfiram; thus, even though the level of RNA synthesis in the disulfiram:2-AAF group did not return to the starch:PG:DMSO level, the absence of any further inhibition in this group relative to the disulfiram control group indicates true prevention. In addition, we studied the effects of 2-AAF in rats pretreated with disulfiram, 0.5 g/kg. As with the higher dose, RNA synthesis was inhibited (50% of control). In these studies, however, the 2-AAF-induced changes were only partially prevented; the level of RNA synthesis in the disulfiram:2-AAF-treated animals was lower than in the disulfiram:solvent group.

Thus, in spite of the large inhibition of RNA synthesis by disulfiram, it is possible for the carcinogen to depress RNA synthesis even further and again, the data obtained with disulfiram, 1 g/kg, indicate true prevention.

Studies with Single Treatments of Selenium or Pyrazole and MAM Acetate in Regenerating Liver. The data in Table 3 show that treatment of rats with selenium, 1 mg/kg i.p., did not prevent the inhibitory effects of MAM acetate, while treatment with pyrazole prior to MAM acetate results in complete prevention of the carcinogen-induced inhibition of hepatic RNA synthesis.

Studies with Selenium or Pyrazole and MAM Acetate in Colon. The data in Table 4 indicate that, in 3 separate experiments, MAM acetate reduced RNA synthesis in colon to 68 to 71% of control values. Treatment with selenium in drinking water for 1 week or given i.p. did not prevent this inhibition. Treatment with pyrazole, however, completely prevented the carcinogen-induced inhibition.

### Table 1

**Effect of selenium in the drinking water on 2-AAF- and MAM acetate-induced inhibition of RNA synthesis in regenerating liver**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>dpm/μmol ribose</th>
<th>% of control</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drinking water + PG:DMSO</td>
<td>10</td>
<td>8,795 ± 397</td>
<td>&gt;0.05</td>
<td>4.5</td>
</tr>
<tr>
<td>Drinking water + 2-AAF (25 mg/kg)</td>
<td>10</td>
<td>5,898 ± 301</td>
<td>67.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Selenium in water + PG:DMSO</td>
<td>6</td>
<td>6,542 ± 132</td>
<td>63.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Selenium in water + 2-AAF (25 mg/kg)</td>
<td>4</td>
<td>4,153 ± 261</td>
<td>63.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Drinking water + 0.9% NaCl solution</td>
<td>3</td>
<td>10,146 ± 839</td>
<td>&gt;0.05</td>
<td>4.5</td>
</tr>
<tr>
<td>Drinking water + MAM acetate (35 mg/kg)</td>
<td>5</td>
<td>2,200 ± 474</td>
<td>21.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Selenium in water + 0.9% NaCl solution</td>
<td>4</td>
<td>7,524 ± 650</td>
<td>&gt;0.05</td>
<td>4.5</td>
</tr>
<tr>
<td>Selenium in water + MAM acetate (35 mg/kg)</td>
<td>4</td>
<td>660 ± 315</td>
<td>8.8</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Mean ± S.E.

### Table 2

**Effect of selenium i.p. and disulfiram p.o. on 2-AAF-induced inhibition of RNA synthesis in regenerating liver**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>dpm/μmol ribose</th>
<th>% of control</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9% NaCl solution + PG:DMSO</td>
<td>6</td>
<td>9,050 ± 744</td>
<td>&gt;0.05</td>
<td>4.5</td>
</tr>
<tr>
<td>0.9% NaCl solution + 2-AAF (25 mg/kg)</td>
<td>6</td>
<td>5,870 ± 128</td>
<td>62.7</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Selenium (1 mg/kg) + PG:DMSO</td>
<td>5</td>
<td>7,864 ± 271</td>
<td>&gt;0.05</td>
<td>4.5</td>
</tr>
<tr>
<td>Selenium (1 mg/kg) + 2-AAF (25 mg/kg)</td>
<td>7</td>
<td>5,384 ± 312</td>
<td>68.5</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>4% Starch + PG:DMSO</td>
<td>3</td>
<td>13,858 ± 861</td>
<td>&gt;0.05</td>
<td>4.5</td>
</tr>
<tr>
<td>4% Starch + 2-AAF (100 mg/kg)</td>
<td>3</td>
<td>7,291 ± 1,403</td>
<td>52.6</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Disulfiram (1 g/kg) + PG:DMSO</td>
<td>3</td>
<td>5,594 ± 473</td>
<td>&gt;0.05</td>
<td>4.5</td>
</tr>
<tr>
<td>Disulfiram (1 g/kg) + 2-AAF (100 mg/kg)</td>
<td>3</td>
<td>5,987 ± 791</td>
<td>107.0</td>
<td>&gt;0.70</td>
</tr>
<tr>
<td>4% Starch + 0.9% NaCl solution</td>
<td>6</td>
<td>7,040 ± 817</td>
<td>&gt;0.05</td>
<td>4.5</td>
</tr>
<tr>
<td>4% Starch + MAM acetate (35 mg/kg)</td>
<td>6</td>
<td>3,403 ± 51</td>
<td>48.3</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Disulfiram (1 g/kg) + 0.9% NaCl solution</td>
<td>5</td>
<td>4,609 ± 459</td>
<td>&gt;0.05</td>
<td>4.5</td>
</tr>
<tr>
<td>Disulfiram (1 g/kg) + MAM acetate (35 mg/kg)</td>
<td>6</td>
<td>2,805 ± 135</td>
<td>60.9</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

* Mean ± S.E.

* A test of the percentage of inhibition (48.3% versus 60.9%) by 2-way analysis of variance with interaction indicates that they are not significantly different (p = 0.23).

### Table 3

**Effect of selenium i.p. and pyrazole i.p. on MAM acetate-induced inhibition of RNA synthesis in regenerating liver**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>dpm/μmol ribose</th>
<th>% of control</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9% NaCl solution + 0.9% NaCl solution</td>
<td>2</td>
<td>10,687 ± 802</td>
<td>&gt;0.05</td>
<td>4.5</td>
</tr>
<tr>
<td>0.9% NaCl solution + MAM acetate (35 mg/kg)</td>
<td>3</td>
<td>3,584 ± 679</td>
<td>33.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Selenium (1 mg/kg) + 0.9% NaCl solution</td>
<td>2</td>
<td>8,912 ± 168</td>
<td>&gt;0.05</td>
<td>4.5</td>
</tr>
<tr>
<td>Selenium (1 mg/kg) + MAM acetate (35 mg/kg)</td>
<td>3</td>
<td>1,904 ± 348</td>
<td>21.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>0.9% NaCl solution + 0.9% NaCl solution</td>
<td>6</td>
<td>6,583 ± 269</td>
<td>&gt;0.05</td>
<td>4.5</td>
</tr>
<tr>
<td>0.9% NaCl solution + MAM acetate (35 mg/kg)</td>
<td>6</td>
<td>2,069 ± 170</td>
<td>31.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pyrazole (180 mg/kg) + 0.9% NaCl solution</td>
<td>6</td>
<td>6,641 ± 156</td>
<td>&gt;0.05</td>
<td>4.5</td>
</tr>
<tr>
<td>Pyrazole (180 mg/kg) + MAM acetate (35 mg/kg)</td>
<td>7</td>
<td>6,145 ± 196</td>
<td>92.5</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

* Mean ± S.E.
animals that had received a single treatment of selenium, 1 mg/kg i.p.

**DISCUSSION**

The administration of selenium in the drinking water can reduce the incidence of tumors in the livers or colons of animals receiving 2-AAF or MAM acetate, respectively (11, 15). It has been suggested that, at least in the case of 2-AAF, selenium exerted this effect by interfering with metabolic activation of the carcinogen (2, 16). The experiments leading to this conclusion were performed *in vitro* and a decreased formation of active metabolite was measured (16). Also, less 2-AAF was bound to DNA and RNA isolated from livers of rats treated with selenium (2). The administration of selenium in the drinking water to rats for several weeks resulted in a decreased metabolism of the colon carcinogen, 1,2-dimethylhydrazine (10). It cannot be presumed, however, that the fraction of active metabolite that was formed or the amount of adduct formation that did occur was insufficient to exert biological activity. In fact, in the study in which selenium did decrease the overall metabolism of 1,2-dimethylhydrazine, an enhanced alkylation of colonic DNA was observed (10). Since the acute and chronic effects of each carcinogen are believed to be mediated via the same metabolic product, we attempted to study this problem by determining whether the antitumor doses of selenium could in fact interfere with the acute biological effects of the carcinogen. Carcinogen-induced inhibition of nucleic acid synthesis was chosen as the end point and, in each case, the dose of carcinogen used was determined to be on the linear portion of the dose-response curve. Any interference with carcinogen activation or activity required to induce inhibition of DNA or RNA synthesis would be easily measurable.

The data indicate that selenium administration in the drinking water at concentrations used in the antitumor studies, or at high doses given acutely in these studies, did not prevent the inhibition of RNA synthesis induced by 2-AAF in regenerating liver or the inhibition of RNA synthesis induced by MAM in either regenerating liver or colon. We have shown that selenium was present in serum at the time that the animals were killed either regenerating liver or colon. We have shown that selenium administration in the drinking water was sufficient to exert biological effects, since the animals did not grow as well as the control animals. Similar effects on growth were reported by others (9, 13, 22).

Disulfiram, an inhibitor of the microsomal mixed-function oxidase system (see Ref. 5) that activates 2-AAF, did block the inhibitory effects of the carcinogen. Also, pyrazole, an inhibitor of alcohol dehydrogenase (7) that prevents MAM-induced tumor development (33) and lethality (8) and enzymatic activation of MAM to an alkylating agent (4), prevented the carcinogen-induced inhibition of RNA synthesis. Thus, known inhibitors of the metabolic pathways responsible for activation of these carcinogens did inhibit the acute toxicity induced by these agents. The data indicate, therefore, that selenium does not interfere with metabolic activation of either 2-AAF or MAM, at least to an extent that would prevent their biological activities.

The data shown in Tables 3, 4, and 5 indicate that the MAM-induced inhibition of RNA synthesis is more dependent on metabolism by alcohol dehydrogenase than is the inhibition of DNA synthesis. The carcinogen-induced inhibition of RNA synthesis is completely prevented by pyrazole, while that of DNA synthesis is not markedly affected (Table 5). Other data confirming these results using different doses of carcinogen and inhibitor and different treatment schedules have been reported previously (30, 34). It is reasonable to speculate that, while the inhibition of both RNA and DNA synthesis is mediated via the carbonium ion released from MAM, DNA synthesis is more sensitive and can be inhibited by the amount of carbonium ion

**Table 4**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>dpm/μmol deoxyribose</th>
<th>% of control</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drinking water + 0.9% NaCl solution</td>
<td>6</td>
<td>3,032 ± 176**</td>
<td>71.0</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Drinking water + MAM acetate (35 mg/kg)</td>
<td>6</td>
<td>2,154 ± 85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selenium in water + 0.9% NaCl solution</td>
<td>6</td>
<td>3,707 ± 301</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selenium in water + MAM acetate (35 mg/kg)</td>
<td>6</td>
<td>2,174 ± 205</td>
<td>58.6</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>0.9% NaCl solution + 0.9% NaCl solution</td>
<td>6</td>
<td>2,674 ± 118</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.9% NaCl solution + MAM acetate (35 mg/kg)</td>
<td>6</td>
<td>1,850 ± 192</td>
<td>69.2</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Selenium (1 mg/kg) + 0.9% NaCl solution</td>
<td>6</td>
<td>3,089 ± 258</td>
<td></td>
<td></td>
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<tr>
<td>Selenium (1 mg/kg) + MAM acetate (35 mg/kg)</td>
<td>6</td>
<td>1,698 ± 87</td>
<td>54.9</td>
<td>&lt;0.001</td>
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<td>0.9% NaCl solution + 0.9% NaCl solution</td>
<td>7</td>
<td>3,154 ± 221</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.9% NaCl solution + MAM acetate (35 mg/kg)</td>
<td>7</td>
<td>2,150 ± 132</td>
<td>68.2</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Pyrazole (180 mg/kg) + 0.9% NaCl solution</td>
<td>7</td>
<td>2,598 ± 143</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrazole (180 mg/kg) + MAM acetate (35 mg/kg)</td>
<td>7</td>
<td>2,843 ± 160</td>
<td>109.4</td>
<td>&gt;0.20</td>
</tr>
</tbody>
</table>

*a Mean ± S.E.

**Table 5**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>dpm/μmol deoxyribose</th>
<th>% of control</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drinking water + 0.9% NaCl solution</td>
<td>10</td>
<td>317 ± 38**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drinking water + MAM acetate (35 mg/kg)</td>
<td>9</td>
<td>123 ± 14</td>
<td>38.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Selenium in water + 0.9% NaCl solution</td>
<td>9</td>
<td>403 ± 33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selenium in water + MAM acetate (35 mg/kg)</td>
<td>10</td>
<td>145 ± 14</td>
<td>36.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Colon</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drinking water + 0.9% NaCl solution</td>
<td>10</td>
<td>1214 ± 85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drinking water + MAM acetate (35 mg/kg)</td>
<td>10</td>
<td>436 ± 47</td>
<td>35.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Selenium in water + 0.9% NaCl solution</td>
<td>10</td>
<td>1414 ± 153</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selenium in water + MAM acetate (35 mg/kg)</td>
<td>10</td>
<td>351 ± 33</td>
<td>24.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Colon</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.9% NaCl solution + 0.9% NaCl solution</td>
<td>2</td>
<td>1888 ± 370</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.9% NaCl solution + MAM acetate (35 mg/kg)</td>
<td>2</td>
<td>480 ± 14</td>
<td>25.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Pyrazole, (360 mg/kg) + 0.9% NaCl solution</td>
<td>2</td>
<td>2303 ± 96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrazole, (360 mg/kg) + MAM acetate (35 mg/kg)</td>
<td>2</td>
<td>899 ± 309</td>
<td>39.0</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*a Mean ± S.E.
released spontaneously from MAM. Inhibition of RNA synthesis appears to require the additional carbonium ions released from the metabolite of MAM following its metabolism by alcohol dehydrogenase (4).

It is conceivable that selenium exerts its antitumor properties by interfering with the effects of the carcinogen on DNA synthesis. The data in Table 5 indicate that selenium did not prevent the carcinogen-induced inhibition of DNA synthesis.

It appears from our studies that selenium can enhance the MAM acetate-induced inhibition of RNA synthesis. Although at the moment we have no explanation for these findings, the data are in keeping with the selenium-enhanced alklylation of DNA by 1,2-dimethylhydrazine, as noted by Harbach and Swenberg (10).

In conclusion, the data suggest that the inhibitory effects of selenium on carcinogen-induced tumor development are due to some mechanism other than inhibition of carcinogen metabolism. Wortzman et al. (29) reported that selenium did not decrease 2-AAF binding to DNA or alter the rate of DNA repair. They did report that selenium prevented DNA strand breakage at low doses of carcinogen.

Selenium can also prevent tumor development when given after carcinogen treatment is complete (26), can prevent viral-induced mammary dysplasia (18), and can decrease the incidence of spontaneous tumors (17). It appears that selenium can inhibit tumorigenesis regardless of the mechanism of tumor initiation; further work is required to fully understand the mechanism whereby selenium can influence cell transformation and tumor development.

REFERENCES

Selenium and the Acute Effects of the Carcinogens, 2-Acetylaminofluorene and Methylazoxymethanol Acetate

William P. Banner, Queng Hui Tan and Morris S. Zedeck


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