Inhibition of Carcinogen-induced Neoplasia by Sodium Cyanate, tert-Butyl Isocyanate, and Benzyl Isothiocyanate Administered Subsequent to Carcinogen Exposure

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ABSTRACT

The effects of sodium cyanate, tert-butyl isocyanate, and benzyl isothiocyanate on carcinogen-induced neoplasia were studied in experiments in which the test compound was fed starting 1 week following completion of carcinogen administration. Under these conditions, all three test compounds exerted an inhibitory effect on the occurrence of 7,12-dimethylbenz(a)anthracene-induced neoplasia of the breast of Sprague-Dawley rats. In a second experiment model, sodium cyanate inhibited the occurrence of 1,2-dimethylhydrazine-induced neoplasia of the large bowel of female CF-1 mice. This, a new group of compounds has been identified which has inhibitory capacities against neoplasia when given subsequent to carcinogen exposure.

INTRODUCTION

An increasing number of compounds are being found to inhibit chemical carcinogenesis (14, 15, 18–22). One of these inhibitors has been identified recently as sodium cyanate (21). This compound is of particular interest because it produces a marked inhibition of protein synthesis in a variety of neoplastic cells under conditions where a corresponding inhibition of protein synthesis in normal tissues of the tumor-bearing animal does not occur. Neoplastic cells in which inhibition of protein synthesis has been observed include hepatomas, MK-3 kidney tumors, primary and transplantable carcinomas of the large bowel of the rat, and Ehrlich ascites cells (1, 4, 5, 12, 13). Sodium cyanate requires metabolic activation in order to inhibit protein synthesis. A phenobarbital-inducible cytochrome P-450 has been implicated as the activating system (5). Data have been obtained indicating that suppression of protein synthesis is the result of inhibition of protein chain initiation (1). In addition to the effects on protein synthesis, sodium cyanate has also been shown to inhibit DNA synthesis in neoplastic cells (10–12). Another property of sodium cyanate is that it carbamylates the amino-terminal residues of both the α- and β-globin chains of hemoglobin S (7). The compound has been found to have anti-sickling properties and has been proposed as a potential treatment for sickle cell disease. Although chronic administration studies in animals have shown low toxicity, some adverse effects in humans have limited its usefulness (2, 3, 6, 7).

Previous work has shown that administration of sodium cyanate in the diet prior to and during the course of administration of polycyclic aromatic hydrocarbons inhibits the occurrence of neoplasia (21). The present investigation was directed towards determining if sodium cyanate would be inhibitory if its feeding were started shortly after carcinogen administration, the period during which neoplasia evolves. Two animal systems have been used, i.e., DMBA2-induced mammary neoplasia in the rat and DMH-induced neoplasia of the large intestine in the mouse. In both, sodium cyanate was fed in the diet starting 1 week after carcinogen exposure and continued throughout the subsequent course of the experiment. In addition to sodium cyanate, 2 other compounds, tert-butyl isocyanate and benzyl isothiocyanate, were investigated.

MATERIALS AND METHODS

Mammary Neoplasms. The procedure for producing mammary tumors was a modification of that described by Huggins et al. (8). Female Sprague-Dawley rats (King Company, Oregon, Wis., or Holtzman Company, Madison, Wis.) were given 12 mg of DMBA in 1.0 ml of olive oil by p.o. intubation when they were 7 weeks old. One week later, the rats were randomized by weight and placed on the experimental diets. These diets consisted of powdered Purina rat chow (Ralston Purina Company, St. Louis, Mo.) containing the test compound or without any additions for the diet fed the control groups. The experimental diets were fed until the experiment was terminated. The rats were weighed at 2-week intervals, and mammary tumors were counted starting 10 weeks after administration of DMBA. The diagnosis was confirmed at autopsy.

Neoplasms of the Large Bowel. The procedure for producing large-bowel neoplasms was a modification of that described by Thurnherr et al. (16). Female CF-1 mice (Charles River Breeding Laboratory, Kingston, N. Y.) were used throughout. When the mice were 10 weeks old, they were randomized by weight. At that time, 0.6 mg of DMH in a 0.2-ml solution of EDTA brought to pH 6.5 with sodium carbonate was injected s.c. A total of 16 DMH administrations, twice a week for 8 weeks, was given. One week following the last injection of DMH, the mice were placed on the experimental diets. These diets consisted of powdered Purina rat chow containing the test compound or without any additions for the diet fed the control groups. The experimental diets were fed until mice were 52 weeks old. At that time, all animals were killed by inhalation of CO2 and autopsied. The large bowel was opened, and the number and location of neoplasms were recorded. Each lesion was studied microscopically.

Chemicals. Sodium cyanate was obtained from the Aldrich

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2 The abbreviations used are: DMBA, 7,12-dimethylbenz(a)anthracene; DMH, symmetrical 1,2-dimethylhydrazine dihydrochloride.
Chemical Company, Milwaukee, Wis. The preparation was assayed as follows: sodium cyanate, 97%; sodium chloride, 3%; and sodium carbonate, trace. Other chemicals used were as follows: DMH and tert-butyl isocyanate (Aldrich); and benzyl isothiocyanate and DMBA (Eastman Organic Chemicals, Rochester, N. Y.).

Statistical Analysis. Student's t test was used to determine the statistical differences in the number of tumors per group between the control and treated groups, and the χ² test was used for the differences of percentage of tumor-bearing animals in these groups.

RESULTS

Data on the effects of sodium cyanate on DMBA-induced mammary neoplasia in the rat are shown in Table 1. In all 3 experiments in which it was used, sodium cyanate administration resulted in fewer rats showing mammary neoplasms and a decrease in average number of tumors per rat. Comparable findings were obtained with rats fed tert-butyl isocyanate and benzyl isothiocyanate. The 4 experiments were carried out over a period of 2 years. As described in "Materials and Methods," Sprague-Dawley rats from 2 different suppliers were used. Those used in Experiments 3 and 4 were more responsive to DMBA. With the mammary tumor count at a shorter time interval, the data become comparable to those in Experiments 1 and 2. When all the rats in the control groups in Experiments 1 to 3 showed mammary tumors (with the exception of one animal in Experiment 1), neoplasms were found in 60 to 63% of rats in the groups fed 0.026 mmol of sodium cyanate per g of diet. The average number of tumors per animal in the sodium cyanate groups was 42 to 43% of that of the corresponding controls. Thus, in the 3 experiments, consistent results were obtained. In Experiment 4, similar inhibitory effects were found with tert-butyl isocyanate and benzyl isothiocyanate as the test compounds.

The experiments determining the inhibitory capacity of sodium cyanate on DMH-induced neoplasia of the large bowel showed that feeding sodium cyanate resulted in fewer mice having demonstrable neoplasms at the conclusion of the experiments and a decrease in the average number of neoplasms per mouse. The magnitude of the protective effect was similar to that found in the mammary tumor protocols. If the results of Experiments 1 and 2 are combined, 39% as many mice fed sodium cyanate (0.026 mmol/g of diet) had neoplasms of the large bowel as compared to the control groups. The average number of neoplasms in the sodium cyanate groups was 38% that of the control (Table 2).

DISCUSSION

Sodium cyanate has been shown to inhibit protein and DNA synthesis in several types of neoplastic cells, including those of the large bowel. It is possible that the mechanism by which the early stages of neoplasia of the large bowel and mammary gland were inhibited in the present study resides in the capacity of sodium cyanate to suppress synthesis of one or both of these macromolecular species. The cyanate anion undergoes activation to an unstable metabolite by a phenobarbital-inducible cytochrome P-450 system (5). Thus, the magnitude of the inhibitory activities observed in the present study might be controlled by the activity of the activating system. If this is the case, the potential would exist for magnifying the inhibitory effects of sodium cyanate by enhancing the activity of the activating system.

The finding that tert-butyl isocyanate and benzyl isothiocyanate cause levels of inhibition of mammary tumor formation comparable to those produced by sodium cyanate suggests

Table 1

Effects of sodium cyanate and related compounds on DMBA-induced mammary tumor formation in female Sprague-Dawley rats

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Additions to the diet</th>
<th>Concentration (mmol/g)</th>
<th>No. of rats</th>
<th>Wt gain (g)</th>
<th>% of rats with tumors</th>
<th>No. of tumors/rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td></td>
<td>16</td>
<td>68</td>
<td>94</td>
<td>2.6 ± 0.3*</td>
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<td></td>
<td>Sodium cyanate</td>
<td>0.026</td>
<td>16</td>
<td>68</td>
<td>63*</td>
<td>1.1 ± 0.2*</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td></td>
<td>16</td>
<td>69</td>
<td>100</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Sodium cyanate</td>
<td>0.026</td>
<td>16</td>
<td>66</td>
<td>63*</td>
<td>1.0 ± 0.3*</td>
</tr>
<tr>
<td>3</td>
<td>None</td>
<td></td>
<td>29</td>
<td>66</td>
<td>100</td>
<td>2.8 ± 0.2</td>
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<td></td>
<td>Sodium cyanate</td>
<td>0.013</td>
<td>13</td>
<td>64</td>
<td>77*</td>
<td>1.2 ± 0.3*</td>
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<tr>
<td></td>
<td>Sodium cyanate</td>
<td>0.026</td>
<td>15</td>
<td>65</td>
<td>60*</td>
<td>1.2 ± 0.5*</td>
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<td>4</td>
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<td>27</td>
<td>53</td>
<td>100</td>
<td>3.4 ± 0.3</td>
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<td></td>
<td>tert-Butyl isocyanate</td>
<td>0.013</td>
<td>16</td>
<td>43</td>
<td>50*</td>
<td>1.2 ± 0.4*</td>
</tr>
<tr>
<td></td>
<td>Benzyl isothiocyanate</td>
<td>0.017</td>
<td>16</td>
<td>48</td>
<td>63*</td>
<td>1.4 ± 0.4*</td>
</tr>
</tbody>
</table>

* Female Sprague-Dawley rats used in Experiments 1 and 2 were from King Company, those in Experiments 3 and 4 were from Holtzman Company. In all experiments, DMBA, 12 mg in 1.0 ml olive oil, was given by p.o. intubation when the rats were 7 weeks old. The experimental diets were begun at 8 weeks of age and continued throughout the remainder of the experiment.

† Weight gain from 7 to 25 weeks of age in Experiments 1 and 2 and from 7 to 23 weeks of age in Experiments 3 and 4.

‡ Mammary neoplasms when rats were 25 weeks old in Experiments 1 and 2 and 23 weeks old in Experiments 3 and 4.

§ Number of neoplasms occurring in the entire group divided by the number of rats at risk.

* Mean ± S.E.

† p < 0.05

§ p < 0.01

h p < 0.001.
that the suppression of early neoplastic events may be brought about by a diverse group of related compounds. It is not known whether the mechanism of inhibition by all 3 compounds is the same or if they differ. The reactive species of both tert-butyl isocyanate and benzyl isothiocyanate can undergo dissociation to form the stable tert-butyl and benzyl carbonium ions. The stability of these ions is a good driving force for the liberation of the isocyanate and isothiocyanate anions. Once these anions are formed, their reactivities would be the same as the corresponding ions from the sodium salt. The isocyanate and cyanate ions are identical because of resonance. The isothiocyanate ion would be expected to have reactivities similar to those of the cyanate ion. Studies of Lea and Koch (10) have shown that sodium thiocyanate resembles sodium cyanate in its inhibitory effects on thymidine incorporation and on the uptake of phosphate and amino acids in transplanted tumors of the rat. Accordingly, it is possible that the 3 compounds found to inhibit neoplasia in the present work act through a common mechanism.

The full variety and interrelationships of cyanates, isocyanates, isothiocyanates, and possibly thiocyanates that will suppress the occurrence of neoplasia warrant further exploration. Benzyl isothiocyanate is of interest in that it is a naturally occurring compound. Benzyl isothiocyanate and the closely related compound, phenethyl isothiocyanate, occur in relatively large amounts in cruciferous vegetables including cabbage, Brussels sprouts, cauliflower, and broccoli (9, 17). Thus, there is consumption of at least one category of food containing an inhibitor(s) of neoplastic events subsequent to carcinogen exposure.

REFERENCES

9. Kjaer, H. Naturally occurring 

Table 2

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Additions to the diet</th>
<th>Concentration (mmol/g)</th>
<th>No. of mice</th>
<th>Wt gain (g)a</th>
<th>% of mice with tumors</th>
<th>No. of tumors/mouseb</th>
<th>% of mice with tumors</th>
<th>No. of tumors/mouseb</th>
<th>% of mice with tumors</th>
<th>No. of tumors/mouseb</th>
</tr>
</thead>
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<tr>
<td>1 None</td>
<td>Sodium cyanate 0.013</td>
<td>17 17 53 1.09 ± 0.22 21 0.21 ± 0.07 41 0.88 ± 0.21</td>
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<td></td>
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</tr>
<tr>
<td>2 None</td>
<td>Sodium cyanate 0.026</td>
<td>16 16 16 0.47 ± 0.28 0 0.00 ± 0.00 18 0.47 ± 0.28</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Combined 1</td>
<td>Sodium cyanate 0.026</td>
<td>16 16 17 0.50 ± 0.27 6 0.06 ± 0.05 17 0.44 ± 0.24</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*a Female CF-1 mice were given 16 s.c. administrations of 0.6 mg DMH (twice a week for 8 weeks) starting when the animals were 10 weeks old. One week after the last administration of DMH, the mice were placed on the experimental diets, and they were continued on these diets until the animals were 52 weeks old. At that time, the experiments were terminated.

*b Weight gain from 10 to 52 weeks of age.

*c Neoplasms of the large bowel when mice were 52 weeks old.

*d Number of neoplasms in the entire group divided by the number of mice at risk.

*e Mean ± S.E.

*f p < 0.05.

*g p < 0.01.


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