Relative Carcinogenic Effectiveness of Derivatives of Nitrosodiethylamine in Rats

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ABSTRACT

The carcinogenicity of five derivatives of nitrosodiethylamine was compared with that of the parent compound by p.o. administration to rats. All were less potent than nitrosodiethylamine. When nitrosobis(2-methoxy-ethyl)amine and nitrosobis(2-ethoxyethyl)amine were administered at equimolar doses in drinking water, there was a high incidence of liver tumors, but the animals died later than they did after nitrosodiethylamine treatment, which also induced esophageal tumors. Nitrosoiminodipropionitrile and nitrosobis(2,2-diethoxyethyl)amine failed to induce tumors at the same dose level. Nitrosobis(2-chloroethyl)amine was administered in oil by gavage at a dose lower than that of nitrosodiethylamine and produced a much weaker tumor response; 5 of 15 treated rats had forestomach papillomas, and 1 had olfactory adenocarcinoma and no other induced tumors.

INTRODUCTION

Among cyclic nitrosamines profound effects have been observed on their carcinogenic effectiveness following replacement of hydrogen atoms at various positions by substituents of several kinds. For example, in nitrosomorpholine, replacement of α-hydrogen atoms by deuterium greatly reduced the number of animals with liver tumors compared to the number of animals with tumors induced by the parent compound (12). Similarly, replacement of hydrogen by deuterium in nitrosodimethylamine reduced its carcinogenic potency (2). We wanted to examine systematically the effect of other substituents on the carcinogenicity of allphatic nitrosamines, as had been carried out with cyclic nitrosamines (3-6, 11). The essence of these studies was to test the compounds by administration of equimolar doses to rats and to observe the animals until death.

The base compound for these studies was nitrosodiethylamine, which can be considered a 1,1′-dimethyl derivative of nitrosodimethylamine; the latter is the simplest nitrosamine possible. The compounds examined in this series are all symmetrically substituted derivatives of nitrosodiethylamine and include the 2,2′-dichloro-, 2,2′-dicyano-, 2,2′-dimethoxy-, 2,2′-diethoxy-, and 2,2′-bis-diethoxy derivatives. With the exception of the dichloro compound, which was relatively unstable and had to be administered in olive oil, the other nitrosamines, including nitrosodiethylamine, were given to rats as solutions in drinking water at 0.68 ml.

Some similar unsymmetrical compounds such as methylmethoxymethylnitrosamine have been tested in rats, but not at equimolar doses, which makes comparison of carcinogenic potency more difficult (16).

MATERIALS AND METHODS

Chemicals. The nitrosamines were all prepared by nitrosation of the corresponding secondary amines, which were obtained from Aldrich Chemical Co., Milwaukee, Wis.

Nitrosodiethylamine (Diethylnitrosamine). This was prepared by reaction of diethylether (35 g, 0.5 mol; 50 ml) (converted to the hydrochloride with 40 ml 10 N HCl cooled in ice) with 50 g of sodium nitrite in 50 ml of glacial acetic acid. After the mixture had stood for 2 hrs at room temperature, the yellow oil was separated off, mixed with 50 ml methylene chloride, and dried with anhydrous sodium carbonate. After filtration the solvent was evaporated from the clear liquid, and the remainder was distilled. Almost all of the oil distilled at 174°-176°, giving 36 g of yellow oil, which was at least 98% pure by nuclear magnetic resonance and thin-layer chromatography. The mass spectrum was that expected for nitrosodiethylamine, with a strong molecular ion at m/e 102 (13).

Nitrosoiminodipropionitrile. This was prepared by dissolving 25 g (0.2 mol) of iminodipropionitrile in 50 ml of of glacial acetic acid and 50 g of ice. Sodium nitrite (30 g) was added, and the solution stood overnight at 4°. The solution was diluted with 50 ml of water and extracted with 2 × 75 ml of methylene chloride. This extract was shaken with 5% sodium carbonate solution and then with 20 ml 5 N HCl. After evaporation to dryness in a stream of nitrogen, the solution left a pale yellow solid that, after crystallization from methanol, weighed 17.6 g and had a melting point of 47°-49°.

$C_6H_8N_2O$

Calculated: C 47.40, H 5.30, N 36.82

Found: C 47.35, H 5.33, N 36.72

The compound gave only 1 spot on a thin-layer chromatography plate. The mass spectrum (13) showed no molecular ion (70 eV), but the fragmentation was consistent with the structure in Chart 1. The molar absorptivity was 80 at 354 nm in water.

Nitrosobis(2-chloroethyl)amine. Bis(2-chloroethyl)amine hydrochloride (18 g, 0.1 mol) was dissolved in 30 ml glacial acetic acid and 50 g ice. Sodium nitrite (15 g) was added, and the mixture was cooled in ice. After 3 hr, an oil separated at the bottom, and 20 ml methylene chloride was added. The lower layer was separated off, washed with 2 × 50-ml portions of water, and dried with anhydrous magne-
There was a prominent molecular ion in the mass spectrum (13), and the fragmentation was consistent with the structure in the chart. The molar absorptivity was 78 at 362 nm in ethanol.

An aqueous solution of the compound showed decomposition, which was considerable after 2 hr and complete after 18 hr.

**Nitrosobis(2-methoxyethyl)amine.** Bis(2-methoxyethyl)amine (5 g, 0.04 mol) was dissolved in 10 g glacial acetic acid and 10 g ice. Sodium nitrite (6 g) was added, and the mixture was allowed to react for 2 hr at room temperature. A thin layer of oil separated and was extracted with methyl chloride (2 x 50 ml). The extract was shaken with water (3 x 25 ml), and the solvent was evaporated in a stream of nitrogen at room temperature, leaving 5.7 g of yellow oil. After evaporation of the solvent in a stream of nitrogen at room temperature, a light yellow-brown oil remained, which showed a main band on thin-layer chromatography and 2 very faint bands due to impurities. The yield was 11.7 g.

**Nitrosobis(chloroethyl)amine was expected to be much more potent than was the unsubstituted nitrosamine, by analogy with that effect in nitrosodichloropiperidine (8), and thus was administered at a lower dose than that of nitrosodiethylamine. The dose chosen was approximately one-fifth of that of nitrosodiethylamine and was administered by gavage in olive oil solution at the rate of 1.15 mg twice weekly for 30 weeks, amounting to a total dose of 0.4 mmol. This treatment was administered to rats in drinking water at a concentration of 0.68 x 10^{-3} M. A stock solution of each compound was prepared in ethanol, and each week 5 ml of it were appropriately diluted with water to 1.5 liters to serve as drinking water. Each group of 3 Sprague-Dawley rats, bred and maintained in the barrier facility of the Biology Division, was given 60 ml of the nitrosamine solution on each of 5 days a week (a total of 300 ml), and tap water was given on the remaining 2 days. The animals were 8 to 10 weeks old at the beginning of the treatment and, after a few days of adjustment, drank all or almost all of the solution offered, so that the dose per group could be well quantified. The animals were given Rockland rat diet ad libitum in pellets. Treatment with the nitrosamine was continued for 50 weeks in all but the group given nitrosodiethylamine, most of which had died before the 29th week when the treatment ceased.

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well tolerated, and only 1 rat died early in the experiment as a result of mechanical damage.

At the end of the treatments, the rats were allowed to die naturally or were killed when moribund. Each was completely necropsied, and all tumors and other lesions were fixed for histological examination.

RESULTS

The treatments of the rats with the various N-nitroso compounds and the pattern of their survival are shown in Table 1. The group of rats treated with nitrosodiethylamine were all dead by the 33rd week of the experiment, and most were dead before the end of the 30-week treatment with nitrosamine. In all of the other groups, survival was much better, a reflection of the lesser carcinogenic potency of the substituted compounds. Nitrosobis(2-chloroethyl)amine was neither very carcinogenic nor very toxic. The tumors induced by the various treatments are listed in Table 2. Only 2 compounds, nitrosodiethylamine and nitrosobis(2-methoxyethyl)amine, induced tumors in all the animals treated. While there were tumors in all of the other groups, many of these were endocrine tumors commonly observed in old Sprague-Dawley rats (10), and only those that appear uncommonly in untreated animals of this strain were considered to be induced. These include liver tumors (hepatocellular carcinomas and hemangioendothelial sarcomas), olfactory adenocarcinomas, esophageal tumors (papillomas and carcinomas), and tumors of the forestomach (papillomas and carcinomas). All of the types of tumor observed in this series of experiments have been described by us previously (3, 7, 9, 15).

DISCUSSION

As measured by the time until death with tumors, nitrosodiethylamine is the most potent carcinogen of the compounds included in this study, since all animals treated with this compound were dead by the 33rd week of the experiment, mostly with hepatocellular carcinomas and hemangioendothelial sarcomas of the liver. There was little difference in the response of the males and females, despite the fact that the females received more than twice as high a dose per unit body weight than did the males. With few exceptions this has been a common finding in our tests of a large number of N-nitroso compounds, therefore, to conserve animals; we have chosen to carry out our recent tests in either males or females, as available. Nitrosobis(2-chloroethyl)amine was a much weaker carcinogen than was nitrosodiethylamine, in contrast with the greatly increased potency of 3,4-dichloronitrosopyrrolidine and 3,4-dichloronitrosopiperidine, both of which were very much more potent than were the parent nitrosamines (5, 8). This was interpreted as an electronic effect of the chlorine substituents on the activity of the hydrogen atoms α to the nitroso function. The low carcinogenic activity of nitrosodiethy

Table 1
Survival of rats treated with nitrosodiethylamine derivatives

<table>
<thead>
<tr>
<th>Nitroso compound</th>
<th>Concentration in (mg/liter) in drinking water</th>
<th>Length of treatment (wk)</th>
<th>Total dose (mg)</th>
<th>No. of rats alive at Wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diethylamine</td>
<td>70 (0.68)</td>
<td>29</td>
<td>203 (2.0)</td>
<td>6 (M) 6 5 3 0</td>
</tr>
<tr>
<td>Iminodipropionitrile</td>
<td>105 (0.68)</td>
<td>50</td>
<td>525 (3.5)</td>
<td>15 (F) 15 15 15 15 15 14 14 12 11 9 4 0</td>
</tr>
<tr>
<td>Bis(methoxyethyl)amine</td>
<td>110 (0.68)</td>
<td>50</td>
<td>550 (3.5)</td>
<td>15 (F) 15 15 15 15 14 12 3 0</td>
</tr>
<tr>
<td>Bis(ethoxyethyl)amine</td>
<td>130 (0.68)</td>
<td>50</td>
<td>650 (3.5)</td>
<td>15 (F) 15 15 14 13 13 13 12 10 8 4 2 0</td>
</tr>
<tr>
<td>Bis(diethoxyethyl)amine</td>
<td>190 (0.68)</td>
<td>50</td>
<td>950 (3.5)</td>
<td>15 (F) 15 15 15 14 14 14 14 10 9 6 2 1 0</td>
</tr>
<tr>
<td>Dichlorodiethylamine (by gavage in oil)</td>
<td>5.75 mg/ml (0.2 ml, 2 times/wk)</td>
<td>30</td>
<td>70 (0.4)</td>
<td>15 (F) 14 14 14 14 13 11 10 8 7 5 0</td>
</tr>
</tbody>
</table>

a Numbers in parentheses, mm.
b Numbers in parentheses, mmol.

Table 2
Tumor induced in rats fed nitrosodiethylamine derivatives

<table>
<thead>
<tr>
<th>Nitroso compound</th>
<th>No. of animals with tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td></td>
<td>No. of animals</td>
</tr>
<tr>
<td>Diethylamine</td>
<td>6 (M)</td>
</tr>
<tr>
<td>Bis(2-chloroethyl)amine</td>
<td>15 (F)</td>
</tr>
<tr>
<td>Bis(2-methoxyethyl)amine</td>
<td>15 (F)</td>
</tr>
<tr>
<td>Bis(2-ethoxyethyl)amine</td>
<td>15 (F)</td>
</tr>
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</table>
bischloroethylamine might be due to its instability in water, although its half-life is several hr and it was administered in solution in oil. This dichloro compound is strongly mutagenic in Salmonella without the need of metabolic activation by liver microsomes.

Nitrosobis(2-hydroxyethyl)amino (nitrosodiiethanolamine) is only weakly carcinogenic, very much weaker than is nitrosodiethyamine, and gives rise to liver tumors only (1). This compound has assumed some importance, since it was found to be a contaminant of cutting oils used in machining, apparently formed by reaction of the sodium nitrite in the cutting oil with tertiary amine triethanolamine (17); it is not known whether nitrosodiiethanolamine is carcinogenic by routes other than ingestion. In contrast to the large reduction in carcinogenic activity of nitrosodiethylamine resulting in a potent carcinogenic activity by routes other than ingestion. In contrast to the carcinogenic nitrosodiethanolamine, nitrosoiminodipropionitrile, is noncarcinogenic, even at maximally tolerated dose (1); it also is nonmutagenic in Salmonella liver microsome system.

REFERENCES

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