Induction of Carcinogenesis in Fischer Rats by Methylalkylnitrosamines

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ABSTRACT

Five nitrosomethyl-n-alkylamines with long aliphatic chains were administered to male F344 rats by gavage for 30 weeks. The rats treated with nitrosomethyl-n-octylamine and nitrosomethyl-n-nonylamine died within one year, while a majority of those given nitrosomethyl-n-decylamine, nitrosomethyl-n-dodecylamine, and nitrosomethyl-n-tetradecylamine lived for more than 80 weeks. Apart from the spontaneous tumors found in untreated rats of this strain, the rats treated with all four compounds containing an even number of carbon atoms in the long chain developed a high incidence of transitional cell carcinoma of the bladder. In addition, the rats treated with nitrosomethylundecylamine developed tumors of the liver (hepatocellular carcinomas and some angiosarcomas), lung, and nasal cavity. Nitrosomethylundecylamine failed to induce tumors in the bladder but induced tumors of the liver (hepatocellular carcinomas and cholangiocarcinomas), lung tumors, and some tumors of the nasal cavity.

INTRODUCTION

One of the early systematic examinations of the results of testing nitrosamines on a large scale was that of Druckrey et al. (4), which concluded that symmetrical aliphatic nitrosamines gave rise to liver tumors in rats whereas asymmetrical nitrosamines induced tumors of the esophagus in rats. Later, our incidental finding that the asymmetrical nitrosomethylalkylamines induced tumors of the bladder (11). Nitrosomethylundecylamine (IV), with an odd-numbered carbon chain which could not be β-oxidized to a nitrosomethylcarboxypropylamine, was a potent carcinogen in the bladder but did not induce bladder tumors in rats (10). This tended to support Okada’s hypothesis. Therefore, we decided to continue to evaluate this hypothesis by studying the carcinogenic effect of a number of nitrosomethylalkylamines administered chronically to rats at equimolar doses. The compounds chosen were nitrosomethyl-n-octylamine (I), nitrosomethyl-n-nonylamine (II), nitrosomethyl-n-decylamine (III), and nitrosomethyl-n-tetradecylamine (VI). Also included was a group of male Fischer rats which were treated with compound V, for comparison with previous tests of this compound (Chart 1).

MATERIALS AND METHODS

Chemicals

Nitroso-N-methyl-n-octylamine (I)

Compound I was prepared by dissolving 29 g (0.2 mol) of N-methyl-n-octylamine (Eastman Organic Chemicals, Rochester, N. Y.) in 20 ml HCl containing 30 g ice. Acetic acid, 30 ml, was added following by 20 g sodium nitrite. The reaction was allowed to proceed for 2 hr at room temperature, after which the upper yellow layer was separated, diluted with 50 ml methylene chloride, and washed with 3 x 30 ml water. The clear, lower methylene chloride layer was evaporated in a rotary evaporator, and the residual oil was distilled under reduced pressure. The yield was 27 g of yellow oil, b.p. 82-83° (0.4 mm). UV λ max (e) 345 nm (91). NMR (CDCl3) δ 0.89 (t, 3H), 1.27 (s, 10H), 1.74 (m, 2H), 3.40 (s, 2.1H), syn CH3, 3.75 (s, 0.9H) anti CH3, 3.6 (t, 0.4H), 4.4 (t, 1.6H). MS, m/z (%), 172 (M+, 4), 155 (50), 142 (10), 84 (23), 74 (49), 73 (65).

Nitroso-N-methyl-n-nonylamine (II)

Compound II was prepared by nitrosation of the amine formed by reducing the corresponding N-methylmethylene, methyl-n-nonylamide, which was prepared from the acid chloride and methylnitrite in 96% yield as described previously (3). A 500-ml flask equipped with a Soxhlet extractor was charged with 10 g (0.26 mol) of finely powdered lithium aluminum hydride, and 260 ml of ether. Inside the extraction thimble were placed 30 g (0.175 mol) of N-methyl-n-nonylamide. The mixture was refluxed for 15 hr and then stirred at 25° for 24 hr. The reduction mixture was cooled to 5° and hydrolyzed with ice water and filtered. The filtrate was washed with anhydrous sodium sulfate, the solvent was evaporated on a rotary evaporator, and the residual oil was distilled under reduced pressure. The yield was 27 g of yellow oil, b.p. 82-83° (0.4 mm). UV λ max (e) 345 nm (91). NMR (CDCl3) δ 0.89 (t, 3H), 1.27 (s, 10H), 1.74 (m, 2H), 3.40 (s, 2.1H), syn CH3, 3.75 (s, 0.9H) anti CH3, 3.6 (t, 0.4H), 4.4 (t, 1.6H). MS, m/z (%), 172 (M+, 4), 155 (50), 142 (10), 84 (23), 74 (49), 73 (65).

C6H15N2O

Calculated: C 62.75, H 11.70, N 16.26
Found: C 62.79, H 11.77, N 16.22

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1288 CANCER RESEARCH VOL. 41
Nitroso-N-methyl-n-decylamine (III)

N-Methyl-n-decanoylamide. This amide was prepared in 90% yield from decanoyl chloride and methylamine as described in the literature: m.p. 57-58° (literature m.p. 57°).

N-Methyldecylamine. A flask equipped with Soxhlet extractor was charged with 9.8 g (0.26 mol) of finely powdered lithium aluminum hydride and 260 ml of ether. The thimble was filled with 38 g (0.21 mol) of N-methyl-n-decanoylamide, and the contents were reduced as described previously. Vacuum distillation of the crude product gave 24 g (68%) of amine: b.p. 74-75° at 1.0 mm Hg; IR (film) 3300, 2920, 1470 cm⁻¹; NMR (CDCl₃) δ 0.90 (t, 3H), 0.92 (s, 1H), 1.24 (s, 14H), 2.41 (s, 3H), 2.53 (t, 2H).

N-Nitroso-N-methyl-n-decylamine. N-Methyldecylamine (18 g, 0.11 mol) was nitrosated as described above; the crude product was vacuum distilled to give 15 g (71%) of the pure nitrosamine: b.p. 181° at 1.5 mm Hg; m.p. 29-30°; IR (film) 2920, 1470, 1440, 1335 cm⁻¹; NMR (CDCl₃) δ 0.87 (t, 3H), 3.55 (t, 0.4H) syn α-CH₃, 4.12 (t, 1.6H) anti α-CH₃, 3.03 (s, 2.5H) syn CH₃, 3.71 (s, 0.5H) anti CH₃, 1.45 (b, 0.4H) syn β-CH₂, 1.70 (t, 1.6H) anti β-CH₂, 1.26 (s, 4H), 2.15 (s, 18H); MS, m/z (%), 256 (0.2 M⁺), 239 (51), 226 (43), 217 (117), 133 (29), 111 (58), 97 (13), 84 (100); UV λ_max (ε), 347 nm (85).

C₁₅H₂₄N₂O
Calculated: C 70.25, H 12.58, N 10.93
Found: C 70.10, H 12.68, N 10.84

N-Nitroso-N-methyl-n-tetradecylamine (VI)

N-Methylmyristoylamide. This compound was prepared in 86% yield as described in the literature: m.p. 78-79° (literature m.p. 78-4°).

N-Methyl-n-tetradecylamine. A lithium aluminum hydride reduction of 21.6 g (0.17 mol) of N-methylmyristoylamide was carried out as described previously. The crude product was vacuum distilled to yield 24 g (62%) of amine: b.p. 1470 cm⁻¹; NMR (CDCl₃) δ 0.87 (t, 3H), 1.25 (s, 24H), 2.41 (s, 3H), 2.53 (t, 2H).

N-Nitroso-N-methyl-n-tetradecylamine. The amine was nitrosated in 94% yield as described above: b.p. 161° at 1.5 mm Hg; m.p. 29-30°; IR (film) 2920, 1470, 1440, 1335 cm⁻¹; NMR (CDCl₃) δ 0.87 (t, 3H), 3.55 (t, 0.4H) syn α-CH₂, 4.12 (t, 1.6H) anti α-CH₂, 3.03 (s, 2.5H) syn CH₃, 3.71 (s, 0.5H) anti CH₃, 1.45 (b, 0.4H) syn β-CH₂, 1.70 (t, 1.6H) anti β-CH₂, 1.26 (s, 4H), 2.15 (s, 18H); MS, m/z (%), 256 (0.2 M⁺), 239 (51), 226 (43), 183 (115), 111 (13), 97 (31), 84 (46), 74 (52), 73 (49), 69 (41), 43 (100); UV λ_max (ε), 347 nm (85).

C₁₅H₃₂N₂O
Calculated: C 70.10, H 12.68, N 10.84
Found: C 70.10, H 12.68, N 10.84

Animal Treatments

Each of a group of 20 male F344 rats from the colony of the Frederick Cancer Research Center, 7 to 8 weeks old at the beginning of the experiment, was given 0.2 ml of a solution of the appropriate nitrosamine in corn oil by gavage, twice a week. The concentrations of the nitrosamines are shown in Table 1. The treatments continued for 30 weeks, after which the animals were allowed to die naturally or were killed when moribund. Each animal was given complete necropsy, and all lesions, major organs, and tissues were fixed for histological examination.
RESULTS

Table 1 shows the pattern of death of animals treated with the 5 nitrosomethylalkylamines. In every group, almost all of the rats died with tumors, mostly tumors not seen in untreated control animals of this strain and, therefore, induced by the nitrosamine treatments. Carcinomas of the liver and urinary bladder and probably carcinomas of the nasal cavity were the cause of death.

All of the groups of treated rats received equimolar doses of the respective nitrosamines except one, compound V, which was given at one-half the dose of the others. This was because in a previous study (9), which involved treatment of Fischer rats with a total dose of 6.5 mmol given twice weekly for 30 weeks, one-half of the treated rats were dead at 50 weeks, all were dead at 60 weeks, and all bore bladder tumors. This result and the data in Table 1 suggest that compounds III and VI are of about equal potency as carcinogens and compound V is somewhat more potent, but not by as much as factor of 2. On the other hand, based on the mortality data, compounds I and II are both considerably more potent than the larger molecules, compound I causing death of the animals somewhat more rapidly than compound II, although there were no bladder tumors in the case of the latter.

Four of the compounds (I, III, V, and VI) induced in a large proportion of the treated rats transitional cell carcinomas of the urinary bladder (Table 2). In Table 2 are listed all of the tumors observed in the rats given the various treatments, or no treatment. Tumors of the liver, bladder, lung, nasal cavity, and forestomach are virtually never seen in untreated Fischer rats of our colony or, indeed, elsewhere (5) and can therefore be considered to have been induced by the treatments. Most of these tumors have been described previously and were characterized as follows.

The carcinomas of the urinary bladder generally were 2.5 to 3.5 cm in largest diameter in rats receiving compounds III, V, or IV; however, some carcinomas in rats of each group were as large as 5 cm in greatest diameter. These carcinomas were not only large but also invasive. By contrast, carcinomas of the urinary bladder were 1.2 to 1.5 cm in diameter in rats given compound I. Some of these bladder tumors were successfully transplanted.

It is worth noting that groups with large numbers of rats that had carcinomas of the liver had either smaller carcinomas of the urinary bladder (compound I) or no carcinomas of the urinary bladder (compound II). Rats in the 2 latter groups also developed more carcinomas of the lung and nasal cavity. Twelve rats given compound II had carcinoma of the lung. By contrast, groups with large numbers of rats with carcinomas of the urinary bladder had either smaller carcinomas of the liver (compounds III, V, and VI). Rats in the latter 2 groups had few neoplasms of the lung and other organs.

Carcinomas of the lung were smaller in rats in the groups with carcinomas of the liver and other organs and larger in rats without carcinomas of other organs. Carcinomas often were multiple. The largest carcinomas of the lung, up to 1 cm in diameter, were observed in rats given compound III. Carcinomas of the lung were often invasive.

Histologically, carcinomas of the urinary bladder were transitional cell, grew in sheets, and were invasive. The pattern varied from well differentiated to poorly differentiated.

Adenocarcinomas of the lung grew in a well-differentiated pattern with papillary projections or in sheets with poorly differentiated cells. They compressed and invaded the adjacent pulmonary tissue.

Carcinomas of the nasal cavity were basal cell or a mixture of basal and squamous cell with keratin in rats given compound I. They filled the nasal cavity as they increased in size. Carcinomas of the nasal cavity in rats given compound II were squamous cell with large amounts of keratin.

Large numbers of rats given compound I or II developed neoplasms of the liver. The cell type varied somewhat in the rats in these 2 groups. Rats given compound I developed mainly hepatocellular carcinomas, as well as a few hemangiosarcomas, whereas rats receiving compound II had hepatocellular and cholangiocellular carcinomas; 3 rats had both types of tumors. Hepatocellular carcinomas were seen in small numbers of rats in the other groups. Both hepatocellular and cholangiocellular carcinomas were well differentiated. The former were made up of cords 2 to several cells thick separated by prominent sinusoids. The latter carcinomas consisted of cells forming ducts with a background of dense fibrous connective tissue. Hemangiosarcomas contained anaplastic cells lining vascular channels.

Rats with hepatocellular carcinomas as a result of treatment with compound II also had hyperplastic nodules and severe diffuse hepatic cell hyperplasia. In addition, rats with cholangiocellular carcinomas in the same groups had cholangiofibrosis and cirrhosis of the liver.

Table 1

Mortality of male Fischer rats treated with nitrosomethylalkylamines by gavage for 30 weeks

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration in oil (mg/ml)</th>
<th>Semi-weekly dose (mg)</th>
<th>Total dose (mg)</th>
<th>Survivors at wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrosomethylhexylamine</td>
<td>60</td>
<td>0.2</td>
<td>1200 (6.5)</td>
<td>0^b</td>
</tr>
<tr>
<td>Nitrosomethyldodecylamine</td>
<td>60</td>
<td>0.2</td>
<td>1200 (6.5)</td>
<td>0^b</td>
</tr>
<tr>
<td>Nitrosomethyldecylamine</td>
<td>93</td>
<td>0.2</td>
<td>1120 (6.5)</td>
<td>0^b</td>
</tr>
<tr>
<td>Nitrosomethylundecylamine</td>
<td>100</td>
<td>0.2</td>
<td>1200 (6.5)</td>
<td>0^b</td>
</tr>
<tr>
<td>Nitrosomethylundecylamine</td>
<td>108</td>
<td>0.2</td>
<td>1300 (6.5)</td>
<td>0^b</td>
</tr>
<tr>
<td>Nitrosomethylundecylamine</td>
<td>140</td>
<td>0.2</td>
<td>1680 (6.5)</td>
<td>0^b</td>
</tr>
<tr>
<td>Untreated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Numbers in parentheses, total dose in mmol.
b Last killed at 43 weeks.
c Last killed at 51 weeks.
d Last killed at 102 weeks.
e Killed at 112 weeks.

Eight survivors were killed at 126 weeks.
<table>
<thead>
<tr>
<th>Compound</th>
<th>No. of tumor-bearing animals</th>
<th>Liver Hepato-cellular</th>
<th>Other sites</th>
<th>Bladder</th>
<th>Adenoma</th>
<th>Adenocarcinoma</th>
<th>Lung</th>
<th>Papilloma Carcinoma</th>
<th>Nasal cavity Papilloma Carcinoma</th>
<th>Forestomach Papilloma Carcinoma</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrosomethyloctylamine (I)</td>
<td>20</td>
<td>13</td>
<td>4 angiosarcomas</td>
<td>14</td>
<td>4</td>
<td>8</td>
<td>3</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Nitrosothylmethylamine (II)</td>
<td>18</td>
<td>14</td>
<td>6 cholangiocarcinomas</td>
<td>0</td>
<td>1</td>
<td>9</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Nitrosothyldecylamine (III)</td>
<td>18</td>
<td>2</td>
<td></td>
<td>17</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Nitrosothylidodecylamine (V)</td>
<td>20</td>
<td>3</td>
<td></td>
<td>19</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>5</td>
<td></td>
<td></td>
<td>18</td>
</tr>
<tr>
<td>Nitrosothyltetradecylamine (VI)</td>
<td>20</td>
<td>0</td>
<td></td>
<td>20</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>No treatment</td>
<td>19</td>
<td>0</td>
<td></td>
<td>0</td>
<td>2</td>
<td>interstitial cell carcinomas</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>11</td>
</tr>
</tbody>
</table>

Tumors in male Fischer rats treated with nitrosomethylalkylamines
DISCUSSION

The results of treating Fischer rats with the 5 nitrosomethylalkylamines described here should be considered together with results with other nitrosomethylalkylamines of shorter chain length which have been tested previously in rats. Thus, the simplest of these compounds, nitrosomethylethylamine, gave rise to tumors of the liver (hepatocellular carcinomas) when this water-soluble compound was administered in drinking water (6) or in food (4). Nitrosomethyl-n-propylamine in drinking water induced esophageal tumors (9), and the next higher homolog, nitrosomethyl-n-butylamine, also induced esophageal tumors in Fischer rats (7) when given either by gavage or in drinking water (the latter being a somewhat more effective treatment based on weight of compound administered). Nitrosomethyl-n-amylamine has also induced tumors of the esophagus in already reported experiments (4). Using time to death with tumors as a criterion, the propyl, butyl, and amyl compounds are considerably more potent carcinogens for rats than are the 5 compounds in the study presented here.

Several differences can be noted between the effects of the smaller molecules described above and the larger molecules. One is the absence of esophageal tumors from the rats treated with Compound I and its higher homologs. A second difference is the induction of a large proportion of bladder tumors in the rats given the octyl, decyl, dodecyl, and tetradecyl compounds, which were not seen in rats treated with the lower homologs, even those having an even-numbered alkyl chain. Compound II, like Compound IV already reported (10), induces tumors of the liver and lung in Fischer rats but no bladder tumors. This accords well with the hypothesis of Okada et al. (11) that nitrosomethylalkylamines are metabolized by $\omega$ oxidation followed by repeated $\beta$ oxidation of the acid (presumably in the liver), in the manner of fatty acid metabolism, giving rise, in the case of those nitrosamines with an even-numbered alkyl chain, to nitrosomethyl-3-carboxypropylamine. This is excreted by the kidneys (and indeed is found to some extent in the urine) and is presumed to be the proximate carcinogen in the bladder. However, nitrosomethyl-$n$-butylamine, which presumably would also be oxidized to the same nitrosamoanoic acid, has not been shown to give rise to bladder tumors in rats. It also remains to be demonstrated that nitrosomethylcarboxypropylamine is a bladder carcinogen in rats. Another gap to be filled is the carcinogenic effect of nitrosomethyl-$n$-hexylamine which, having an even-numbered alkyl chain, would be expected to induce bladder tumors in rats.

While the substantiation of the $\beta$ oxidation hypothesis of bladder tumor induction in rats by asymmetrical nitrosamines remains an important goal and is supported by the present results, there are other results of these experiments which might be important in understanding nitrosamine carcinogenesis. Four of the 5 nitrosomethylalkylamines are mutagenic in the Ames test when activated by rat liver microsomes (2). The exception is nitrosomethyltetradecylamine (VI), which is as potent a carcinogen as the mutagenic nitrosomethyldecylamine (III), which is mutagenic to Salmonella under those conditions.

Compounds I and II both gave rise to a high proportion of liver tumors, lung tumors, and tumors of the nasal cavity, which suggests that they have a somewhat similar mechanism of action at those sites. The failure of Compound II to induce bladder tumors, on the other hand, indicates that the mechanism by which tumors are induced in this organ is quite different. It will be important to compare the metabolism of some of these compounds in the liver, lung, and bladder to gain some insight into their mechanism of induction of tumors at these sites.

The absence of tumors of the testis from the rats treated with Compounds I and II can be explained by the early deaths of those animals, which precluded development of these tumors of old age. Most of the other tumors usually seen in untreated Fischer rats appeared at a similar incidence in those animals treated with the nitrosamines which lived long enough. However, it is not clear why 50% of the rats treated with Compound V developed islet cell carcinomas of the pancreas. It is conceivable that the nitrosamine potentiated development of this "spontaneous" tumor in rats.

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