Carcinogenicity of 3-Chloronitrosopiperidine, 4-Chloronitrosopiperidine, and 3,4-Dichloronitrosopiperidine in Fischer Rats

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ABSTRACT

Three chlorinated nitrosopiperidines, 3-chloro-, 4-chloro-, and 3,4-dichloronitrosopiperidine, were administered to groups of 20 male Fischer 344 rats at a concentration of 0.17 mM in drinking water. Treatment with the monochloro compounds lasted for 30 weeks, while treatment with the dichloro compound lasted for 21 weeks. Almost all of the animals died with esophageal tumors. There was also a significant incidence of tumors of the forestomach and tongue in the rats treated with the monochloro compounds. Using the rate of death of the animals with tumors as an index, the relative potency of the three compounds increases from 3-chloro- to 4-chloro- to 3,4-dichloronitrosopiperidine.

INTRODUCTION

Previous tests of halogenated cyclic nitrosamines in rats have shown that the presence of the halogen greatly increases the carcinogenic potency of the molecule (1-3). In the case of nitrosopiperidine, the 3,4-dichloro derivative caused the death of Sprague-Dawley rats from esophageal tumors much more quickly than did nitrosopiperidine (1), even at much lower doses (4). Nitroso-4-chloropiperidine was also a more potent carcinogen than was nitrosopiperidine, although less potent than nitroso-3,4-dichloropiperidine, and it induced esophageal tumors as well as many liver tumors in Sprague-Dawley rats (3), which were not seen with nitrosopiperidine itself. The reasons for these differences are not clear, although they could be due to a combination of electronic and conformational effects of the substituents on the reactivity or activation of the nitrosamine. At the time of the previous experiments, it was not possible to prepare nitroso-3-chloropiperidine, but satisfactory methods have been developed for its synthesis since. Therefore, it was decided to study the 3 chlorinated derivatives of nitrosopiperidine further; to begin, the relative carcinogenic effectiveness of the 3 compounds was compared by administering equimolar doses in drinking water to Fischer 344 rats.

MATERIALS AND METHODS

Chemicals. 3,4-Dichloronitrosopiperidine was prepared as described previously (1). 4-Chloronitrosopiperidine was initially prepared by nitrosative dealkylation of 4-chloro-N-methylpiperidine as previously described (3). Further quantities were prepared from 4-hydroxypiperidine (4-piperidinol) as follows. A well-stirred mixture of 5 g (0.036 mol) of 4-hydroxypiperidine hydrochloride in 20 ml of methylene chloride was cooled to 0°. When thionyl chloride (7 ml; 0.05 mol) was added dropwise, a homogeneous solution resulted. The ice bath was removed, and the reaction mixture was stirred at 25° overnight. The solvent was removed under vacuum, and the residual oil was cooled to 5°. The crude product was dissolved in 25 ml of ice-cold water and treated with 4.9 g (0.072 mol) of sodium nitrite in 50 ml of water. After all the nitrite had been added, the reaction mixture was stirred at 25° for 1 hr. The crude nitrosamine was extracted into methylene chloride, washed with 5% aqueous sodium bicarbonate solution, dried over sodium sulfate, and filtered through a layer of magnesium sulfate. Evaporation of the solvent gave 2.9 g of a yellow oil. The crude oil was filtered through a silica gel column and eluted with methylene chloride to give 1.88 g (35%) of 4-chloronitrosopiperidine. NMR (CDCl₃): δ 1.9 (m, 2H) syn β-H, δ 2.20 (m, 2H) anti β-H, δ 3.64 (m, 1H) syn α-H axial, δ 4.16 (m, 1H) anti α-H axial, δ 4.32 - δ 4.6 (m, 3H) α-H equatorial and CHCl₃; UV (ether) λ 355 nm (72); IR (film) 2982 cm⁻¹, 1445, 1360, 740; mass spectrum m/e 148 (40%), m/e 118 (4%), m/e 82 (33%); isotope cluster M: 148 (100%), M + 1; 149 (8.5%), M + 2; 150 (30%).

Calculated: C 40.41, H 6.11, N 18.85, Cl 23.86
Found: C 39.97, H 6.12, N 18.45, Cl 26.01

3-Chloronitrosopiperidine was also prepared by nitrosative dealkylation of 3-chloro-N-ethylpiperidine (Schuchardt, Munich, Germany), but the yield was very low (2 to 3%), and the product was a dark oil which when purified by chromatography to a yellow liquid was characterized as 3-chloronitrosopiperidine. The compound was quite stable in aqueous solution. A second, and improved, synthesis started with 3-hydroxypiperidine (3-piperidinol) as follows. A partial solution of 13 g (0.094 mol) of 3-hydroxypiperidine hydrochloride in 100 ml of methylene chloride was cooled to -5°. Thiouyl chloride (15.4 ml; 0.11 mol) was added dropwise, the ice bath was removed, and the solution was stirred at room temperature overnight. The solvent was removed on a rotary evaporator, and the last traces of thiouyl chloride were removed under high vacuum (0.2 mm Hg). The residual oil was cooled to 5°, and ice water was added until all of the residue had dissolved. A solution of 13.2 g (0.2 mol) of sodium nitrite in 100 ml of water was added dropwise; once addition was completed, the ice bath was removed, and the mixture was stirred for 1 hr at 25°. The mixture was extracted with dichloromethane. The organic layer was washed in 5% sodium bicarbonate solution, dried over anhydrous potassium carbonate, and filtered through a pad of magnesium sulfate. Evaporation of the solvent under vacuum gave 6.9 g of a brown oil. The crude mixture was filtered through a silica gel column and eluted with methylene chloride, giving 3.15 g (23%) of 3-
chloronitrosopiperidine. NMR (CDCl₃): δ 1.8 – 2.5 (m, 4H), δ 3.5 – 4.7 (m, 5H); UV λ_{max} (e), 355 nm (85); mass spectrum m/e 148 (100%), m/e 118 (31%), m/e 82 (60%); isotope cluster M: 148 (100%), M + 1; 149 (12%), M + 2; 150 (33%).

Animal Treatments. Groups of 20 male 8-week-old, Fischer 344 rats of the Frederick Cancer Research Center colony were housed, 4 to a cage, in cages with wire mesh bottoms. The animals were fed Wayne rat chow ad libitum. Instead of drinking water, each cage of animals was supplied with 80 ml of a solution of the appropriate nitrosamine each night, 5 nights/week. After a few days, during which the animals became accustomed to the taste of the nitrosamine, all of the solution was consumed each day, so that the dose administered could be quantified. For the remaining 2 days each week, the rats were given tap water ad libitum to make up any water deficit. The rats were approximately 8 weeks old at the beginning of treatment. Treatment continued for the number of weeks stated in Table 1, after which the animals were observed until they died. The last 3 surviving animals were killed at Week 74.

Complete gross necropsies were carried out on all animals. Tissues were fixed in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following tissues were examined histopathologically: brain, pituitary, thyroid, parathyroid, adrenal, salivary gland, heart, thymus, lung, aorta, trachea, liver, spleen, esophagus, stomach, duodenum, colon, lymph nodes, pancreas, kidney, urinary bladder, testes, mammary gland, skin, and femur, as well as other abnormal tissues.

RESULTS AND DISCUSSION

The mortality of the rats treated with the chlorinated nitrosopiperidines is described in Table 1. Treatment of all of the animals was originally scheduled to last 30 weeks. However, several rats treated with 3,4-dichloronitrosopiperidine had died by the 20th week, and treatment with that compound stopped at Week 21 of the experiment. Therefore, the rats given dichloronitrosopiperidine received smaller doses than did those given the other 2 compounds. However, most of these rats died earlier with tumors, suggesting that the dichloro compound is a more potent carcinogen than is either monochloro compound. This confirms the previous finding in Sprague-Dawley rats that 3,4-dichloronitrosopiperidine is one of the most potently carcinogenic nitrosamines (1).

The rats treated with dichloronitrosopiperidine had fewer carcinomas and more papillomas in the esophagus than did rats treated with 3-chloro- or 4-chloro-nitrosopiperidine. In addition, the carcinomas in the latter 2 groups tended to be larger and more invasive.

Some of the rats with esophageal or forestomach carcinomas died from emaciation. Other rats with esophageal carcinomas died with bronchitis and abscesses of the lung which developed terminally. The cause of acute inflammation of the lungs was aspiration of keratin or food particles or squamous metaplasia of the trachea and bronchi. Of the rats given 3-chloronitrosopiperidine, 10 had bronchitis or abscesses in the lung, while 9 rats given dichloronitrosopiperidine had bronchitis or lung abscesses. On the other hand, only 2 animals given 4-chloronitrosopiperidine had bronchitis, and rats in this group with carcinomas of the esophagus and tongue died from emaciation.

As measured by the mortality of the animals from tumors, 4-chloronitrosopiperidine appears to be somewhat more potent than 3-chloronitrosopiperidine, although the difference is not large. All 3 chlorinated nitrosopiperidines are much more potent carcinogens than is nitrosopiperidine itself. This has been observed previously in Sprague-Dawley rats (1, 3), when mortality with the same type of tumors was similar after treatment of rats with 0.88 mmol nitrosopiperidine solution for 28 weeks, for a total dose of 2.5 mmol/rat. 4

Almost all of the treated rats died with tumors, which are listed in Table 2. Neoplasms of the esophagus, forestomach, and tongue were papillomas or carcinomas. The carcinomas usually were sessile growths with invasion. The papillomas were papillary growths with a stalk. Histopathologically, the carcinomas were well- or poorly differentiated basal cell carcinomas with areas of squamous cell carcinomas and keratin. Poorly differentiated carcinomas often were invasive. The papillomas were basal or squamous cell neoplasms. Rats with carcinomas of the esophagus generally also had papillomas and basal cell hyperplasia. Occasionally, rats developed squamous metaplasia of the trachea and/or bronchi. The most commonly observed tumor was basal cell carcinoma of the esophagus, together with some papillomas. These were virtually the only tumors induced by dichloronitrosopiperidine. Both monochloronitrosopiperidines also induced significant numbers of carcinomas and papillomas of the forestomach and tongue. Noticeably absent were tumors of the liver, which were induced in Sprague-Dawley rats by 4-chloronitrosopiperidine (3). This high susceptibility to tumors of the upper gastrointestinal tract and lower susceptibility to liver tumors is typical of the response of Fischer rats, as compared with that of Sprague-Dawley rats (4). 4

Table 1
Mortality of male Fischer rats treated with 3-chloro-, 4-chloro-, and 3,4-dichloronitrosopiperidine in water

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
<th>Duration of treatment (wk)</th>
<th>Total dose</th>
<th>No. of survivors at</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/liter</td>
<td>mm</td>
<td>mg</td>
<td>10 wk 15 wk 20 wk 25 wk 30 wk 35 wk 40 wk 45 wk 50 wk 55 wk</td>
</tr>
<tr>
<td>3-Chloronitrosopiperidine</td>
<td>25</td>
<td>0.17</td>
<td>30</td>
<td>75 0.5 20 20 20 20 15 9 4 3 0</td>
</tr>
<tr>
<td>4-Chloronitrosopiperidine</td>
<td>25</td>
<td>0.17</td>
<td>30</td>
<td>75 0.5 20 20 20 20 16 13 3 2 1 1 1</td>
</tr>
<tr>
<td>3,4-Dichloronitrosopiperidine</td>
<td>31</td>
<td>0.17</td>
<td>21</td>
<td>65 0.35 20 20 20 17 9 4 3 2 2 2 2 2 2</td>
</tr>
</tbody>
</table>

* Killed at 74th week.

4 W. Lijinsky and M. D. Reuber, unpublished information.
Chlorinated Nitrosopiperidine Carcinogenicity in Fischer Rats

Table 2

Neoplasms induced in Fischer rats by chlorinated nitrosopiperidines

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. of animals</th>
<th>No. of tumor bearing animals</th>
<th>No. of rats with tumors of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Esophagus</td>
</tr>
<tr>
<td>3-Chloronitrosopiperidine</td>
<td>20</td>
<td>19</td>
<td>16 3 4 0 6 2</td>
</tr>
<tr>
<td>4-Chloronitrosopiperidine</td>
<td>20</td>
<td>18</td>
<td>15 3 5 1 12 0</td>
</tr>
<tr>
<td>3,4-Dichloronitrosopiperidine</td>
<td>20</td>
<td>17</td>
<td>9 8</td>
</tr>
</tbody>
</table>

*C; carcinomas; P, papillomas.

Chart 1. Structures of 3-chloronitrosopiperidine (A), 4-chloronitrosopiperidine (B), and 3,4-dichloronitrosopiperidine (C).

Dawley rats, to nitrosamines. The reasons for the differences in susceptibility are not known but might lie in the readiness with which the nitrosamines are metabolized by Sprague-Dawley rat liver as compared with Fischer rat liver.

The conclusions from this study are that substitution of chlorine for hydrogen at either the 3- or 4-position in nitrosopiperidine greatly increases carcinogenic effectiveness, more so in the 4- than in the 3-position (Chart 1). The effect of chlorine substitution at both 3- and 4-positions is to increase carcinogenic potency still more, such that a total dose of one-third mmol of 3,4-dichloronitrosopiperidine per rat leads to death of almost all of the rats with esophageal tumors within 35 weeks. Examination of the NMR spectra of the 3 chlorinated compounds indicates that 3,4-dichloronitrosopiperidine exists almost entirely as 1 chair form with the chlorine atoms trans-diaxial, which possibly make all of the molecules favorably oriented for enzymic activation. On the other hand, both monochloronitrosopiperidines exist in more than one form, although one predominates in each case. The effects of chlorine substitution on the carcinogenicity of nitrosopiperidine might be electronic, leading to increased activation of the nitrosamine supposedly at the carbon atom α to the nitroso function. There is some indication that this might be so in the considerably increased mutagenicity of the chloronitrosopiperidines, as compared with nitrosopiperidine, in the Ames test. (5). However, a determination as to whether this is the reason for the increased carcinogenicity will depend on studies of activation and metabolism of the compounds in the rat esophagus, difficult experiments because it is not easy to obtain sufficient active tissue fractions from the rat esophagus.

REFERENCES


A

B

C

Chart 1. Structures of 3-chloronitrosopiperidine (A), 4-chloronitrosopiperidine (B), and 3,4-dichloronitrosopiperidine (C).
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