The Effect of Substituents on the Carcinogenicity of N-Nitrosopyrrolidine in Sprague-Dawley Rats

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SUMMARY

N-Nitrosopyrrolidine and two of its derivatives were prepared and fed in drinking water to Sprague-Dawley rats to compare the effects of substituents on the carcinogenicity of the N-nitrosopyrrolidine molecule. 3,4-Dichloro-N-nitrosopyrrolidine induced esophageal tumors in 13 of 14 animals, olfactory carcinomas in 4, and a hepatocellular tumor in 1. All animals that received this compound were dead at 55 weeks after the start of the experiment. N-Nitrosopyrrolidine induced hepatocellular tumors in 26 of 29 animals and induced 1 olfactory carcinoma. Not all animals in this group were dead until 104 weeks of the experiment. 2,5-Dimethyl-N-nitrosopyrrolidine induced only 2 hepatocellular tumors in 29 animals. The α-methyl substitution diminished the liver carcinogenicity, while the β chloride substitution affected a different target organ, the esophagus, and greatly reduced the time to death with tumors.

INTRODUCTION

The study of the effects of a variety of substituents on the carcinogenicity of 6-membered cyclic nitrosamines has yielded insight into the mechanism of action of this type of cyclic compound. These studies in rats demonstrated several effects of such substituents, including changes in potency and in organ specificity (6-10). N-Nitrosopyrrolidine, a 5-membered cyclic compound, has been tested for carcinogenicity in rats and found to induce primarily hepatocellular tumors in this species (1, 5). Some carboxyl derivatives of N-nitrosopyrrolidine have been reported to be inactive, however (1, 3).

This study investigated the effects on carcinogenicity of halogen and methyl substitution in the N-nitrosopyrrolidine molecule. The compounds included here were the new compound 3,4-dichloro-N-nitrosopyrrolidine and 2,5-dimethyl-N-nitrosopyrrolidine.

MATERIALS AND METHODS

N-Nitrosopyrrolidine. N-Nitrosopyrrolidine was prepared by the reaction of pyrrolidine with sodium nitrite in acetic acid solution according to standard methods. It was a yellow oil, b.p. 78-79° (8 mm). Thin-layer chromatography, NMR, and mass spectrometry confirmed the structure and showed the absence of significant impurities.

2,5-Dimethyl-N-nitrosopyrrolidine. 2,5-Dimethylpyrrolidine (Aldrich Chemical Co., Milwaukee, Wis.; 35 g, 0.35 mole) was dissolved in acetic acid (70 ml) + ice (200 g). Sodium nitrite (50 g) was added, and the mixture was allowed to stand for 3 hr at room temperature. The upper yellow oil was separated and combined with an ether extract of the aqueous layer (2 x 100 ml). The ether solution was washed with water (50 ml), dried (anhydrous Na₂CO₃), and evaporated in a stream of nitrogen. The almost yellow oil distillate at a constant temperature in a vacuum to give 37 g (83%) of the nitroso compound: b.p. 86-88° (12 mm); UV₅₀ (H₂O) 342 nm (ε 86).

The NMR and mass spectra of the compound were consistent with the assigned structure and showed the absence of significant impurities.

Mass spectrum (70 eV): m/e (relative intensity) 128 (63%, M⁺), 113 (11%, M⁺ - CH₃), 99 (5%, M⁺ - C₂H₅), 96 (4%, M⁺ - NO), 83 (19%), 82 (28%), 81 (10%).

3,4-Dichloro-N-nitrosopyrrolidine. To a solution of N-nitroso-3-pyrroline (3 g) in methylene chloride (40 ml) was added chlorine, prepared by dropping 10 N HCl (60 ml) onto potassium permanganate (10 g). The nitrosopyrroline solution was cooled in ice water. After consumption of all of the Cl₂, the weight of the methylene chloride solution had increased by 8 to 9 g. The solvent was removed at room temperature in a stream of nitrogen and the residual brown oil was kept at -20° until it crystallized (2 days). Approximately 15 ml of cold methanol were added with stirring, and the solid was filtered off and washed with cold methanol. Recrystallization from about 20 ml of warm ethanol yielded 4 g (40%) of almost colorless crystals, m.p. 54-55°; UV₅₀ (H₂O) 344 nm (ε 92). A further 1.2 g were obtained by evaporation of the mother liquors.

C₆H₆N₂Cl₂O

Calculated: C 28.42, H 3.58, N 16.58, Cl 41.95
Found: C 28.52, H 3.61, N 16.45, Cl 42.22

The compound gave only 1 spot, on thin-layer chromatography, and the mass spectrum was consistent with the assigned structure.

Mass spectrum (15 eV): m/e (relative intensity) 172 (5%, M⁺), 170 (33%, M⁺ - CH₃), 168 (56%, M⁺), 138 (27%, M⁺ - NO), 133 (5%, M⁺ - Cl), 103 (6%, M⁺ - NO, Cl), 102 (22%).

Animal Treatments. The compounds were administered as solutions in drinking water to male or female Sprague-Dawley rats.
Dawley rats that were born and maintained in a closed colony in this laboratory. Animals were segregated by sex, housed 3 to a cage, and fed Purina laboratory chow ad libitum. All animals were 8 weeks of age at the start of the experiment. Drinking water solutions were given at the rate of 20 ml/rat/day, for 5 days each week. Regular tap water was given on the remaining 2 days. All of the solution was drunk each day, and the doses consumed by the rats could be quantified. Solutions were prepared weekly by dissolving each compound in 10 ml of ethanol and diluting with distilled water to required volume. All of the compounds were stable in aqueous solution, as determined by unchanged absorbance after standing for several weeks. Solutions of N-nitrosopyrrolidine (200 mg/l) and 2,5-dimethyl-N-nitrosopyrrolidine (250 mg/l) were given for 50 weeks, and 3,4-dichloro-N-nitrosopyrrolidine (100 mg/l) was given for 34 weeks, at which time several animals had died with tumors.

Animals were allowed to die naturally or were killed when moribund, and they were submitted to complete postmortem examination. Major organs and all lesions were examined microscopically.

**RESULTS**

All animals that received the 3,4-dichloro compound were dead at Week 55 of the experiment (Table 1). Of the 14 animals in this group, 13 died with tumors, the first at 31 weeks, and several had more than 1 type of tumor present. All 13 animals had tumors present in the esophagus. Eight animals had esophageal papillomas, while 5 animals had invasive squamous cell carcinomas of the esophagus. Four animals had olfactory carcinomas that arose from the nasal turbinates and invaded posteriorly into the brain; laryngeal papillomas were present in 2; papillomas of the nonglandular stomach and a hepatocellular tumor were each present in 1 animal. The hepatocellular tumor was present in the animal that lived longest (55 weeks).

In the rats that received nitrosopyrrolidine, all females were dead at 85 weeks, and all males, at 104 weeks of the experiment (Table 1). Of the 14 animals in this group, 13 died with tumors, the first at 14 weeks, and all but 1 had hepatocellular tumors. The remaining tumor-bearing animal was the first to die (45 weeks) and had an olfactory carcinoma. In addition to hepatocellular tumors, 2 animals had cholangiocarcinomas in their livers. There were 14 of 15 tumor-bearing females in this group, and all but 1 of these had hepatocellular tumors; this animal had a cholangiocarcinoma in the liver. In addition to an hepatocellular tumor, 1 animal had an olfactory carcinoma (67 weeks) and 1 had a cholangiocarcinoma, and the 1 animal without a liver tumor died at 62 weeks.

There were no differences in male and female death rates in the group that received 2,5-dimethyl-N-nitrosopyrrolidine, and there was only 1 hepatocellular tumor in each sex (Table 1). The 1 hepatocellular tumor in the group of females was present in the longest-lived animal (134 weeks) and the 1 in the male group occurred in a death at 111 weeks.

Various benign endocrine tumors occurred in most ani-
mals over 60 weeks of age, but were similar in incidence to those in age-matched controls (Table 1).

DISCUSSION

2,5-Dimethyl-N-nitrosopyrrolidine gave rise to only 2 tumors in this experiment, while N-nitrosopyrrolidine induced hepatocellular tumors in 27 of 29 animals after a total dose of 10 mmoles. This is similar to the situation with N-nitrosopiperidines, where methyl groups substituted in both positions α to the nitroso function appeared to eliminate carcinogenic activity (9). N-Nitrosopyrrolidine appears to be a weaker carcinogen than its higher homologs, N-nitrosopiperidine and N-nitrosohexamethylenimine, since smaller doses of these compounds caused death of animals with tumors in considerably less time (4, 9). Dimethylnitrosamine is also a more potent liver carcinogen than is N-nitrosopyrrolidine, although it induces liver tumors other than hepatocellular tumors in our rats (11). 2,5-Dimethyl-N-nitrosopyrrolidine can be considered to have the same structural relationship to N-nitrosopyrrolidine as does diisopropylnitrosamine to diethylnitrosamine, a methyl substituent on each of the α-carbon atoms. The effect is similar, since diisopropylnitrosamine is a much weaker carcinogen than is diethylnitrosamine (1). The lesser activity of 2,5-dimethyl-N-nitrosopyrrolidine may additionally be influenced by conformational factors because of the ring structure.

3,4-Dichloro-N-nitrosopyrrolidine was a more potent carcinogen than N-nitrosopyrrolidine in that it killed the animals with tumors in a shorter time and after administration of one-fifth of the dose. In this respect, it resembles its homolog, 3,4-dichloro-N-nitrosopiperidine, although it is less potent than the latter (8). As suggested earlier, the effect of the chlorine substitution could be to increase the activation of the α hydrogen atoms, facilitating cleavage of a carbon-hydrogen bond, thereby enhancing carcinogenic activity (8). This does not explain the change in the primary target organ of the carcinogenic molecule, however.

REFERENCES

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