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Increased Carcinogenicity of 2,6-Dimethylnitrosomorpholine Compared with Nitrosomorpholine in Rats[1]

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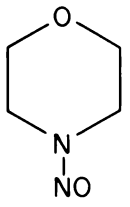
SUMMARY

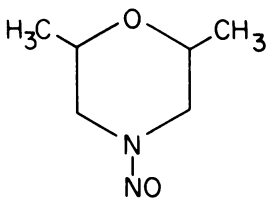
Nitrosomorpholine was given to rats in drinking water at the rate of 4 mg/week for 30 weeks. Tumors of the liver were induced in 53% of treated animals and were of both hepatocellular and Kupffer cell origin. One-half of the treated animals were alive 75 weeks after the beginning of treatment, but only 2 survived to 104 weeks. 2,6-Dimethylnitrosomorpholine was given to rats at the same molar concentration in drinking water for 30 weeks (5 mg/week). All of these animals died with tumors within 34 weeks after the beginning of treatment; these tumors were mainly in the esophagus and nasal turbinates, and only 1 liver tumor was seen. This greatly changed tumorigenicity of the dimethyl compound might be due to steric factors or to enhanced reactivity at the positions α to the nitroso function induced by the methyl groups at the β position.

INTRODUCTION

A pronounced enhancement of the carcinogenicity of dinitrosopiperazine by substitutions of methyl groups at the 2-positions was seen (3), while no such effect was observed with methyl substitution in nitrosopiperidine (W. Lijinsky and H. W. Taylor, unpublished data). As part of a series of studies into the effect of replacing the hydrogen atoms of cyclic nitrosamines with various substituent groups, 2,6-dimethylnitrosomorpholine was prepared and given to rats in drinking water at the same molar dose as was nitrosomorpholine and for the same period of time.



Nitrosomorpholine



2,6-Dimethylnitrosomorpholine

MATERIALS AND METHODS

Chemicals. Nitrosomorpholine and 2,6-dimethylnitrosomorpholine were prepared by reaction of morpholine or 2,6-dimethylmorpholine (Eastman Organic Chemicals, Rochester, N. Y.) with sodium nitrite in aqueous acetic acid, as in the preparation of most *N*-nitroso compounds. The conditions were: 0.5 mole of amine dissolved in 50 ml 10 N HCl + 150 g ice and 60 ml acetic acid. Approximately 1 mole (70 g) of sodium nitrite was added with gentle shaking. Within 2 hr at room temperature, a layer of oil had formed above the aqueous solution. The solution was made strongly alkaline by the slow addition of solid KOH, while cooling in water, and was extracted twice with 150 ml of methylene chloride. The combined extracts were shaken with 20 ml of 5 N HCl (to remove unreacted amine), and the solvent was removed in a stream of nitrogen at room temperature. The residual oil was distilled under reduced pressure and a constant-boiling center cut was collected as a yellow oil.

The yield of nitrosomorpholine was 85% of the theoretical. The compound crystallized on standing as a pale yellow solid, m.p. 27-29°.

The yield of 2, 6-dimethylnitrosomorpholine was 78% of the theoretical. The compound crystallized at −20° but was liquid at room temperature.

$C_6H_{12}N_2O_2$

Calculated: C 49.98, H 8.39 N 19.43
Found: C 49.96, H 8.26, N 19.59

Both nitrosamines gave good mass spectra at 70 eV, with strong parent ions and no detectable impurities. The molar absorptivities in water were 93 at 340 nm for nitrosomorpholine and 93 at 343 nm for 2,6-dimethylnitrosomorpholine. The nuclear magnetic resonance spectrum of 2,6-dimethylnitrosomorpholine was complex and indicated the presence of more than 1 conformer, but no significant impurity was detected.

Animal Treatments. Animals were male and female Sprague-Dawley rats of the colony of this laboratory, bred and maintained in a barrier facility. They were housed 3/cage on Sanicel bedding and were fed Purina rat chow. When they were about 8 weeks old, treatment with the nitrosomorpholines began and consisted of 60 ml of a solution of either compound (at the same molar concentration, 0.34 mM) per cage 5 days a week; on the remaining 2 days of the week tap water was given. The animals drank all, or almost all, of the solutions offered to them, so that the dose received by each cage of animals was quantified. Treatment was continued for 30 weeks, after which the

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animals were kept until they died or were killed when moribund. Complete necropsy was performed on each animal, and all tissues with tumors or other lesions were fixed for histological examination.

RESULTS

Animals treated with 2,6-dimethylnitrosomorpholine were all dead at 35 weeks after initiation of treatment, while those that received nitrosomorpholine lived much longer. One-half of the latter group were dead at 80 weeks, and the last 2 survivors were killed at 104 weeks of the experiment (Table 1).

The 2 compounds caused different tumor responses in the rats. 2,6-Dimethylnitrosomorpholine induced tumors mainly in the turbinates, esophagus, trachea, and stomach. Twenty-eight animals had tumors in both the turbinates and the esophagus, 5 had tumors in both the nonglandular stomach and the trachea, and a hepatocellular carcinoma was present in the liver of 1 animal (Table 2). Adenocarcinomas in the turbinates arose from epithelium in the olfactory region. All were malignant and invaded caudally into the olfactory bulb of the brain. These tumors consisted of epithelial cells that formed acini and "rosettes," which were separated by scant mature stroma. These tumors closely resembled those reported in hamsters after administration of di-*n*-propylnitrosamine (4). In the esophagus and nonglandular stomach, mostly benign squamous papillomas were present, with occasional squamous cell carcinomas. All tracheal tumors were benign squamous papillomas.

Nitrosomorpholine induced tumors in the liver that were of both hepatocellular and vascular origin. These tumors were morphologically very similar to those described after administration of aminopyrine plus nitrite and after carbon tetrachloride (5). A total of 16 hepatocellular tumors were seen; some appeared benign and others seemed malignant, although this characteristic was difficult to assess. Hepatocellular tumors metastasized to the lung in 3 animals (Table 2). Kupffer cell sarcomas were seen in the livers of 2 animals, and there were metastases to the lung in 1 of these (Table 2). The livers of 2 animals contained tumors of hepatocellular and Kupffer cell origin. One animal had a benign cholangioma in the liver. In addition to the tumors, there was liver necrosis in the nitrosomorpholine-treated group, often with massive scarring, biliary hyperplasia, and telangiectasis. There was no correlation between time of death and the type of tumor induced.

DISCUSSION

Nitrosomorpholine has been tested previously in rats, and our findings did not differ from these reports (1) except that some liver tumors were of Kupffer cell origin in our case. Nitrosomorpholine seemed to be a considerably weaker carcinogen than dimethylnitrosamine in our rats, the animals having survived much longer than those that received a slightly higher dose (1.5 times) of dimethylnitrosamine for the same time (5). Our results illustrate the large variation in susceptibility to a carcinogen even among this closely related (but not inbred) group of rats. The 1st rat of the 30

Table 1

Mortality and tumor incidence in rats fed nitrosomorpholines in drinking water for 30 weeks

Chemical	Concentration	No. of animals	Survivors at Wk										No. of tumor-bearing animals
			10	20	30	40	50	60	70	80	90	100	
Nitrosomorpholine	40 mg/liter	30M	30	29	27	27	26	24	18	14	6	2[a]	16
2,6-Dimethylnitroso-morpholine	50 mg/liter	30 {15M	15	15	7	0 } 34							15
		30 {15F	15	15	7	0 }							15

[a] Animals were killed at 104 weeks.

Table 2

Incidence and distribution of tumors in rats treated with nitrosomorpholine or 2,6-dimethylnitrosomorpholine

Compound	No. of animals treated	No. of animals with tumors of					
		Liver					
		Hepato-cellular	Kupffer cell	Eso-phagus	Nasal turbinates	Stomach	Trachea
Nitrosomorpholine	30 M	16[a]	2[b]	0	0	0	0[c]
2,6-Dimethylnitroso-morpholine	15 M	0	0	13	15	4	3
	15 F	1	0	15	13	1	2

[a] Three metastasized to the lungs.

[b] One metastasized to the lung.

[c] Other tumors were 1 cholangioma, 1 meningiosarcoma, 1 kidney carcinoma, 1 generalized lymphosarcoma, 1 squamous-cell carcinoma of the axilla, and 1 mixed glioma of brain.

male rats given nitrosomorpholine died with a liver tumor at 17 weeks. Although the treatment ceased at 30 weeks, animals continued to die with the same kind of tumor during the subsequent 1.5 years and 2 were alive 2 years after the beginning of the experiment.

The homolog, 2,6-dimethylnitrosomorpholine, appeared to be a more potent carcinogen than nitrosomorpholine was. The reason for the greatly increased carcinogenic activity of nitrosomorpholine by the substitution of 2 methyl groups in the β position to the *N*-nitroso function is unclear. The explanation might lie in some favorable steric configuration due to the methyl groups, enhancing reaction with some cellular macromolecules. Another explanation might be that the methyl groups on the β-carbon atoms enhance reactivity at the α-carbon atoms. There is evidence that the breaking of a carbon-hydrogen bond at the α-carbon atom of a nitrosamine is a possible rate-limiting step in the chain of reactions leading to carcinogenesis, since replacement of hydrogen by deuterium at these positions diminishes by severalfold the carcinogenic activity of dimethylnitrosamine (2) and of nitrosomorpholine (W. Lijinsky, H. W. Taylor, and L. K. Keefer, unpublished data). We have no explanation of the change in site of tumor formation by nitrosomorpholine when methyl groups are present in positions 2 and 6. With the analogous 2,6-dimethyldinitrosopiperazine the tumors appeared at the same site, esophagus and nasal turbinates, as those induced by dinitrosopiperazine, while the carcinogenic potency of the dimethyl compound was greatly enhanced (3).

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