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Comparative Carcinogenicity of Some Derivatives of Nitrosodi-*n*-propylamine in Rats

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It was suggested some years ago that β-oxidation of nitrosodi-*n*-propylamine might play a part in the carcinogenic action of this compound (Pour *et al.*, 1975). So far there has been no indication that this is so from the tests of two β-oxidized derivatives, nitrosobis(2-hydroxypropyl)amine and nitrosobis(2-oxypropyl)amine, in Syrian golden hamsters (Krüger *et al.*, 1974; Pour *et al.*, 1977), in which the tumorigenic response is quite different from that of nitrosodi-*n*-propylamine. We have carried out a comparative test of the parent compound and those two β-oxidized derivatives in Sprague–Dawley rats, together with the halogenated derivative nitrosobis(2-chloropropyl)amine. All four compounds were tested at approximately equimolar doses which were administered to the rats for a fixed period.

MATERIALS AND METHODS

Chemicals

N-Nitrosobis(2-hydroxypropyl)amine was prepared by dissolving 13 g (0.1 mol) of bis(2-hydroxypropyl)amine (di-isopropanolamine, Eastman Organic Chemicals, Rochester. N.Y.) in 10 ml of 10 N HCl containing 20 g of ice. Acetic acid, 20 ml, was added followed by 15 g of sodium nitrite. After reacting 3 hr at room temperature, solid potassium hydroxide was added until the solution was strongly alkaline; the solution became quite hot, but this ensured decomposition of esters that might be present. After cooling the solution was diluted with an equal volume of water which was sufficient to dissolve the solids and was extracted with 3 × 100 ml of ethyl acetate. The combined extracts were shaken twice with 30 ml of water and dried with anhydrous sodium sulfate and the solvent was removed at moderate temperature in a rotary evaporator. The residue was 10 g of a yellow oil, which did not crystallize.

Anal. Calcd for $C_6H_{14}N_2O_3$: C, 44.43; H, 8.70; N, 17.27. Found: C, 44.24; H, 8.58; N, 17.06.

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The mass spectrum showed no significant molecular ion, but was consistent with the assigned structure of the compound (Rainey *et al.*, 1978), as was the NMR spectrum. The uv molecular absorptivity in ethanol was 84 at 353 nm. The above preparation differed somewhat from that described by Krüger *et al.* (1974), which utilized extraction of the aqueous solution with ether; our compound, which seems to have the correct structure, was essentially unextractable from water with ether.

N-nitrosobis(2-oxopropyl)amine was kindly provided by Mr. H. V. Braun of the Deutsches Krebsforschungszentrum, Institut für Toxikologie und Chemotherapie (Director, Dr. D. Schmähl), Heidelberg, to whom we are most grateful.

N-nitrosobis(2-chloropropyl)amine was prepared by nitrosation of bis(2-chloropropyl)amine which was synthesized by treating 17 g (0.1 mol) of bis(2-hydroxy-*n*-propyl)amine hydrochloride dissolved in methylene chloride with 25 g of thionyl chloride added dropwise, while the reaction mixture was cooled in ice and stirred. After stirring 1 hr the solvent was evaporated, leaving a colorless crystalline solid, which was bis(2-chloropropyl)amine hydrochloride.

Four grams of the hydrochloride was dissolved in 10 ml of acetic acid containing 2 g of ice. The solution was cooled and 4 g of sodium nitrite was added. After standing 2 hr, 100 ml of methylene chloride was added, followed by 30 ml of water. The lower layer was separated, washed four times with 30 ml of water, and evaporated in a stream of nitrogen. The yield was 2.2 g (57% theoretical) of yellow oil, which crystallized at −20°C, but was liquid above 4°C. The mass spectrum was consistent with the structure dichloronitrosodipropylamine (Rainey *et al.*, 1978). The molar absorptivity was 87 at 364 nm in ethanol.

Anal. Calcd for $C_6H_{12}N_2Cl_2O$: C, 36.20; H, 6.08; N, 14.07; Cl, 35.62. Found: C, 36.12; H, 6.44; N, 14.05; Cl, 35.10.

As measured by the decrease in absorption of an aqueous solution, the half-life of the compound in water at 20°C was approximately 2 hr.

Animal Treatments

Nitrosobis(2-hydroxypropyl)amine and nitrosobis(2-oxopropyl)amine were administered as solution in drinking water equimolar with that used in a previous test of nitrosodi-*n*-propylamine, 0.7 mM (Lijinsky and Taylor, 1978), to groups of 15 female Sprague–Dawley rats. These rats were of the closed colony of the Biology Division and were 6 to 8 weeks old at the beginning of the treatment. The rats were housed three to a plastic cage containing Sanicel bedding. Rockland rat diet was fed to the rats *ad libitum*. Each cage of rats was given 60 ml of the solution of nitrosamine as drinking water 5 days each week; tap water was given the remaining 2 days. The solutions were completely consumed after a few days initially while the animals became used to the taste. The length of treatment is given in Table 1.

Nitrosobis(2-chloropropyl)amine was too unstable in water to be administered in aqueous solution and was given by gavage, 0.2 ml to each of 15 female Sprague–Dawley rats twice a week of a solution of 34 mg of the nitrosamine/ml of olive oil. Treatment lasted 20 weeks.

After cessation of treatment the animals were maintained until natural death, at which time complete necropsy was performed and all tumors and other lesions were fixed for microscopic examination.

TABLE 1

MORTALITY OF RATS TREATED WITH NITROSODI-*n*-PROPYLAMINE DERIVATIVES

	Concentration (mg/liter)	Duration of treatment (weeks)	Total dose [mg (mmol)]	Survivors at Week										
				0	10	20	30	40	50	60	70	80	90	100
Nitrosodi-*n*-propylamine	90	30	270 (2.1)	15♂	15	15	15	13	6	0				
Nitrosobis(2-hydroxypropyl)amine	108	50	540 (3.3)	15♀	15	15	15	15	15	12	10	5	2	0
Nitrosobis(2-oxopropyl)amine	106	30	318 (2.0)	15♀	15	15	14	11	8	5	2	0		
Nitrosobis(2-chloropropyl)amine	34 mg/ml	20	272 (1.4)	15♀	11	11	10	10	10	4	2	1	1	0

RESULTS AND DISCUSSION

In Table 1 are listed the treatments of the animal groups and the total dose of each compound given to the animals. Figure 1 shows the structures of these compounds. The mortality of the animals is compared with that of a previous test of the parent compound, nitrosodi-*n*-propylamine, in these rats (Lijinsky and Taylor, 1978). In the latter test only male rats were used, but our observations, after an extensive series of studies of nitrosamine carcinogenesis in Sprague–Dawley rats, is that there is little difference in response between male and female rats to a given dose of most nitrosamines.

Immediately apparent is that nitrosodipropylamine led to earlier death with tumors than any of the other compounds and it must be considered the most potent carcinogen of the four. Conversely, nitrosobis(2-hydroxypropyl)amine had the smallest effect on

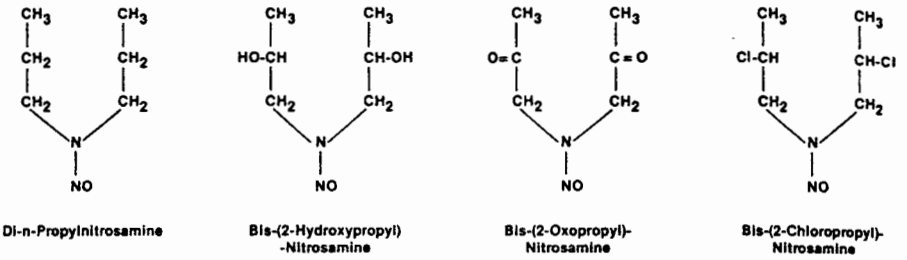

FIG. 1. Structures of the four nitrosodi-*n*-propylamine derivatives administered to rats in this experiment.

survival and seems to be a relatively weak carcinogen, even though more than half of the animals developed tumors. The effectiveness of nitrosodichlorodipropylamine is difficult to assess since the compound was fairly toxic, leading to death of four animals during the first few weeks of treatment; most of these animals lived little more than a year, even though very few died with induced tumors. It is quite possible that the toxicity of the compound was partly due to its instability and that most of the administered dose decomposed in the stomach before being absorbed. It might, then, be very difficult to test this compound adequately for carcinogenicity; it is a very potent mutagen in the Ames test without metabolic activation (Rao *et al.*, 1978). In contrast, nitrosodi-*n*-propylamine was a relatively weak mutagen, even with microsomal activation, and, in the same series of tests, neither bis-2-hydroxy- nor bis-2-oxo nitrosodi-*n*-propylamine was significantly mutagenic with activation by microsomes from the liver of Sprague–Dawley rats. This is very surprising, since this diketone is a potent liver carcinogen in Sprague–Dawley rats.

In Table 2 are listed the tumors found in the rats treated with the four nitroso derivatives of di-*n*-propylamines. While nitrosodipropylamine was a broadly acting carcinogen, inducing a high incidence of tumors in the liver, nasal turbinates, and esophagus, the three derivatives appeared to be less broadly acting. In these experiments, nitrosobis(2-oxopropyl)amine induced only tumors in the liver. This

TABLE 2

TUMORS IN SPRAGUE/DAWLEY RATS TREATED WITH NITROSODI-*n*-PROPYLAMINES

			Number of animals with tumors of				
			Liver	Nasal turbinates	Esophagus	Stomach	Other
Nitrosodi-*n*-propylamine	15♂	14	9 hepatocellular carcinomas	8 adenocarcinomas	6 papillomas 8 carcinomas	1 papilloma	1 tongue carcinoma 1 zymbal gland carcinoma
Nitrosobis(2-hydroxypropyl)amine	15♀	9	3 hepatocellular carcinomas	5 adenocarcinomas 1 squamous cell carcinoma		1 papilloma	5 mammary fibroadenoma 1 ovary granulosal cell tumor 1 thyroid adenoma
Nitrosobis(2-oxopropyl)amine	15♀	13	8 hepatocellular carcinomas 3 cholangiocarcinomas 2 hemangioendothelial sarcomas				1 lung alveolar cell adenocarcinoma
Nitrosobis(2-chloropropyl)amine	15♀	7	1 hepatocellular carcinoma (1 hemangioma)			1 carcinoma	2 mammary fibroadenomas 1 pituitary adenoma 1 subcutis lymphangioma 1 reticulum cell sarcoma 1 adrenal cortex carcinoma 1 ovary hemangiosarcoma 1 ovary adenocarcinoma

contrasts with the recent report of Pour (1978) of the induction of colorectal tumors as well as liver tumors in MRC/Wistar rats. This difference in response might reflect either a difference in susceptibility between the two strains of rat or a difference in response to different routes of administration, subcutaneous injection in Pour's experiments. Additional experiments would be needed to elucidate this.

Nitrosobis(2-hydroxypropyl)amine was a much weaker carcinogen than either nitrosodipropylamine or its 2-keto derivative and, even though the dose administered was higher, induced tumours in only 9 of the 15 rats, including only three liver tumors and six tumors of the nasal cavity. A more extensive test of this compound by subcutaneous injection in Sprague–Dawley rats (Mohr *et al.*, 1977) was reported towards the end of our experiment and showed essentially the same pattern of tumors, except that esophageal tumors were also found. The doses used in those experiments, however, were very much higher than ours and there is a common finding that this compound is a relatively weak carcinogen.

A comparison of carcinogenic effectiveness of nitrosodipropylamine and its two oxygenated derivatives, as can be made in our experiments in which equimolar doses were administered to rats, indicates that the hypothetical formation of oxygenated derivatives of nitrosodipropylamine *in vivo* is not a likely mechanism of carcinogenesis. This was suggested by Krüger and his colleagues (Pour *et al.*, 1977) as a metabolic route whereby nitrosodipropylamine was activated. This is unlikely to be related to carcinogenesis because, in our experiments, neither oxygenated derivative was more potent than the parent compound and, indeed, the bishydroxy compound was very much weaker. Also, the spectrum of tumors induced by the parent compound was much broader than that induced by the oxygenated compounds. It seems, therefore, that all three compounds are likely to act as carcinogens through independent and possibly unrelated mechanisms of activation.

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