Metabolism of Three Cyclic Nitrosamines in Sprague-Dawley Rats

C. M. Snyder, J. G. Farrelly, and W. Lijinsky

Carcinogenesis Program, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

SUMMARY

The metabolism of three cyclic nitrosamines has been studied in Sprague-Dawley rats. The compounds were nitrosopyrrolidine, nitrosohexamethyleneimine, and nitrosoheptamethyleneimine and were labeled at the α carbon with 14C. At low doses (2 to 4 mg/animal) the compounds were metabolized to 14CO2 to the extent of 77, 43, and 27%, respectively, after 24 hr. At doses closer to the 50% lethal dose of the compounds (70 to 160 mg/animal) the metabolism values were only 14, 4, and 8%, respectively, after 24 hr. The significance of these results is discussed.

INTRODUCTION

Since the discovery of the potent carcinogenicity of N-nitroso compounds more than 20 years ago, exploration of the mechanism of their carcinogenic action has been largely devoted to studies of their conversion to alkylation intermediates that alkylate DNA, RNA, and proteins. Because such reactions are easy to observe with simple aliphatic dialkynitrosamines and nitrosamides, such as dimethyl- and diethylnitrosamine and nitrosomethylurea, almost all the investigations have dealt with these compounds that alkylate nucleic acids extensively in vivo. Little attention has been given to the mechanism of action of cyclic nitrosamines which, while being carcinogens of equal or greater potency than the simpler compounds, do not seem to be converted to macromolecule-alkylating moieties to more than a very small extent (9).

There have been few pharmacological studies of the distribution and metabolism of any N-nitroso compounds, since the exemplary studies of Heath (5) with dimethylnitrosamine and other aliphatic nitrosamines, although this has been the classical way of investigating the mechanism of action of biologically active agents. We decided, therefore, to examine the metabolism of carcinogenic cyclic nitrosamines, which have widely different target organ specificities. The compounds were: nitrosopyrrolidine, which induces hepatocellular carcinomas in rats (4); nitrosohexamethyleneimine, which induces hemangioendothelial tumors of the liver and esophageal tumors (2); and nitrosoheptamethyleneimine, which induces squamous carcinomas of the lung and esophageal tumors in rats (8).

All 3 compounds were synthesized with 14C in the carbon atoms α to the nitroso group, and the syntheses were carried out by A. R. Jones of this laboratory, following established synthetic routes.

MATERIALS AND METHODS

The 3 cyclic nitrosamines were prepared as previously described (2, 4, 8). The 3 14C-labeled cyclic nitrosamines were synthesized starting with barium [14C]carbonate that was converted into potassium cyanide and used to prepare, respectively, succinonitrile, pimelonitrile, and suberonitrile, by reaction with the corresponding α,ω-dibromoalkane. All 3 compounds were shown by thin-layer chromatography to contain more than 98% of the radioactivity within the nitrosamine band.

The radioactive and nonradioactive compounds were dissolved in olive oil to give from 10 to 25 μCi at the appropriate concentration for the particular experiment. They were administered by gavage to 250-g female Sprague-Dawley rats of the specific-pathogen-free colony of this laboratory. The experiments were divided into 2 groups. In the 1st group, low doses of nitrosamine were used, which approximated the doses used in the long-term testing, in this laboratory, of the compounds for carcinogenicity (2, 4, 8). The 2nd group received doses approaching the dose of the nitrosamines lethal to 50% of the population, 160 mg nitrosopyrrolidine, 130 mg nitrosohexamethyleneimine, and 70 mg nitrosoheptamethyleneimine. Before the animals were given the higher doses of nitrosamines, they were anesthetized with pentobarbital (24 mg/kg) to avoid death of the animals in convulsion that would occur otherwise. Immediately after receiving the labeled carcinogen, the animals were maintained in a CO2 collection chamber designed by R. B. Cumming of this laboratory. The apparatus consisted of a cylindrical glass cage supplied with 3 fittings for incoming air, incoming water, and exiting air and urine. The air inlet was connected to a manometer gauged to deliver 100 ml breathing air per min to the chamber. The urine-air outlet was fitted with a stopcock and universal joint that accepted a graduated urine-collecting tube, in turn supplied with an air outlet at the top which automatically collected 1-hr samples over a 24-hr period. Complete trapping of CO2 as ethanolate carbonate took place in 5 ml ethanolamine. We tested our procedure for collecting radioactive CO2 by introducing breathing air containing 14CO2 (62 mCi/mole) into the glass chamber at the rate of 100 ml/min and collecting it diluted with CO2 expired by an untreated rat. The CO2 was collected for varying time periods, up to 2 hr, and the radioactivity was proportional to the time of trapping. A
single trap of 5 ml ethanolamine collected 99.8% of the total introduced radioactivity during a 1-hr period. In separate experiments on rats that had received radioactive nitrosamines, double traps were used to ensure that all the radioactivity collected was in the form of \(^4\text{CO}_2\). The 1st trap contained 5 ml cold (−20°) methanol, and the 2nd one contained 5 ml ethanolamine. The radioactivity trapped in the methanol during the 1st 6 hr of each experiment was never more than 0.16% of the total label, suggesting that more than 99% of the radioactivity trapped in the organic base was in the form of CO\(_2\). Ten ml of methanol were used to transfer and solubilize the samples in scintillation vials containing 5 ml fluor [10:1 PPO:p-bis(o-methylstyril)benzene at a concentration designed to give 6 g fluor per liter in the counting vial]. Counting efficiency was 58% for this system. An aliquot of the material received by the rat was counted in the same manner, and internal standards of [\(^{14}\text{C}\)]toluene were used occasionally to check the homogeneity of the counting efficiencies.

Our CO\(_2\) collection chamber for rats delivered the urine from the animals, free of fecal material, to a graduated centrifuge tube from which samples were taken at suitable intervals. Aliquots (usually 0.1 ml) were removed every 4 hr and were solubilized in 1 ml NCS (Amersham/Searle Corp., Arlington Heights, Ill.) and then counted in fluor as above. Internal standards were used to determine dpm.

**RESULTS**

**Low Dose.** The rates of excretion as \(^4\text{CO}_2\) of the 3 cyclic nitrosamines given at low doses (2 to 4 mg/rat) are shown in Chart 1. Nitrosopyrrolidine is metabolized to CO\(_2\) very rapidly. After only 1 hr, more than 10% of the administered dose was excreted, whereas more than 60% had been excreted after 6 hr. By 24 hr, approximately 70% had been metabolized to \(^4\text{CO}_2\). Nitrosohexamethyleneimine and nitrosoheptamethyleneimine, however, are excreted as CO\(_2\) at lower rates and to lesser extents after 24 hr. After 6 hr, 31% of nitrosohexamethyleneimine and 17% of nitrosoheptamethyleneimine had been excreted, whereas 45 and 28% had been metabolized, respectively, after 24 hr. The cumulative \(^4\text{CO}_2\) excretion as well as the elimination of radioactivity in the urine is shown in Chart 2. After 24 hr, 11% of the starting radioactivity of nitrosopyrrolidine had been eliminated in the urine, whereas 33 and 43% of the nitrosohexamethyleneimine and nitrosoheptamethyleneimine, respectively, had been excreted by this route.

**High Dose.** The 3 nitrosamines were administered to rats at concentrations approaching the dose lethal to 50% of the rats of each, and their metabolism to \(^4\text{CO}_2\) was measured. At high doses (nitrosopyrrolidine, 648 mg/kg; nitrosohexamethyleneimine, 576 mg/kg; and nitrosoheptamethyleneimine, 280 mg/kg), the conversion to \(^4\text{CO}_2\) was markedly decreased from that measured at low doses. Chart 3 shows the rate of \(^4\text{CO}_2\) formation over a 24-hr period for all 3 compounds. After 24 hr, 14% of the nitrosopyrrolidine was metabolized to CO\(_2\), whereas 4 and 8%, respectively, of the nitrosohexamethyleneimine and nitrosoheptamethyleneimine were converted.

**DISCUSSION**

In the past it has been assumed that the cyclic nitrosamines are not metabolized to an appreciable extent when administered to rats. For instance, Stewart et al. (10) estimated that 3.3% of N-nitrosomorpholine was converted to CO\(_2\) during 24 hr. In their study, the dose of nitrosamine was high (400 mg/kg body weight), so the results are not surprising in light of our findings. In a number of more recent reports, however, smaller doses of nitrosamines were administered and the expired CO\(_2\) was measured. The exhalation of radioactivity as CO\(_2\) in all of these studies was lower.
Our results indicate that cyclic nitrosamines are indeed metabolized to a large extent when fed to rats. However, it is obvious that the animal can metabolize only a limited quantity in a given time. At high doses, the metabolism to carbon dioxide of the cyclic nitrosamines used in this study is very small. It is possible that the enzyme systems necessary to convert the compounds to metabolic product exist in limited quantity. This is supported by the limitation on metabolism of these cyclic nitrosamines by liver microsomal enzymes demonstrated in their conversion to bacterial mutagens (1).

When the concentration of nitrosamine is too high, it is excreted in the urine instead of being metabolized to compounds leading to CO₂. In other experiments we fed low doses of nonradioactive nitrosamines daily for 2 weeks. The rats were then fed radioactive compounds, and the CO₂ excretion was followed. The pretreatment did not change the pattern of CO₂ excretion. This indicates that the metabolic enzymes are capable of metabolizing a large proportion of nitrosamines when they are encountered in small doses and that more of the nitrosamine-metabolizing enzymes were not induced by pretreatment with nitrosamine.

**REFERENCES**

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