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Brief Communication

Reaction of Drugs with Nitrous Acid as a Source of Carcinogenic Nitrosamines[1]

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SUMMARY

Twelve common drugs that are tertiary amines react with nitrite in aqueous solution at ph 3 to 4 to form dialkylnitrosamines that are known carcinogens. Aminopyrine gave dimethylnitrosamine in 30% yield or higher at all concentrations down to 50 ppm (with 25 ppm nitrite); the other product of this reaction was the nitrite salt of 4-hydroxyantipyrine. The other drugs, when present at 0.01 M with 0.04 M nitrite, formed nitrosamines in yields ranging from 0.03% from dextropropoxyphene to 2.4% from lucanthone in 4 hr at 37°.

INTRODUCTION

Some preliminary studies of the interaction of tertiary amines with nitrous acid have indicated that formation of dialkylnitrosamines by nitrosative dealkylation is a reaction usually undergone by tertiary amines at moderately acid pH (6). Such reactions taking place in the stomach of man might be a source of carcinogenic nitrosamines (3), since nitrite is commonly used as a curing and preserving agent in meat and fish and can be formed by reduction of nitrate. A significant exposure of man to tertiary amines is in the form of the many drugs commonly taken (a large proportion of which are tertiary amines). A short report of reactions of a few drugs with nitrous acid has been made (4), and a biological study of the reaction of one tertiary amine, aminopyrine, has demonstrated the formation of large amounts of dimethylnitrosamine in this way (5).

Here are reported further studies on the reactions of aminopyrine and other commonly used drugs with nitrous acid, with special regard to the extent of interaction at rather low concentrations, to offer some indication of the magnitude of the hazard to man from such interactions.

The drugs examined were aminopyrine (analgesic), chlorpheniramine and methapyrilene (antihistaminics), chlorpromazine and dextropropoxyphene (tranquilizers), tolazamide (hypoglycemic), quinacrine (antimalarial), lucanthone (antischistosomiasis), cyclizine (for motion sickness), disulfiram (antialcoholic), and methadone (narcotic).

MATERIALS AND METHODS

Aminopyrine (dimethylaminoantipyrine) was from Aldrich Chemical Co. (Milwaukee, Wis.), quinacrine hydrochloride and methapyrilene hydrochloride were from Sigma Chemical Co. (St. Louis, Mo.), and methadone hydrochloride was from Mallinckrodt Chemical Works (St. Louis, Mo.). The remaining compounds were kindly supplied gratis by the manufacturers as follows: chlorpheniramine (Schering Corp., Bloomfield, N. J.), chlorpromazine (Smith, Kline and French, Philadelphia, Pa.), dextropropoxyphene (Eli Lilly and Co., Indianapolis, Ind.), lucanthone (Sterling-Winthrop Pharmaceutical Co., Rensselaer, N. Y.), tolazamide (Upjohn Co., Kalamazoo, Mich.), disulfiram (Ayerst Laboratories, New York, N. Y.), cyclizine (Burroughs-Wellcome, Research Triangle Park, N. C.), and oxytetracycline hydrochloride (Pfizer Pharmaceuticals, Groton, Conn.).

Reactions were carried out in 20- to 40-ml volumes of aqueous solution containing the drug, 0.5 to 1.0 ml acetic acid (as a convenient acidifying agent), and sodium nitrite (4 to 8 moles/mole of amine). The pH of the solution was taken at the beginning and end of the reaction. Reactions were carried out at 90° under reflux or at 37° in corked flasks. At the end of the allotted time the reactions were stopped by addition of a few pellets of sodium hydroxide (this made the solutions alkaline), and the aqueous solutions were extracted twice with double volumes of methylene chloride; this was adequate to extract almost completely all of the nitrosamine present (the partition coefficient for dimethylnitrosamine between methylene chloride and water, or dilute alkali, is 3.5:1, and higher than this for other nitrosamines). The methylene chloride extracts were back-extracted with a small volume of 5 N hydrochloric acid and were evaporated to a small volume at room temperature in a stream of nitrogen. The residual solutions were made to 5 or 10 ml in volumetric flasks, and the nitrosamines were estimated by gas-liquid chromatography on 8.4% diethyleneglycol succinate on Chromosorb (analyses were carried out by the Analytical Chemistry Division, Oak Ridge National Laboratory, and by Dr. George Singer of the

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Biology Division). Identification of the product as the nitrosamine was based on the retention time in a gas-liquid chromatogram and was in most cases confirmed by mass spectrometry carried out on the products of the reactions on a larger scale, as for cyclizine (Table 1) and in Ref. 4. A Coulson nitrogen-specific detector was used for the estimation of concentration; this eliminated possible over-estimation of nitrosamine because of the presence of by-products having the same retention time, among which is acetic acid with the same retention time as dimethylnitrosamine. The yields given are minimal because of inevitable losses in the extraction and evaporation procedures. Recovery of the most easily lost nitrosamine, dimethylnitrosamine, was 75 to 80%.

RESULTS

The yields of nitrosamine by reaction of the several drugs with nitrite at arbitrary concentrations and with a ratio of drug to nitrite of 1:1 or 1:2 are given in Table 1; the concentration in the reaction mixture and the fraction of the theoretical yield are given. In all cases the expected volatile nitrosamine was formed from the tertiary amine. No attempt was made to identify the other products of the reactions, among which must have been the other possible nitrosamine formed by cleavage of one of the small alkyl groups.

In Table 2 are the results of similar reactions of the drugs with nitrite, but under identical conditions of concentration of amine and of nitrite, each solution being 0.01 M in amine and 0.04 M in nitrite. The acid was acetic acid, and the pH's of the solutions were all 3.3 to 3.4. Again the yields of volatile nitrosamine are given in μg/ml of solution and as a percentage of theoretical.

Because the reaction of aminopyrine with nitrous acid was so much more vigorous than that of any other tertiary amine we have examined, this reaction was explored more thoroughly. Table 3 shows the yield of dimethylnitrosamine at quite low concentrations of aminopyrine with various

Table 1

Nitrosamines formed by interaction of drugs with nitrite in acetic acid

Drug	Concentration of drug (mg/ml)	Concentration of $NaNO_2$ (mg/ml)	Temperature	Time (hr)	pH	Yield of nitrosamine		
						μg/ml		% of theoretical
Aminopyrine	0.25	0.25	37°	2	3.2	33	DMN[a]	40
Oxytetracycline	8	16	37	4	3.0	20	DMN	15
	1	1	37	2	3.2	0.5	DMN	0.3
Chlorpromazine	5	10	37	4	3.4	10	DMN	0.88
	5	10	90	1	3.4	38	DMN	3.3
Dextropropoxyphene	5	10	37	4	3.3	2	DMN	0.16
	5	10	90	4	3.5	17	DMN	1.5
Chlorpheniramine	5	10	37	4	3.5	1	DMN	0.07
	5	10	90	4	3.5	25	DMN	1.8
Methadone	5	10	37	4	3.3	2	DMN	0.18
	5	10	90	1	3.3	26	DMN	2.4
Methapyrilene	5	10	37	4	3.4	9	DMN	0.7
	5	10	90	1	3.4	175	DMN	14
Disulfiram	5	10	37	2	3.6	4.5	Diethylnitrosamine	0.3
Quinacrine	5	10	37	4	3.5	1.5	Diethylnitrosamine	0.15
	5	10	90	4	3.5	12	Diethylnitrosamine	1.2
Lucanthone	5	10	37	4	3.6	10	Diethylnitrosamine	0.7
	5	10	90	4	3.6	40	Diethylnitrosamine	3.0
Tolazamide	5	10	37	3	3.1	70	NHMI	3.4
Cyclizine	40	36	90	4	3.7	155	DNP	8.2

[a] DMN, dimethylnitrosamine; NHMI, nitrosohexamethyleneimine; DNP, dinitrosopiperazine.

Table 2
Formation of nitrosamines from drugs at 0.01 M amine and 0.04 M nitrite

Drug	Temperature	Time (hr)	pH	Yield of nitrosamine[a]		
				μg/ml		% of theoretical
Chlorpromazine	90°	1	3.4	0.5	DMN	0.06
	37	4	3.4	0.35	DMN	0.05
Chlorpheniramine	90	1	3.4	5	DMN	0.7
	37	4	3.4	1.6	DMN	0.2
Dextropropoxyphene	90	1	3.3	3	DMN	0.4
	37	4	3.3	0.2	DMN	0.03
Methadone	90	1	3.4	3	DMN	0.4
	37	4	3.4	0.3	DMN	0.04
Methapyrilene	90	1	3.4	5	DMN	0.7
	37	4	3.3	0.6	DMN	0.08
Quinacrine	90	1	3.3	1.5	Diethylnitrosamine	0.15
	37	4	3.3	1	Diethylnitrosamine	0.1
Lucanthone	90	1	3.3	19	Diethylnitrosamine	1.9
	37	4	3.3	24	Diethylnitrosamine	2.4
Disulfiram	37	3	3.3	0.8	Diethylnitrosamine	0.08
Tolazamide	37	3	3.3	8	NHMI	0.6
Trimethylamine	37	4	3.3	10.5	DMN	1.4

[a] DMN, dimethylnitrosamine; NHMI, nitrosohexamethyleneimine.

Table 3
Reaction of aminopyrine with nitrite in acetic acid at 37° in 1 hr

Concentration of aminopyrine		Concentration of $NaNO_2$		Dimethylnitrosamine yield (% of theoretical)
mg/ml	mM	mg/ml	mM	
0.1	0.43	0.1	1.45	55
0.1	0.43	0.075	1.1	54
0.1	0.43	0.05	0.7	48
0.1	0.43	0.025	0.35	26
0.1	0.43	0.012	0.17	13
0.05	0.22	0.05	0.7	52
0.05	0.22	0.025	0.35	35
0.05	0.22	0.012	0.17	22

concentrations of nitrite. At high concentrations of aminopyrine (0.2 M) with 1.6 M sodium nitrite, the yield of dimethylnitrosamine was more than 75% theoretical within 30 min. From the reaction mixture there separated a yellow crystalline solid which melted at 87–88° with decomposition and which must have been a major reaction product, since it almost equalled in weight the initial aminopyrine (2.15 g from 2.3 g aminopyrine). The UV absorption spectrum in acid solution was that of nitrous acid, indicating that the compound was a nitrite salt. The mass spectrum of the compound showed strong ions at *m/e* 220, 219, 192, 191, 176, 164, 163, 148, and 121; accurate mass measurement gave a composition of $C_{11}H_{12}N_2O_3$ for the ion at *m/e* 220, which must be considered a fragment derived from decomposition of the yellow compound. Elemental analysis of the yellow product, which could not be recrystallized without decomposition, gave 53.15% C, 4.49% H, 16.65% N, which corresponds quite well with the composition 52.59% C, 5.21% H, 16.72% N for $C_{11}H_{13}N_3O_4$. It is reasonable to deduce that the yellow product of reaction of aminopyrine with nitrous acid is the nitrite salt of the compound shown below (4-hydroxyantipyrine), formed by oxidative deamination of aminopyrine (Chart 1). A yellow compound was precipitated by addition of sodium nitrite to an acid solution of 4-hydroxyantipyrine. This had a UV absorption spectrum similar to that of the compound derived from aminopyrine and had the same melting point, a mixture of the 2 products showed no depression of melting point, and the mass spectra of the 2 products were the same. The 2 products seem identical.

DISCUSSION

The finding that dialkylnitrosamines are formed by interaction of the several drugs examined with nitrite in mildly acid solution is unexceptional, since this is a common reaction of tertiary amines (6). However, the variation in yield of nitrosamine from the various compounds is quite large, ranging from 40% for aminopyrine to 0.03% for dextropropoxyphene at 37°. In most cases yields were considerably higher at 90° than at 37°, as is normal with reactions of this type. The reason for the wide variation in yield of dialkylnitrosamine is not clear, since the part of the molecule containing the dialkylamino function was very similar in many cases; yet the yield of dialkylnitrosamine in a given time could be greatly different, as in the case of quinacrine and lucanthone.

The significance of the reactions described here is that the

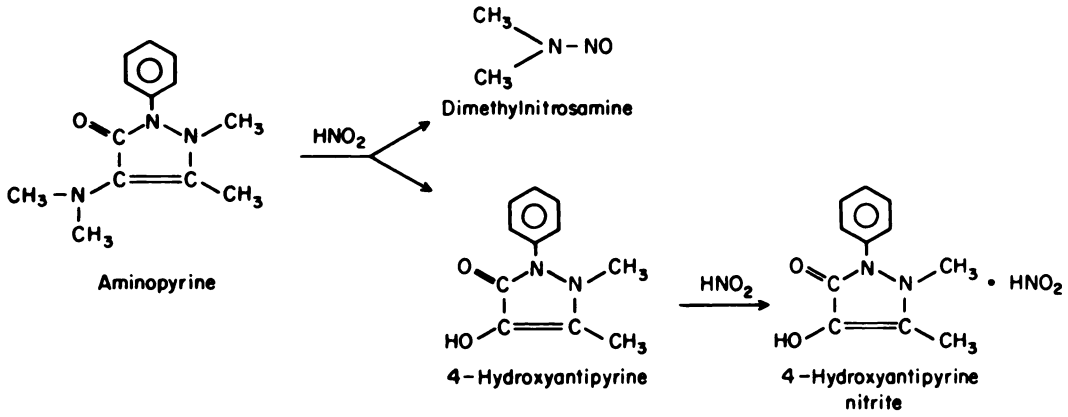

Chart 1.

products include nitrosamines that are highly potent carcinogens, such as dimethylnitrosamine and diethylnitrosamine. The drugs examined are only a few examples of the many hundreds that could react similarly (2), and they were chosen because they are widely used. Many of them are taken almost chronically by large numbers of people. In circumstances in which people taking these drugs also consume diets containing nitrites [as, for example, preserved meat or fish, or crops grown on mineral-deficient soil (1)], such a combination might pose a significant carcinogenic hazard, since nitrosamines could be formed in the stomach. We are examining this possibility by feeding nitrite together with several of these drugs to rats; aminopyrine with nitrite has produced liver tumors (7).

Although the amount of nitrosamine formed from these drugs at low concentrations is often small, it cannot be considered insignificant, particularly when balanced against other sources of carcinogens (8). The amounts of nitrosamine formed from a drug and nitrite in the stomach could be on the order of micrograms. This emphasizes the need to remove nitrite from the diet whenever it is present for reasons other than health.

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