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Protective Effect of Ascorbic Acid on Hepatotoxicity Caused by Sodium Nitrite Plus Aminopyrine

(rats/serum alanine aminotransferase)

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ABSTRACT **The oral treatment of rats with sodium ascorbate in combination with sodium nitrite and aminopyrine prevents the rise in serum alanine aminotransferase (EC 2.6.1.2) observed when nitrite and aminopyrine are given alone. Ascorbic acid also affords protection, whereas dehydroascorbic acid exerts no protective effect.**

Sodium nitrite is extensively used in meat processing to produce a characteristic color and to suppress bacterial activity (1). Nitrate, which is converted to nitrite by microorganisms (2), is a contaminant of some water supplies and vegetables as a result of the extensive use of nitrogenous fertilizers and the decomposition of plant and sewage wastes, followed by leaching of nitrates into ground water (1). Concern has been expressed in recent years because nitrite reacts with various naturally occurring and synthetic secondary and tertiary amines to form nitroso derivatives, which may possess carcinogenic activity (3, 4).

Recently, Lijinsky and Greenblatt (5) reported that the oral administration of sodium nitrite and aminopyrine (a tertiary amine) to rats results in hepatic necrosis, which is accompanied by an elevation in alanine aminotransferase activity. This hepatotoxicity is presumably due to the formation of dimethylnitrosamine from the reaction of nitrous acid, formed from sodium nitrite, with aminopyrine in the acidic environment of the rat's stomach (5). The nitrosative cleavage of tertiary amines by nitrous acid has been described by Smith and Loeppky (6) and the acute hepatotoxicity of dimethylnitrosamine is well documented (7).

The present report describes a protective effect of sodium ascorbate and ascorbic acid on the acute hepatotoxicity that follows the oral administration of sodium nitrite and aminopyrine to rats.

METHODS

Male, Sprague–Dawley rats (250–280 g) were housed in individual cages and had free access to food and water

TABLE 1. *Effect of sodium ascorbate on elevation of serum alanine aminotransferase activity in rats caused by sodium nitrite plus aminopyrine*

Exp. no.	Group	Compound administered (mg)			Change in body weight (g)	Enzyme	
		Sodium nitrite	Amino-pyrine	Sodium ascorbate		IU/liter	% Protection
1	A	0	0	0	+25.5 ± 3.0	13.6 ± 2.6	—
	B	30	35	0	−28.5 ± 10.2*	87.1 ± 38.5*	—
	C	0	35	0	+23.7 ± 3.2†	14.0 ± 1.5†	99.5
	D	30	0	0	+27.0 ± 12.5†	12.0 ± 1.7†	100
	E	0	0	70	+18.1 ± 12.9†	11.5 ± 1.7†	100
	F	30	35	70	+12.9 ± 6.5‡	12.4 ± 3.7†	100
2	A	0	0	0	+25.6 ± 1.7	17.4 ± 3.7	—
	B	30	35	0	−37.0 ± 15.8*	104.8 ± 61.0*	—
	C	30	35	60	+12.0 ± 7.2‡	18.3 ± 2.7†	100
	D	30	35	35	+23.3 ± 4.2‡	23.7 ± 6.4†	95.9
	E	30	35	20	+16.8 ± 3.0‡	20.0 ± 5.3†	95.9
	F	30	35	15	−10.0 ± 17.2‡	23.9 ± 13.4†	92.6
	G	30	35	10	−10.7 ± 20.5‡	45.8 ± 35.0§	67.5
	H	30	35	5	−17.0 ± 7.2‡	62.9 ± 24.9¶	47.9

Each compound was administered to 5–7 rats, according to the protocol described in the text, as a single saline solution (1.5 ml). The sodium ascorbate dose is expressed in terms of free acid. Control animals (Group A) received 1.5 ml of saline. The numbers for body weight changes and activity are means ± the standard deviation. Data for enzyme activity are expressed as international units (IU)/liter of serum. The percent protection was calculated from: $100–100[\Delta_1 \div \Delta_2]$, where Δ_1 is the mean for the protected group minus the mean for the controls (Group A) and Δ_2 is the mean for the positive controls (Group B) minus the mean for the controls (Group A).

* Differs from control (Group A), $P < 0.05$. † Not different from controls (Group A); differs from positive control (Group B), $P < 0.05$. ‡ Differs from both control groups (A and B), $P < 0.05$. § Not different from either control group (A and B). ¶ Differs from control (Group A), $P < 0.05$ but not from positive control (Group B).

TABLE 2. *Effect of sodium ascorbate, ascorbic acid, and dehydroascorbic acid on elevation of serum alanine aminotransferase activity in rats caused by sodium nitrite plus aminopyrine*

Treatment	Group	Enzyme	
		IU/liter	% Protection
Saline	A	16.1 ± 3.3	—
Sodium nitrite + aminopyrine	B	86.2 ± 49.2*	—
Sodium nitrite + aminopyrine + sodium ascorbate	C	17.5 ± 4.5†	98.0
Sodium nitrite + aminopyrine + ascorbic acid	D	16.1 ± 5.4†	100
Sodium nitrite + aminopyrine + dehydroascorbic acid	E	65.4 ± 16.7‡	29.7

Each compound was administered to 7–8 rats, according to the protocol described in the text. Two solutions were used: the first contained 30 mg of sodium nitrite and 35 mg of aminopyrine in 0.5 ml of saline. The second, administered immediately after the first, contained 20 mg of the compound being tested for a protective effect in 1.0 ml of saline. Control animals (Group A) received 1.5 ml of saline. Doses of compound being tested for protection are expressed as the free acid. The enzyme amounts are means ± the standard deviation. The percent protection was calculated from: $100 - 100[\Delta_1 \div \Delta_2]$, where Δ_1 is the mean for the protected group minus the mean for the controls (Group A) and Δ_2 is the mean for the positive controls (Group B) minus the mean for the controls (Group A).

* Differs from control (Group A), $P < 0.05$. † Not different from controls (Group A); differs from positive control (Group B), $P < 0.05$. ‡ Differs from control (Group A), $P < 0.05$ but not from positive control (Group B).

throughout the experiments. The compounds were administered orally as saline solutions between 8:00 and 8:30 a.m., alone or in various combinations, once a day for 2 consecutive days. 48 hr after the last dose, the animals were lightly anesthetized with carbon dioxide and blood was obtained by cardiac puncture. Serum was prepared by centrifugation and the amount of alanine aminotransferase was determined on the same day by use of a commercial diagnostic kit (Determatube-SGP, Worthington Biochemical Corp., Freehold, N.J.). The animals were weighed at the start of the experiment and once again before blood was drawn.

RESULTS

Rats that were administered sodium nitrite and aminopyrine, according to the schedule described above, had marked elevations in enzyme activity (Table 1, Exp. 1) as reported by Lijinsky and Greenblatt (5). In addition, these animals lost a considerable amount of body weight. When either sodium nitrite or aminopyrine was administered alone, there was no effect on either alanine aminotransferase or body weight (Table 1, Exp. 1). The elevation of enzyme activity caused by the administration of sodium nitrite and aminopyrine was completely blocked when 70 mg of sodium ascorbate per animal was added to the regimen, and the loss in body weight was almost completely prevented (Table 1, Exp. 1).

Table 1 (Exp. 2) shows the dose–response effect of sodium ascorbate on the elevation of enzyme activity and body weight loss. As little as 15 mg of sodium ascorbate (0.85×10^{-4} mol) in combination with 30 mg of sodium nitrite (4.35×10^{-4} mol) and 35 mg of aminopyrine (1.15×10^{-4} mol) inhibited the rise in enzyme activity by 93% ($P < 0.05$), and 5 mg of sodium ascorbate partially blocked the toxic effect (Table 1, Exp. 2). In addition to its effect on alanine aminotransferase, sodium ascorbate prevented the weight loss, associated with the administration of sodium nitrite and aminopyrine, in a dose-related manner.

Ascorbic acid and dehydroascorbic acid were investigated for their protective effects on the rise in enzyme activity caused by the administration of sodium nitrite and aminopyrine. Ascorbic acid was as effective as sodium ascorbate in preventing the rise in activity (Table 2). In contrast, dehydroascorbic acid did not prevent the elevation in activity.

DISCUSSION

The mechanism by which ascorbate protects rats from hepatotoxicity due to the administration of sodium nitrite and aminopyrine is not completely understood. Recently, Mirvisch *et al.* (8) demonstrated that ascorbate blocks the formation of nitrosamines *in vitro* by competing for available nitrite. The reaction between ascorbate and nitrous acid has been described by Dahn *et al.* (9). A similar competition, which would prevent the formation of dimethylnitrosamine from sodium nitrite and aminopyrine, may occur in rat stomach. Preliminary studies in our laboratory suggest that ascorbate may also afford protection by another mechanism. In rats, the administration of 70 mg/rat of sodium ascorbate together with an oral dose of dimethylnitrosamine (20 mg/kg of body weight) partially prevents the elevation in serum alanine aminotransferase activity caused by the administration of dimethylnitrosamine alone. Lower doses of ascorbate (20 mg per animal) afford no protection.

Aminopyrine reacts with sodium nitrite in the acidic environment of a rat's stomach to form dimethylnitrosamine; the nitrosamine causes an acute toxicity, which is characterized by hepatic necrosis and an elevation in alanine aminotransferase in the serum (5). Experiments described in this communication demonstrate that in rats, ascorbate can prevent the rise in enzyme activity that follows the oral administration of nitrite and aminopyrine. The livers from the animals used in the experiments shown in Table 1 were examined for signs of toxicity. The livers from animals that had received aminopyrine and sodium nitrite showed marked necrosis, as has been reported by Lijinsky and Greenblatt (5). In contrast, the livers from animals that had received as little as 20 mg of sodium ascorbate, in addition to the aminopyrine and nitrite, were microscopically normal in appearance.

The significance of our findings with respect to man is not known since the toxicity resulting from the interaction of amines and nitrite, which has been demonstrated in rats, has not been adequately investigated in humans. The *in vivo* experiments described in this communication, as well as the *in vitro* studies by Mirvisch *et al.* (8), suggest a need for further research to determine whether ascorbate can prevent a possible toxicity in man resulting from the ingestion of certain foods, particularly cured meats and fish, that contain nitrite and naturally occurring secondary or tertiary amines.

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