N-Nitrosodiethylamine Induced Changes in Levels, Metabolism, and Urinary Excretion of Ascorbic Acid in Guinea Pigs

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Summary Treatment of male guinea pigs with the potent chemical carcinogen N-nitrosodiethylamine (NDEA) at an intraperitoneal dose of 40 mg/kg body weight for three consecutive days resulted in a significant decrease in the levels of ascorbic acid in liver, lungs, kidneys, and adrenal glands. The decrease in the brain and plasma was, however, not significant. Radiorespirometric, distribution, and urinary excretion studies conducted with the use of L-[carboxyl- ^{14}C]ascorbic acid revealed that a possible mechanism responsible for depletion of the body ascorbate store by NDEA might be the enhanced metabolic degradation of ascorbic acid into CO_2 , and other metabolites that are excreted through urine.

Key Words: N-nitrosodiethylamine, ascorbic acid, metabolism, radiorespirometry

Human studies have revealed that plasma ascorbate reserves are depleted in a number of conditions such as inflammation [1, 2], surgical trauma [3, 4], myocardial infarction [5], smoking [6], use of oral contraceptives [7-9], and thermal burns [10]. Similarly, estrogen administration to female guinea pigs reduced the plasma ascorbate levels [11]. It is likely that there may be a large number of other conditions that might affect the body ascorbate status.

N-Nitroso-compounds, including N-nitrosodiethylamine, are widely distributed in the environment, and induce a wide variety of tumors in many species and strains of animals [12]. It has been suggested that these compounds may also be involved in a number of human cancers. In the present study we found that N-nitrosodiethylamine administration to male guinea pigs resulted in decreased

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body ascorbic acid levels. We further evaluated the mode of action of this carcinogen in reducing the levels of the vitamin.

MATERIALS AND METHODS

L-[carboxyl- ^{14}C]Ascorbic acid (^{14}C -AA, 21 mCi/mmol) was purchased from the Radiochemical Centre, UK. N-Nitrosodiethylamine (NDEA), PPO, and POPOP were purchased from Sigma Chemicals, St. Louis, MO. All other chemicals and reagents of analytical grade were purchased locally.

Male guinea pigs, 300–350 g, were used for various studies. They were maintained on an ascorbic acid-free stock diet and given water *ad libitum*. In addition, the animals orally received 2 mg ascorbic acid/100 g body weight/day in 10% sucrose solution. The animals were divided into two groups, each containing 5 guinea pigs, and one was intraperitoneally administered NDEA at a dose of 40 mg/kg body weight/day in normal saline (1 ml/kg) for 3 days. The second group received the equivalent dose of normal saline. After an overnight fasting, ^{14}C -AA (0.2 μCi /100 g body weight) was administered orally by intubation to all the animals. Soon after, radiorespirometric studies were conducted for 200 min by a modified method of Basu *et al.* [11]. In short, individual animals were kept in an airtight perspex chamber. A steady flow of dry air at a rate of 80 ml/min was maintained throughout the experiment. Carbon dioxide exhaled by the animals was trapped in test tubes containing 1 ml of a mixture of ethanalamine-ethyleneglycol monomethylether (3 : 1, v/v) and 0.125 ml of a saturated solution of sodium hydroxide. In each tube $^{14}\text{CO}_2$ was trapped for 10 min. The trapping mixture from each tube was decanted into a scintillation vial containing 10 ml of scintillation fluid (5 g PPO and 125 mg POPOP in 1 liter of toluene: ethyleneglycol monomethylether [5 : 2, v/v]). Radioactivity was counted in a LKB scintillation counter having inbuilt provision for conversion of CPM into DPM. At the end of the experiment, the animals were sacrificed, and their liver, lungs, adrenal glands, brain, kidneys, spleen, and blood were removed immediately. Tissues were homogenized in 5% trichloroacetic acid. After centrifugation at $300\times g$ for 10 min, 0.1 ml of supernatant was neutralized with 1 N KOH, and counted for radioactivity. The ascorbic acid content in various organs and serum was estimated by the method of Zannoni *et al.* [13].

In another experiment, guinea pigs were intraperitoneally administered ^{14}C -AA (0.5 μCi /100 g body weight). After equilibration of labelled ascorbic acid in the body for 24 h NDEA (40 mg/kg body weight) was injected intraperitoneally, and the animals were immediately put in the radiorespirometric chamber. $^{14}\text{CO}_2$ was trapped as described above for 10 min after 1, 3, 5, 7, 9, and 24 h of NDEA administration.

For determination of the effect of NDEA administration on urinary excretion of ascorbic acid, 5 guinea pigs were intraperitoneally injected with ^{14}C -AA (0.5 μCi /100 g body weight). They were put in metabolic chambers, and urine was

collected in 5 ml of 20% sulfuric acid over a period of 24 h. The total radioactivity of ascorbic acid was determined by combustion of urine in sulfuric acid at 100°C for 3 h. Carbon dioxide was collected in saturated sodium hydroxide solution and counted for radioactivity.

Statistical analysis of the results was done by Student's *t*-test.

RESULTS

Treatment of guinea pigs with NDEA at an intraperitoneal dose of 40 mg/kg for 3 days did not produce any change in the body weight.

Table 1 shows that NDEA treatment resulted in a significant decrease in the level of ascorbic acid in liver, lungs, kidneys, and adrenal glands. However, the

Table 1. Effect of NDEA (40 mg/kg/day for 3 days) on ascorbic acid content ($\mu\text{g/g}$ or ml) in various organs of the guinea pig.

	Liver	Lung	Kidney	Adrenal	Brain	Plasma
Normal	165 $\pm$ 10	68.2 $\pm$ 7.09	59.8 $\pm$ 10.5	134 $\pm$ 6.07	123 $\pm$ 8.40	2.56 $\pm$ 0.23
NDEA-treated	72.1 $\pm$ 3.57*	35.6 $\pm$ 4.07*	30.1 $\pm$ 3.66*	71.9 $\pm$ 6.55*	116 $\pm$ 7.10	2.34 $\pm$ 0.15

Mean $\pm$ SD ($n=5$); * $p<0.05$ vs. corresponding normal value.

Fig. 1. $^{14}\text{CO}_2$ exhalation pattern in control (a) and NDEA-pretreated (b) guinea pigs after the oral administration of ^{14}C -AA ($0.2 \mu\text{Ci}/100 \text{ g}$ body weight).

decrease in ascorbic acid content in the brain and plasma was not significant.

Immediately after oral administration of radioactive ascorbic acid, expiration of $^{14}\text{CO}_2$ took place. The peak values occurred at 18 ± 4.5 and 76 ± 11.0 min. A similar pattern of $^{14}\text{CO}_2$ exhalation was observed in all the control animals. However, we found that NDEA pretreatment for 3 days nonsignificantly decreased the rate of $^{14}\text{CO}_2$ exhalation, resulting in only one peak of $^{14}\text{CO}_2$ at 70 ± 21 min (Fig. 1). Cumulative $^{14}\text{CO}_2$ exhaled in 200 min (% of administered dose) dropped

Table 2. Effect of NDEA treatment (40 mg/kg/day for 3 days) on the distribution of ^{14}C -AA in tissues of the guinea pig.

	Normal		NDEA-treated	
	% of administered dose per g tissue or ml plasma	% of administered dose per tissue	% of administered dose per g tissue or ml plasma	% of administered dose per tissue
Liver	0.22 ± 0.079	2.43 ± 0.747	$0.65 \pm 0.288^*$	$4.36 \pm 1.57^*$
Lung	0.312 ± 0.037	0.708 ± 0.078	$0.422 \pm 0.089^*$	0.938 ± 0.238
Kidney	0.33 ± 0.076	0.726 ± 0.105	$0.455 \pm 0.078^*$	0.945 ± 0.225
Spleen	0.268 ± 0.041	0.078 ± 0.022	0.506 ± 0.315	$0.272 \pm 0.142^*$
Adrenal	0.778 ± 0.548	0.124 ± 0.102	0.46 ± 0.236	0.07 ± 0.024
Brain	0.276 ± 0.008	0.942 ± 0.078	0.308 ± 0.123	0.912 ± 0.223
Heart	0.12 ± 0.084	0.118 ± 0.81	0.198 ± 0.085	0.135 ± 0.031
Blood	0.255 ± 0.066	—	0.355 ± 0.133	—

Mean $\pm$ SD ($n=5$); $^*p < 0.05$ vs. corresponding normal value.

Fig. 2. Effect of intraperitoneal administration of NDEA (40 mg/kg body weight), after the equilibration of ^{14}C -AA for 24 h, on $^{14}\text{CO}_2$ exhalation at different periods. $^*p < 0.05$ in comparison with the zero time value.

Table 3. Effect of NDEA (40 mg/kg body weight) on the urinary level of ^{14}C -AA.

Animal number	Weight (g)	Total ^{14}C -AA administered (DPM)	^{14}C -AA (% of administered dose) in urine		
			0-24 h	25-48 h	49-72 h (24 h after NDEA treatment)
1	300	692,112	0.53	0.10	0.15
2	280	645,971	0.51	0.06	0.19
3	300	692,112	0.48	0.11	0.16
4	400	922,816	0.96	0.06	0.14
5	340	784,393	0.57	0.11	0.19
$\bar{X} \pm \text{SD}$			0.61 ± 0.19	0.09 ± 0.03	$0.17 \pm 0.02^*$

* $p < 0.001$ in comparison to 25-48 h value.

from 30 ± 4.29 in the controls to $27.1 \pm 4.9\%$ in the NDEA-treated animals.

Distribution of radioactivity in the tissues of control and NDEA-pretreated guinea pigs 200 min after the oral administration of ^{14}C -AA is shown in Table 2. There was an overall increase in the tissue retention of radioactive ascorbic acid in the tissues except in the adrenal glands of the animals pretreated with NDEA.

Figure 2 depicts the effect of NDEA administration on $^{14}\text{CO}_2$ exhalation after the equilibration of ^{14}C -AA in the body for 24 h. There was a significant increase ($p < 0.05$) in the $^{14}\text{CO}_2$ exhalation up to 1 h of NDEA administration, and thereafter a decrease in $^{14}\text{CO}_2$ was observed. The rate of formation of $^{14}\text{CO}_2$ after 24 h of NDEA treatment was less than the rate immediately before the carcinogen administration.

Data on urinary excretion of radioactive ascorbic acid are given in Table 3. In control animals $0.61 \pm 0.19\%$ of the administered dose was excreted during the first 24 h, whereas during the next 24 h $0.09 \pm 0.03\%$ of the dose was excreted. Thus, under normal conditions, the urinary level of radioactivity in the first 24 h was almost 7-fold the level of radioactive ascorbic acid excreted during the 25-48 h period. NDEA treatment after 48 h of ^{14}C -AA administration significantly elevated the urinary radioactive levels from $0.09 \pm 0.03\%$ in 25-48 h to $0.17 \pm 0.02\%$ ($p < 0.001$) of the administered dose during 49-72 h.

DISCUSSION

We found that intraperitoneal administration of NDEA, a cancer-causing agent, for three consecutive days significantly decreased ascorbic acid levels in various organs and plasma. We considered it due to multiple reasons such as impaired absorption of ascorbic acid from the gastrointestinal tract and faster degradation and/or elimination of ascorbic acid from the body. To test these speculations we evaluated the problem in steps.

It is well known that the conversion of ascorbic acid into carbon dioxide is the main pathway of its metabolism in guinea pigs [14]. About 60-70% of the vitamin is believed to be metabolized to CO_2 and expired. The monitoring of

exhaled CO_2 , therefore, provides a good way by which the metabolism of ascorbic acid and its interactions with other agents can be studied in guinea pigs. Using radiolabelled ascorbic acid we observed two peaks of exhaled $^{14}\text{CO}_2$ at 18 ± 4.5 and 76 ± 11.0 min, after an oral administration of $^{14}\text{C-AA}$ to the control animals. The first peak possibly demonstrates a very rapid absorption of the vitamin from the gastrointestinal tract and its accumulation in the liver through the portal system where it starts getting metabolized. The subsequent appearance of the second peak reflects that $^{14}\text{C-AA}$ achieved the state of dynamic unequilibrium in the body only after this time. The disappearance of the first peak in the NDEA-pretreated guinea pigs was possibly due to slower absorption of the tracer from the gastrointestinal tract, which might be responsible for the nonsignificant decrease in the amount of $^{14}\text{CO}_2$ exhaled in 200 min. Another reason for the decreased cumulative $^{14}\text{CO}_2$ value seems to be the greater retention of the tracer in the body of the animals that had been depleted of ascorbate by the pretreatment with the carcinogen. Thus, it seems that the treatment with NDEA did not affect the overall absorption of ascorbic acid from the gastrointestinal tract. Therefore, we believe that an increased rate of catabolism of ascorbic acid might account for the decreased tissue and plasma levels of the vitamin. This was confirmed by our experiment showing that administration of NDEA, after the equilibration of the radioactive tracer in the body pool of ascorbic acid had been achieved, significantly increased the exhalation of $^{14}\text{CO}_2$ up to 1 h after the carcinogen administration. Subsequent decrease in the rate of $^{14}\text{CO}_2$ exhalation thereafter indicates the constant decrease in the body pool of ascorbate due to faster metabolic degradation and urinary excretion of ascorbic acid and its metabolites. However, we still do not know the enzymatic/nonenzymatic parameters which are stimulated by NDEA to increase the metabolic degradation of ascorbic acid.

In conclusion, the chemical carcinogen NDEA depletes the body ascorbate store. The possible mechanism may be the enhanced metabolic degradation of ascorbic acid into CO_2 , and other metabolites that are excreted from the body through the urine.

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