Effect of Ascorbic Acid on Antioxidant Defense Systems and Lipid Peroxidation in Guinea Pig

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(Received July 30, 1988)

Summary In view of the antioxidant properties of ascorbic acid (AA), effects of inadequate and excessive doses of AA on hepatic and pulmonary antioxidant enzymes and NADPH-dependent lipid peroxidation were investigated in the present study. Male guinea pigs, dosed daily with 0.2 mg AA/100 g b wt (inadequate) or 50 mg AA/100 g b wt (excessive) for 8 weeks, demonstrated no difference in body growth, liver and lung weights, and post-10,000×g supernatant protein contents as compared with the control group, which was daily fed with 2 mg AA/100 g b wt. Inadequacy of AA decreased the hepatic and pulmonary contents of catalase, glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD), but it significantly increased glutathione reductase (GR) activity ($p < 0.005$) in lung. However, levels of hepatic and pulmonary NADPH-dependent lipid peroxidation remained unaltered when the supply of AA was inadequate. Excessive doses of AA did not influence any pulmonary antioxidant enzyme, level of NADPH-dependent lipid peroxidation and content of reduced glutathione (GSH), but increased the hepatic GSH-Px and GR activities. Hepatic SOD activity showed a significant decrease ($p < 0.01$), whereas NADPH-dependent lipid peroxidation and GSH contents remained unchanged. It appears that the changes in antioxidant enzymes may be a nonspecific response to AA or these changes may not be sufficient to bring about any shift in the levels of NADPH-dependent lipid peroxidation in the presence of unaltered GSH contents or other biomolecules which may act as antioxidants or free radical scavengers in the cell system.

Key Words: ascorbic acid, antioxidant enzymes, lipid peroxidation, guinea pig

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During the course of aerobic metabolic reactions a considerable amount of reactive oxygen species such as superoxide anions (O_2^-) and hydrogen peroxide (H_2O_2) are generated [1-3]. H_2O_2 and O_2^- further undergo a variety of chain reactions and produce free radicals such as $OH^\cdot$ and $HO_2^\cdot$. These radicals are capable of abstracting hydrogen atoms from polyunsaturated fatty acid and thereby initiate the process of lipid peroxidation [4, 5], which results in oxidative degradation and inactivation of various important biomolecules. A normal cell may control or prevent such adverse reactions with the help of nonenzymatic and enzymatic antioxidant defense systems. These include vitamin E, which is capable of scavenging active oxygen species, such as, peroxy radicals, superoxide anions and singlet oxygen. Superoxide dismutase (SOD) is the principal protective enzyme which dismutates O_2^- to H_2O_2 and O_2 [6]. Hydrogen peroxide is detoxified by catalase and glutathione peroxidase (GSH-Px) [7, 8]. GSH-Px is further capable of reacting with lipid peroxides to control the process of peroxidation [9].

It has been suggested that ascorbic acid (AA) can also play the role of an antioxidant [10]; and a recent proposal has been made that ascorbate scavenges $OH^\cdot$, resulting in the formation of the ascorbate free radical (AFR), which is further converted into dehydroascorbic acid and ultimately to diketogulonic acid [11]. However the antioxidant properties of AA have not been clearly established experimentally in a well-controlled *in vivo* system. As a reducing compound, AA maintains sulphhydryl compounds including glutathione in a reduced state and participates in many redox reaction. It has also been shown that AA has a synergistic effect on the antioxidant functions of vitamin E and selenium [12, 13]. In this study we present evidence that a large intake of AA failed to inhibit NADPH-dependent lipid peroxidation in guinea pigs. The effect of oral intake of different amounts of AA on antioxidant enzymes is also presented.

MATERIALS AND METHODS

Male weanling guinea pigs (250-300 g) of the institute colony were used in the experiments. All the animals were maintained on an AA-free stock diet plus water *ad libitum*. Animals were divided into three groups following a period of equilibration in chambers bedded with rice husk. AA dissolved in 1 ml of freshly prepared 10% sucrose was administered orally by a blunt tip syringe. The control group received 2 mg AA/100 g b wt/day, the inadequate AA group, 0.2 mg AA/100 g b wt/day, and the excessive AA group, 50 mg AA/100 g b wt/day. This treatment with AA was continued for 8 weeks. After this period, animals were sacrificed by exsanguination under light ether anaesthesia between 7-8 h. Thoracic and peritoneal cavities of the animals were opened and lung and liver were perfused immediately *in situ* with cold 0.15 M KCl containing 0.05 M phosphate buffer, pH 7.4. Liver and lung were diced with scissors, homogenized with a Potter-Elvehjem homogenizer and diluted to a concentration of 1 g wet weight/4 ml with cold 0.05 M phosphate buffer, pH 7.4. Homogenates were then centrifuged

at $10,000\times g$ for 20 min at 4°C . The post- $10,000\times g$ supernatant was used for the estimation of enzymatic activities of GSH-Px, catalase, SOD, and glutathione reductase (GR). GSH-Px was measured according to the method of Paglia and Valentine [14]. Catalase activity was determined by the method described by Luck [15]. SOD was measured according to Kono [16]. GR activity was measured following the method of Carlberg and Mannervik [17]. Reduced Glutathione (GSH) was estimated in the whole tissue homogenate by the method of Moron *et al.* [18]. NADPH-dependent lipid peroxidation was assayed by the method of Trush *et al.* [19] in lung and by that of Pederson *et al.* [20] in liver. With 20, 40, or 60 nmol of AA in the reaction mixture, lipid peroxidation was also assayed in liver preparations of control animals.

Protein was determined by the method of Lowry *et al.* [21] using bovine serum albumin as standard.

Statistical significance of differences between groups was determined by unpaired Student's *t*-test.

RESULTS

Oral administration of excessive or inadequate doses of AA had no effect on body growth, organ weights, or post- $10,000\times g$ supernatant protein contents. The influence of inadequate doses of AA on pulmonary antioxidant enzymes and on NADPH-dependent lipid peroxidation is depicted in Table 1. The activities of catalase, GSH-Px, and SOD decreased significantly. In contrast, AA deficiency increased GR activity, whereas the concentration of GSH increased non-significantly and levels of NADPH-dependent lipid peroxidation remained unaltered. In the case of liver, administration of inadequate doses of AA had effects similar to those in lung in all respects except for GR (Table 2) whose activity remained unaltered.

Influence of excessive doses of AA on lung antioxidant enzymes and NADPH-dependent lipid peroxidation is shown in Table 3. It was surprising to

Table 1. Effect of inadequate daily intake of ascorbic acid on pulmonary antioxidant defense system and lipid peroxidation in guinea pigs.

Parameter	Control	Inadequate
Catalase	217 ± 21.5	$140 \pm 10.4^{**}$
GSH-Px	53.1 ± 4.91	$38.9 \pm 3.18^{*}$
GR	20.5 ± 1.36	$40.1 \pm 2.61^{***}$
SOD	5.15 ± 0.32	$3.80 \pm 0.48^{*}$
GSH	1.63 ± 0.13	1.82 ± 0.20
Lipid peroxides	42.5 ± 0.63	41.9 ± 1.67

Mean $\pm$ SE ($n=5-6$). Units are expressed as follows: Catalase, I.U./mg protein; GSH-Px and GR, nmol of NADPH consumed/min/mg protein; SOD, units/mg protein; GSH, $\mu\text{mol/g}$ tissue; lipid peroxides, terms of nmol of malondialdehyde/g tissue/60 min. *p* values:

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$.

Table 2. Effect of inadequate daily intake of ascorbic acid on hepatic antioxidant defense system and lipid peroxidation in guinea pigs.

Parameter*	Control	Inadequate
Catalase	2,168 ± 135	1,366 ± 112***
GSH-Px	264 ± 6.70	218 ± 20.5*
GR	42.6 ± 1.16	35.8 ± 4.20
SOD	5.25 ± 0.55	3.27 ± 0.31**
GSH	9.18 ± 0.97	8.80 ± 0.94
Lipid peroxides	226 ± 21.0	214 ± 25.5

Mean ± SE (*n* = 5–6).

Units and significance are the same in Table 1, except for lipid peroxide level (in terms of nmol of malondialdehyde/g tissue/10 min).

Table 3. Effect of excessive daily intake of ascorbic acid on pulmonary antioxidant defense system and lipid peroxidation in guinea pigs.

Parameter*	Control	Excessive
Catalase	204 ± 15.8	200 ± 21.4
GSH-Px	52.5 ± 4.87	55.3 ± 3.90
GR	21.2 ± 2.37	23.6 ± 2.38
SOD	5.24 ± 0.53	5.02 ± 0.19
GSH	1.59 ± 0.13	1.60 ± 0.19
Lipid peroxides	41.6 ± 3.10	41.3 ± 3.21

Mean ± SE (*n* = 5–6).

Units and significance are the same as in Table 1.

Table 4. Effect of excessive daily intake of ascorbic acid on hepatic antioxidant defense system and lipid peroxidation in guinea pigs.

Parameter*	Control	Excessive
Catalase	2,168 ± 201	2,423 ± 174
GSH-Px	263 ± 10.0	358 ± 7.45***
GR	41.6 ± 2.68	55.9 ± 6.87*
SOD	4.95 ± 0.27	3.49 ± 0.36**
GSH	9.79 ± 0.42	9.42 ± 0.17
Lipid peroxides	238 ± 14.8	223 ± 12.8

Mean ± SE (*n* = 5–6).

Units and significance are the same as in Table 1, except for lipid peroxide level (in terms of nmol of malondialdehyde/g tissue/10 min).

find that AA was unable to induce any significant change in the NADPH-dependent lipid peroxidation or antioxidant enzymes. As shown in Table 4, in liver of excessive AA fed guinea pigs, activities of GSH-Px and GR significantly increased. On the contrary, SOD activity decreased ($p < 0.01$). All these changes, however, could not induce any change in the basal level of lipid peroxidation. Addition of AA at a level of 20, 40, or 60 nmol in the reaction mixture (1 ml) increased non-enzymatic lipid peroxidation, but NADPH-dependent lipid peroxidation still remained unaltered (data not included).

DISCUSSION

SOD is known to be one of the key enzymatic defense factors in the lung against a variety of toxic agents. Catalase and GSH-Px are also important, as they are responsible for the detoxification of peroxides. Our finding of decreased pulmonary and hepatic activities of SOD, GSH-Px, and catalase due to dietary deficiency of AA might have led to an increased formation of lipid peroxidation products because of impairment of the pathway of conversion of O_2^- to $O_2 + H_2O_2$ and H_2O_2 to $H_2O + O_2$. In turn, H_2O_2 and O_2^- can undergo a variety of chain reactions to produce $HO_2^{\cdot}$ and $OH^{\cdot}$ radicals which initiate the process of lipid peroxidation. However, we could not detect any significant increase in the levels of NADPH-dependent lipid peroxidation in lung and liver in spite of impaired activities of the above mentioned enzyme. The nonsignificant ($p > 0.05$) increase in pulmonary GSH content in guinea pigs fed an inadequate amount of AA may be attributed to elevated ($p < 0.005$) activity of GR, causing a changed redox state of GSH and GSSG.

Excessive intake of AA was also found to have no effect on NADPH-dependent lipid peroxidation in lung and liver. Pulmonary antioxidant enzymes were not altered when the animals consumed excessive amounts of AA. However, in liver, the activity of SOD decreased and GSH-Px and GR activities increased in animals given excessive doses of AA. Addition of 20, 40, or 60 nmol of AA to the reaction mixture of lipid peroxidation, which also did not result in any change in NADPH-dependent lipid peroxidation in liver coincides with our findings of no change in NADPH-dependent lipid peroxidation in liver of excessive AA-fed animals. Wartanowicz *et al.* [22] have also reported a nonsignificant decrease (8%) in lipid peroxide levels in serum of elderly subjects after supplementation of 400 mg AA/day for 4 months.

The content of GSH, the principal non-protein sulfhydryl compound in tissues [23] as well as a cofactor for multiple glutathione-S-transferases and GSH-Px, was not altered in lung or liver due to inadequate or excessive intake of the vitamin. GSH is necessary for the stability of sulfhydryl containing enzymes and protects hemoglobin and many other cofactors from oxidation. The depletion of hepatic glutathione *in vivo* by phorone, vinylidene chloride, or diethyl maleate has been shown to enhance lipid peroxidation *in vitro* [24, 25], without affecting the GSH-Px activity. Thus GSH depletion appears to be one of the mechanisms which may cause peroxidative damage to the cellular membrane. From our results it looks as if the unchanged level of GSH was sufficient to maintain control over the levels of NADPH-dependent lipid peroxidation in spite of decreased activities of antioxidant enzymes in AA deficiency. It is also likely that a number of other enzymatic/nonenzymatic factors present in the cell may function as free radical scavengers or antioxidants under certain conditions. Therefore, under normal circumstances the metabolic machinery of the cell is capable of reversing any

adverse effects of continuous oxidative stress in the cellular environment. Under conditions when the capacity of the cellular reducing power to reverse oxidative damage is decreased or when the oxidative stress is increased above the threshold, an irreversible injury to the cell may occur.

In conclusion, excessive intake or deficiency of AA did not cause any change in NADPH-dependent lipid peroxidation. The changes in antioxidant enzymes appear to be a nonspecific response or may not be sufficient to show a relationship between the enzymes and NADPH-dependent lipid peroxidation.

This work was financed by a research grant (IRIS ID NO. 8305240) from Indian Council of Medical Research, New Delhi, India.

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