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The potentiating and protective effects of ascorbate on oxidative stress depend upon the concentration of dietary iron fed C3H mice

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Abstract

Ascorbic acid (AA) is an antioxidant that, in the presence of iron and hydrogen peroxide, increases the production of hydroxyl radicals in vitro. Whether AA has similar pro-oxidant properties in vivo may depend upon the relative balance of iron and AA concentrations. In this study, C3H mice were fed diets supplemented with 100 or 300 mg/kg iron, with or without AA (15 g/kg), for 12 months. Liver AA concentrations were greater in mice fed AA-supplemented diets with either low or high iron ($P=.0001$), while the high-iron diet was associated with a significantly lower liver AA concentration regardless of AA supplementation ($P=.0001$). Only mice fed the high-iron diet with AA had a significantly greater liver iron concentration ($P=.05$). In the high-iron group, AA reduced oxidative stress, as measured by greater activities of glutathione peroxidase, superoxide dismutase (SOD) and catalase and by significantly lower concentrations of 4-hydroxylalkenal (HAE) and malondialdehyde (MDA). In mice fed the low-iron diet, AA was associated with greater concentrations of HAE and MDA and with lower activities of SOD. However, AA did not increase the concentrations of modified DNA bases with the low-iron diet but was associated with significantly lower concentrations of modified DNA bases in mice fed the high-iron diet. In conclusion, dietary AA appears to have mild pro-oxidant properties at low-iron concentrations but has a strong antioxidant effect against oxidative stress and DNA damage induced by dietary iron in mouse liver.

Keywords: Iron; Ascorbic acid; DNA damage; Lipid peroxidation

1. Introduction

Iron is an essential nutrient for normal cellular functions. Its capacity to accept and donate electrons allows it to function in cytochromes, oxygen-binding molecules and many enzymes. However, redox cycling of iron is closely associated with the production of reactive oxygen species (ROS). Free iron, if it exists in vivo, can therefore be quite harmful, particularly in the presence of hydrogen peroxide, with the production of hydroxyl radicals. Iron-induced oxidative stress has one of two consequences: (a) redox regulation failure, which leads to lipid peroxidation and oxidative DNA and protein damage, or (b) redox regulation, which modifies a variety of protective mechanisms against ROS.

Ascorbic acid (AA), a potent antioxidant, decreases the adverse effects of reactive oxygen and nitrogen species that cause oxidative damage to macromolecules such as lipids, proteins and DNA [1]. However, AA enhances the availability and absorption of iron, potentially leading to greater body iron stores. Furthermore, in vitro AA can maintain iron and other transition metals in a reduced state and result in the production of hydroxyl radicals and lipid alkoxyl radicals [2,3]. Whether AA supplementation has a pro-oxidant effect in vivo continues to be debated [4,5]. Previous studies from our laboratory have shown that, in C3H mice, excessive AA supplementation does not significantly increase liver lipid peroxidation or other markers of oxidative stress induced by high dietary iron over a 3-week period [6].

In the present study, we hypothesized that AA has a net antioxidant effect when present in sufficient excess so that concentrations are not limiting. Furthermore, we

Abbreviations: AA, ascorbic acid; MDA, malondialdehyde; HAE, 4-hydroxylalkenal; GPx, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase.

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hypothesized that, at these concentrations, AA is protective against the long-term effects of oxidative damage induced by dietary iron. We investigated this hypothesis by studying the effects of iron and AA on hepatic oxidative stress and DNA damage in C3H mice using a two-by-two factorial design varying dietary iron with or without AA supplementation for a period of 12 months.

2. Materials and methods

2.1. Chemicals and reagents

Reagents for measuring malondialdehyde (MDA) and 4-hydroxylalkenal (HAE), glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) were purchased from Calbiochem (San Diego, CA, USA). All other reagents were of analytic grade and were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Animals

Weanling male C3H/HeJ mice were obtained from The Jackson Laboratory (Bar Harbor, ME, USA) and housed in plastic cages with a 12-h light/dark cycle and with free access to standard laboratory diet (Lab Diet 5K52; Nutrition International) and water ad libitum. They were acclimatized for 1 week before use.

2.3. Study protocol

At 4 weeks of age, mice ($n=33$) were randomly assigned in a two-by-two study design to receive one of four diets supplemented with iron: 100- or 300-mg Fe/kg diet, with or without 15 g AA/kg diet (Table 1). In separate experiments, we have observed that consumption of these diets does not differ significantly (data not shown). Food and demineralized water were supplied ad libitum for 12 months. Each experimental group consisted of a minimum of seven mice.

2.4. Tissue collection and sample preparation

The mice were killed after the experimental period. Their livers were excised, cut into fragments, rinsed with ice-cold phosphate-buffered saline, blotted and processed immediately for DNA isolation and AA estimation. The remaining liver fragments were stored at −80°C. Body and liver weights were recorded. All experiments were performed in compliance with federal veterinary guidelines and were approved by the Animal Use and Care Administrative Advisory Committee at the University of California Davis (Sacramento, CA, USA).

2.5. Measurement of hepatic lipid peroxidation

Total MDA and HAE were assayed by their reaction with 10.3 mM *N*-methyl-2-phenylindole in acetonitrile and methanesulfonic acid, and absorbance was measured at 586 nm [7].

2.6. Estimation of hepatic antioxidant enzyme activities

A 10% (wt/vol) liver homogenate was prepared using 0.1 M Tris–HCl buffer (pH 7.4). GPx activity was determined by a modification of the glutathione-reductase-coupled assay of Paglia and Valentine [8] using *tert*-butyl hydroperoxide and 1 mM glutathione. GPx units were expressed as micromoles of nicotinamide adenine dinucleotide phosphate (NADPH) oxidized per minute at pH 7.5 and 25°C. Three hundred fifty microliters of assay buffer (50 mM Tris–HCl, pH 7.6, 5 mM EDTA) was added to 350 μl of the test mix (3 mM glutathione, 0.6 mM NADPH and 1.2 U/ml glutathione reductase) and 70 μl of homogenate. The mixture was preincubated at 37°C for 10 min. The reaction was started by the addition of 350 μl of *tert*-butyl hydroperoxide (70% aqueous solution), and NADPH consumption rate was measured spectrophotometrically at 340 nm.

SOD activity was measured by the auto-oxidation of 5,6,6*a*,11*b*-tetrahydro-3,9,10-trihydroxybenzo[*c*]flurone in

Table 1
Composition of the experimental diets fed C3H mice for 12 months

	0 g/kg AA		15 g/kg AA	
	100 mg/kg iron	300 mg/kg iron	100 mg/kg iron	300 mg/kg iron
Ingredient (g/kg diet)				
Casein	200	200	200	200
dl-methionine	3	3	3	3
Sucrose	549.5	548.5	534.5	533.5
Corn starch	150	150	150	150
Corn oil	50	50	50	50
Mineral mixture[a]	35	35	35	35
Vitamin mixture[b]	10	10	10	10
Choline bitartrate	2	2	2	2
Ferrous sulfate ($FeSO_4 \cdot 7H_2O$)	0.5	1.5	0.5	1.5
AA	0	0	15	15

[a] Minerals supplemented (per kilogram of diet): calcium phosphate, dibasic, 17.5 g; sodium chloride, 2.6 g; potassium citrate, monohydrate, 7.7 g; potassium sulfate, 1.8 g; magnesium oxide, 0.8 g; manganese carbonate, 0.12 g; zinc carbonate, 0.06 g; cupric carbonate, 0.01 g; potassium iodate, 0.35 mg; sodium selenite, 0.35 mg; chromium potassium sulfate, 0.02 g.

[b] Vitamins supplemented (per kilogram of diet): thiamin HCl, 6 mg; riboflavin, 6 mg; pyridoxine HCl, 7 mg; niacin, 0.03 g; calcium pantothenate, 0.16 g; folic acid, 2 mg; biotin, 0.2 mg; vitamin B12, 0.1 g; vitamin A palmitate, 4000 U; vitamin E, 50 U; vitamin D3, 1000 U; menadione sodium bisulfite, 1.5 mg.

the presence and absence of tissue homogenates at 525 nm [9]. Forty microliters of the sample and 30 μl of 33.3 mM 1-methyl-2-vinylpyridinium trifluoromethane sulfonate in 1 mM HCl were added to 900 μl of 50 mM 2-amino-2-methyl-1,3-propanediol, 3.3 mM boric acid and 0.11 mM diethylenetriaminepentaacetic acid (DTPA; pH 8.8), and incubated for 1 min at 37°C. Thirty microliters of 0.66 mM 5,6,6*a*,11*b*-tetrahydro-3,9,10-trihydroxy-benzo[*c*]fluorine, 0.5 mM DTPA in 32 mM HCl and 2.5% ethanol were added and immediately read at 525 nm.

CAT activity was assayed by adding 30 μl of homogenates to 500 μl of 10 mM H_2O_2 and by incubating at 37°C for 1 min. The reaction stopped by the addition of 500 μl of sodium azide, and the remaining H_2O_2 was measured by the methods described by Fossati et al. [10]. Activities were normalized to sample protein content and expressed in units per milligram of protein.

2.7. AA measurement

AA was measured by the method of Omaye et al. [11]. To 500 μl of homogenates, 500 μl of water and 1 ml of 5% trichloroacetic acid were added, mixed thoroughly and centrifuged. To 1 ml of the supernatant, 200 μl of DTC (dinitrophenyl hydrazine, thiourea, copper sulphate) reagent [3% (wt/vol) 2,4-dinitrophenyl hydrazine, 0.4% (wt/vol) thiourea and 0.05% (wt/vol) copper sulphate in 9 N sulfuric acid] was added and incubated at 37°C for 3 h. Sulfuric acid (1.5 ml) was added, mixed well, incubated at room temperature for 30 min and read at 520 nm. AA was corrected for protein concentration and expressed as micrograms per milligram of protein.

2.8. Tissue iron analysis

The livers were digested in 16 M HNO_3 for 7 days and analyzed for iron by atomic absorption spectrophotometry, as previously described [6].

2.9. Tissue selenium analysis

Hepatic selenium was measured by high-performance liquid chromatography determination of the fluorophore formed by the reaction of Se(IV) with 2,3-diaminonaphthalene, as described by Hawkes and Kutnink [12].

2.10. Measurement of modified DNA bases

nDNA was isolated from mouse livers by NaI, as previously described [13]. Formation of modified DNA adducts was measured by gas chromatography–mass spectroscopy (GC-MS) [14–16]. Following the isolation of DNA, it was hydrolyzed with formic acid (60%, 0.5 ml) at 140°C for 40 min and then lyophilized to remove formic acid. Free bases were derivatized with BSTFA:acetonitrile (4:1; Pierce Chemical Co.) at 90°C for 1 h. Derivatized samples were analyzed by GC-MS with selected ion monitoring (Hewlett Packard 6890 GC/5972 MSD with ChemStation software). For the analysis, an HP-5MS capillary column (15 m×0.25 mm×0.25 μm; Agilent Inc., Little Falls, DE, USA) was used with helium carrier gas at a flow rate of 0.8 ml/min. The injection port and mass selective detector interface temperatures were 250°C and 280°C, respectively. The injector was operated in split mode with a split ratio of 10:1. The oven temperature was programmed from 80°C to 200°C at 7°C/min and at 12°C/min to 270°C after holding for 1 min at 80°C. Pure reference compounds for modified bases and internal standards (dihydrothymine, 5-hydroxyuracil, 5-hydroxymethyluracil, hypoxanthine, fapyadenine, 8-hydroxyguanine, 6-azathymine and 2,6-diaminopurine) were obtained commercially (Cambridge Isotopes, Sigma/Aldrich and ICN) and used to prepare standard curves. Major fragmentations of dihydrothymine [mass/charge ratio (*m*/*z*)=257, 271], 5-hydroxyuracil (*m*/*z*=329, 330, 344), 5-hydroxymethyluracil (*m*/*z*=358, 343, 359), hypoxanthine(*m*/*z*=265, 280, 266), fapyadenine (*m*/*z*=354, 369), 8-hydroxyguanine (*m*/*z*=440, 455), 2,6-diaminopurine (*m*/*z*=351, 366) and 6-azathymine (*m*/*z*=256, 271) were monitored in selective ion monitoring mode. Quantitation was based on the measured peak area of each base relative to the peak area of the internal standard (either 6-azathymine or 2,6-diaminopurine, depending on the elution time of the base). A standard curve for each base was prepared with seven data points in the concentration range of 0.1–140 nM.

2.11. Statistical analysis

Treatment effects were analyzed by two-way ANOVA using GraphPad Prism version 4.0 (GraphPad Software, San

Table 2
Body weight, liver weight and liver iron concentrations of mice fed diets with two levels of iron (100 or 300 mg/kg), with or without AA supplementation[a,b,c]

	0 g AA/kg diet		15 g AA/kg diet		ANOVA (*P*)		
	100 mg/kg iron (*n*=9)	300 mg/kg iron (*n*=9)	100 mg/kg iron (*n*=8)	300 mg/kg iron (*n*=7)	Fe	AA	Fe×AA
Body weight g	31.0±1.5[a]	29.4±2.6[a]	28.5±1.7[a]	29.3±2.3[a]	NS	NS	NS
Liver weight (g)	1.5±0.2[a]	1.5±0.3	1.4±0.1[a]	1.4±0.1[a]	NS	NS	NS
Iron concentration (nmol/g wet weight)	3295±977[a]	4576±1401[a]	4503±1354[a]	5041±1303[b]	<.05	NS	NS

[a] Results are presented as mean±S.D.
[b] Means in a row without a common letter differ significantly (*P*<.05).
[c] NS, nonsignificant (*P*≥.05).

Table 3
Concentrations of AA, selenium, MDA and antioxidant enzymes in the livers of mice fed diets with two levels of iron (100 or 300 mg/kg), with or without AA supplementation[a,b,c]

	0 g AA/kg diet		15 g AA/kg diet		ANOVA (*P*)		
	100 mg/kg iron (*n*=9)	300 mg/kg iron (*n*=9)	100 mg/kg iron (*n*=8)	300 mg/kg iron (*n*=7)	Fe	AA	Fe×AA
AA (μg/mg protein)	3.2±0.2[a]	2.2±0.3[b]	4.0±0.2[c]	2.6±0.4[d]	<.0001	<.0001	NS
Selenium (μg/g wet weight)	1.1±0.1[a]	1.1±0.1[a]	1.2±0.1[a]	1.1±0.2[a]	NS	NS	NS
MDA and HAE (nmol/mg protein)	10.7±0.7[a]	15.0±0.8[b]	12.9±0.8[c]	11.0±1.3[a]	<.001	<.01	<.0001
GPx (U/g protein)	496±65[a]	357±45[b]	432±29[a]	503±48[a]	NS	<.05	<.0001
SOD (U/mg protein)	28.3±6.0[a,b]	21.5±2.9[a,c]	22.6±1.1[c]	29.9±5.2[b]	NS	NS	<.0001
CAT (U/mg protein)	21.7±1.9[a]	13.5±2.6[b]	17.8±3.7[a]	18.3±4.3[a]	<.005	NS	<.005

[a] Results are presented as mean±S.D.
[b] Means in a row without a common letter differ significantly (*P*<.05).
[c] NS, nonsignificant (*P*≥.05).

Diego, CA, USA). When the *P* value obtained from ANOVA was significant, the Tukey test was applied to test for differences among groups. Differences were considered significant if *P*<.05. Values in the text are presented as mean±S.D.

3. Results

Body and liver weights did not differ significantly among the groups (Table 2). Liver iron concentration was significantly greater in the high-iron AA-supplemented group than in other groups (*P*<.05). ANOVA results show that iron, but not AA, had a significant effect (*P*<.05) on hepatic iron concentrations and that there was no interaction between iron and AA (Table 2).

Dietary iron and AA had significant (*P*<.0001) and opposing effects on tissue AA levels. Tissue AA levels differed significantly in all groups and increased in the following order: high iron, high iron with AA, low iron, low iron with AA (*P*<.05; Table 3). Thus, unlike AA, which did not affect tissue iron, high dietary iron significantly decreased tissue AA. No significant effect of either iron or AA on liver selenium was found (Table 3).

We measured the levels of MDA and HAE as index of lipid peroxidation. Both dietary iron (*P*<.001) and AA (*P*<.05) had significant effects, and there was a highly significant interaction between iron and AA (*P*<.0001). MDA and HAE concentrations were greatest in the high-iron group and were significantly lower when AA supplementation was added to the high-iron diet. Interestingly, AA supplementation in the

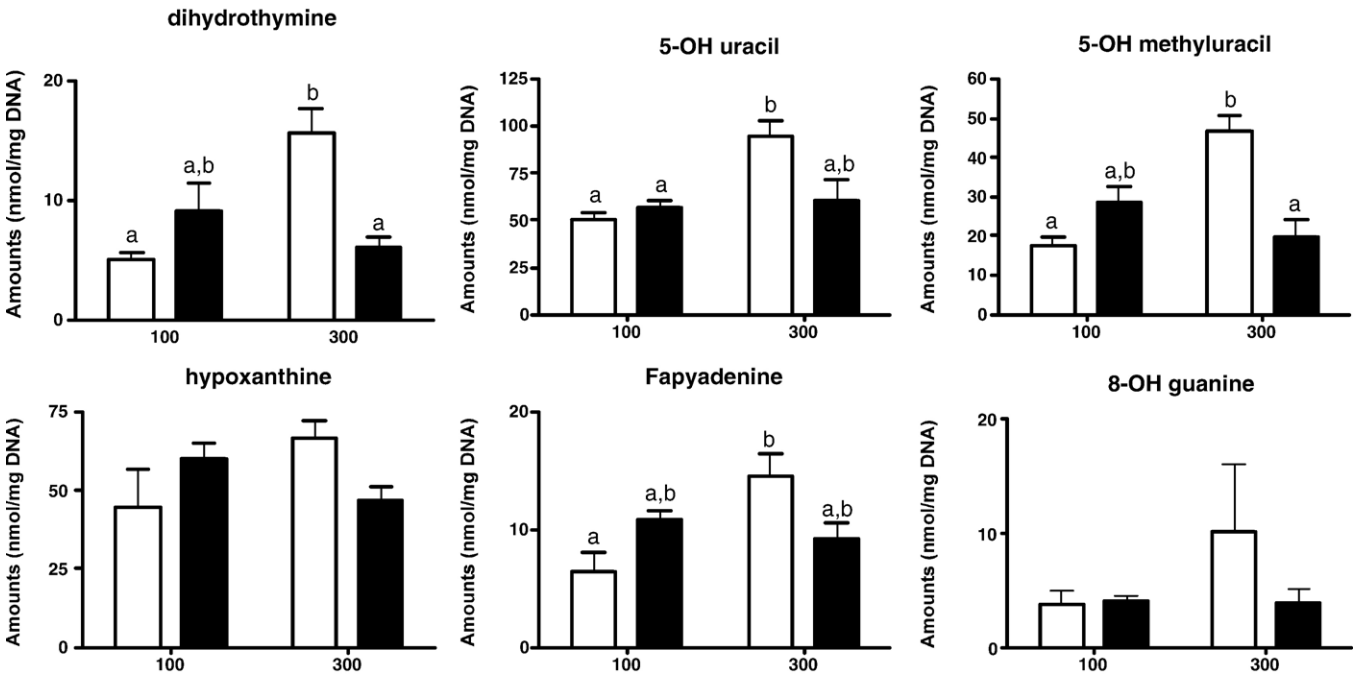

Fig. 1. AA reduces the concentration of iron-induced DNA base modifications. Mice were fed diets containing 100 or 300 mg Fe/kg diet, without AA (open bars) or with 15 mg AA/kg diet (closed bars). Modified DNA bases extracted from the liver were measured by GC-MS. Bars represent means and S.D. Bars not sharing a letter are significantly different (*P*<.05) [*n*=4 for each group; except for the 300-mg Fe/kg group without AA (*n*=3)].

low-iron group was associated with greater MDA and HAE than the low-iron group without AA (Table 3).

Because antioxidant enzymes comprise an important defense against oxidative stress, we measured GPx, SOD and CAT activities. Although no significant effects on liver selenium levels were detected, altered activity of the selenium-dependent enzyme GPx was found. Specifically, AA was found to affect GPx activity, and there was a highly significant interaction between iron and AA. As previously observed [6], the high-iron diet was associated with significantly lower GPx activity, whereas addition of AA to the high-iron diet was associated with GPx activities similar to the those in the low-iron diet.

Liver SOD activity was significantly different between several groups, and ANOVA suggested an interaction between iron and AA. At a low dietary iron concentration, AA supplementation lowered SOD activity, while at a high dietary iron concentration, AA increased SOD activity. In contrast, CAT activity at the low-iron concentration was not significantly affected by AA. However, CAT activity was significantly lower in the high-iron group compared to the low-iron group, and AA supplementation restored CAT activity in the high-iron group.

Oxidative damage to DNA by iron can cause multiple modifications of DNA bases [17,18]. We measured dihydrothymine, 5-OH uracil, 5-OH methyluracil, hypoxanthine, fapyadenine and 8-OH guanine as indices of DNA damage (Fig. 1). The concentrations of dihydrothymine, 5-OH uracil, 5-OH methyluracil and fapyadenine were significantly greater in mice fed the high-iron diet than in mice fed the low-iron diet. Concentrations of hypoxanthine and 8-OH guanine tended to be greater in the high-iron group but did not reach statistical significance. In contrast, the concentration of modified bases was not significantly different in mice fed the high-iron diet with AA supplementation.

4. Discussion

AA is a critical antioxidant that acts as a free radical scavenger and may regenerate other antioxidants, including vitamin E [19]. However, the reducing capacity of AA can potentially lead to redox cycling of transition metals, which in turn can generate hydroxyl radicals in the presence of hydrogen peroxide through Fenton chemistry [20]. The addition of AA to iron in the form of $FeSO_4$ or ferric nitrilotriacetate (Fe–NTA) greatly increases oxidative damage in vitro. Markers of lipid peroxidation, protein modification and DNA damage are all enhanced by AA. In addition, AA has been suggested to directly produce genotoxic lipid hydroperoxides in the absence of metals.

These findings have led to recommendations that AA be limited in individuals with clinical iron overload, despite evidence that these individuals are relatively AA-deficient [21]. However, unlike in vitro systems, iron in vivo is largely bound by transferrin and ferritin, and is not freely available for redox reactions. In human plasma, neither endogenous nor exogenous AA promotes the lipid peroxidation or protein oxidation induced by the addition of ferrous ammonium sulfate [22]. In addition, in plasma with elevated levels of nontransferrin-bound iron, AA is protective against lipid peroxidation [23]. Although supplementation studies with AA alone have shown a significant increase in the concentration of 8-OH adenine [4], trials of AA and iron cosupplementation have failed to reveal significant changes in the level of oxidized DNA bases in peripheral blood cells [24,25].

Limitations in human studies as to the use of peripheral blood have prompted studies in animals. Collis et al. [26] found that, in guinea pigs, which are dependent upon dietary AA, orally administered iron supplementation resulted in greater MDA during auto-oxidation of liver microsomes and that AA cosupplementation was protective against this effect. Chen et al. [27] also compared lipid peroxidation in the liver of guinea pigs fed high or low doses of AA, with or without iron loading, by intraperitoneal injection of iron dextran. The animals that received a high AA dose had reduced liver F_2 isoprostanes regardless of iron treatment. However, a low AA dose produced liver AA levels an order of magnitude lower than those of the high AA dose. Therefore, the effects of AA deficiency may have confounded the lipid peroxidation induced by iron loading and any interaction between iron and AA.

Our study aimed at determining whether AA in the setting of AA sufficiency enhances the pro-oxidant properties of iron. Thus, our use of mice, which are not dependent upon dietary AA, is not confounded by the pro-oxidant effects of AA deficiency. Yet, AA supplementation did increase liver concentrations of AA by approximately 20% regardless of dietary iron content. It is also notable that increased dietary iron reduced tissue AA concentrations, suggesting that iron may deplete AA reserves and that dietary requirements of AA may be greater with increasing dietary iron.

In addition, our model demonstrates the propensity of even relatively small changes in iron status to increase biomarkers of oxidative stress. The minimum recommended concentration of iron for mice is 35 mg Fe/kg diet [28], but commercial rodent chows contain 10 times or more this concentration. The doses of iron used in this study are therefore not excessive, yet the high-iron diet is sufficient to increase products of lipid peroxidation (namely, MDA and HAE) and to decrease the activities of SOD, CAT and GPx. Under these conditions, AA was associated with a decrease in MDA and HAE and with increases in the activities of SOD, CAT and GPx in mice fed the high-iron diet, despite a significant increase in liver iron concentration. However, it should also be noted that AA in the low-iron diet slightly but significantly increased lipid peroxidation and decreased SOD activity without significantly changing liver iron concentration.

The effects of iron on DNA include oxidation of bases, adducts derived from lipid peroxidation, and DNA scission

and strand breaks [29]. Previously, we have reported that AA reduces the frequency of micronucleated erythroblasts in the bone marrow of mice similarly treated with iron and AA, suggesting that AA protects from DNA strand breaks induced by iron [30]. In the present study, we measured DNA bases previously shown to be modified by iron. Abalea et al. [17] treated primary rat hepatocytes with Fe–NTA and found a predominant effect on purines rather than on pyrimidines. The greatest increase occurred in fapyadenine, and the least increase occurred in 5-OH-cytosine. In contrast, in mouse hybridoma cells treated with $FeSO_4$, the greatest relative increase in oxidative bases occurred with 8-OH-adenine, 5-OH-cytosine, 5-OH-uracil and 8-OH-guanine [18]. We found that 5-OH methyluracil, dihydrothymine, 5-OH uracil and fapyadenine were most sensitive to iron treatment in vivo.

Podmore et al. [4], in their study of subjects supplemented with 500 mg/day AA, first suggested that AA supplementation could have pro-oxidant effects on normal human volunteers. They reported a decrease in the levels of 8-oxo-guanosine but an increase in the levels of 8-oxo-adenine compared to baseline. We also observed a trend toward an increase in some DNA bases, including dihydrothymine, 5-OH methyluracil and fapyadenine, in mice fed the AA-supplemented low-iron diet.

Human studies of iron and AA cosupplementation have failed to show any pro-oxidant effects of AA on DNA base damage. Rehman et al. [25] compared the cosupplementation of iron with either 60 or 260 mg/day AA. They found that the higher AA dose led to increased thymine glycol and 5-hydroxycytosine, but decreased 8-oxoguanine, 8-oxoadenine, 5-hydroxyuracil and 5-chlorouracil, in peripheral blood cells. The same investigators found that iron and AA cosupplementation did not lead to significantly greater total oxidative DNA damage compared to AA alone or placebo [24].

In contrast, our results suggest that AA is strongly protective against several oxidative modifications of DNA induced by dietary iron supplementation. Several differences in our studies may account for these disparities. First, based upon the weight of diet consumed by mice, the dose of AA on a per-kilogram weight basis is much greater in our experiment (data not shown). Second, the effect of AA may be tissue-specific. Whereas human studies have been limited to studying peripheral blood cells, we have focused on the liver — the major site of iron storage. Third, unlike humans, these mice are not dependent upon dietary AA and, therefore, the changes in tissue AA levels may have been less than those in humans. Future studies in *Gulo*-deficient mice may clarify these questions.

Several possible mechanisms may be involved in the protective effects of AA against DNA damage. First, AA may scavenge free hydroxyl radicals that may react with nuclear DNA. Whether sufficient hydroxyl radicals are produced in the nucleus or can traverse into the nucleus without being scavenged has been questioned. Second, scavenging of free radicals may prevent the oxidation of other biomolecules that can then oxidize DNA bases or form DNA adducts. Third, AA may regenerate other antioxidants that then prevent DNA base oxidation. Finally, AA has been suggested to induce DNA repair mechanisms that excise modified DNA bases.

From the results of the present study, we conclude that long-term supplementation of AA in the setting of dietary iron loading inhibits lipid peroxidation and oxidative modification of DNA. In the absence of excess iron, AA was associated with a small increase in lipid peroxidation and a decreased activity of SOD, suggesting that AA can have mild pro-oxidant properties under certain conditions in vivo. The present observations, together with our previous findings, demonstrate that AA has potent antioxidant properties against oxidative damage induced by dietary iron loading. However, the exact mechanisms of this protection and whether they are operative in other forms of iron overload remain to be elucidated.

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