Iron loading and large doses of intravenous ascorbic acid promote lipid peroxidation in whole serum in guinea pigs

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Large doses of ascorbic acid may mobilise Fe from Fe-binding proteins in vivo which in turn could catalyse lipid peroxidation, a process associated with degenerative diseases. This hypothesis was tested in vitro in the serum of Fe-loaded animals. Eighteen male guinea pigs weighing about 500 g on arrival were allocated to two groups of nine. Fe loading was induced in one group by two intraperitoneal injections of 200 mg iron dextran given on days 1 and 5. Blood (6 ml) was drawn from all animals on day 12 by cardiac puncture. Serum and LDL were separated. Serum was tested for loosely-bound Fe (bleomycin assay) and lipid peroxidation (thiobarbituric acid reactive substances (TBARS) assay) and LDL for susceptibility to in vitro oxidation (TBARS and conjugated diene assays). On day 12, another intraperitoneal injection of 200 mg iron dextran was given to the animals in the Fe-loaded group. On day 19, all animals were given 75 mg ascorbic acid by intraperitoneal injection. Blood (6 ml) was drawn 4 h later by cardiac puncture. Serum and LDL assays were repeated. Ascorbic acid increased loosely-bound Fe and in vitro oxidation in the serum from animals of the Fe-loaded group but not in the serum from animals of the control group. Susceptibility of LDL to in vitro oxidation increased after the ascorbic acid injection in the control group but there was no further increase in the Fe-loaded group. These data suggest that large doses of ascorbic acid promote Fe mobilisation and in vitro oxidation in the serum of Fe-loaded animals.

Iron: Ascorbic acid: Oxidation

Peroxidation of lipids in blood and tissues may be involved in degenerative processes associated with chronic diseases such as atherosclerosis (Steinberg et al. 1989). It is therefore pertinent to study lipid peroxidation in vivo, especially factors that may affect the rate of peroxidation. Although the mechanism of lipid peroxidation in vivo is not fully understood, it has been suggested that free Fe may catalyse its initiation (Kanner et al. 1988; Halliwell & Chirico, 1993). In biological systems, Fe is not free, but bound to biomolecules such as the proteins transferrin, haemoglobin, and ferritin. Mobilisation of Fe from these biomolecules may be required before it can catalyse lipid peroxidation. In vitro experiments suggest that this may occur under certain conditions such as presence of protonating, chelating, or reducing agents including ascorbic acid (Aust, 1987; Gutteridge, 1987). It is uncertain whether Fe mobilisation occurs in vivo, whether ascorbic acid is involved, and whether mobilised Fe is subsequently engaged in pro-oxidant activity. Epidemiological observations, however, associate high-Fe stores with heart disease (Salonen et al. 1992). Moreover, it has been suggested that large doses of ascorbic acid may be detrimental to haemochromatosis patients (Herbert, 1983). It is therefore important to elucidate the role of ascorbic acid under the conditions of Fe overload in order to better understand the behaviour of ascorbic acid toward oxidation.

The objective of this present study was to test the hypothesis that large doses of ascorbic acid may mobilise Fe from biomolecules in vivo thus promoting in vitro lipid peroxidation in serum, particularly in LDL, in Fe-loaded animals.

Materials and methods

Study protocol

The study protocol was approved by the Institutional Animal Care and Use Committee at Cornell University.

Eighteen male guinea pigs (Hartley strain; Camm Research Institute, Wayne, NJ, USA), weighing about

Abbreviations: i.p., intraperitoneal; TBARS, thiobarbituric acid reactive substances.

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were delivered into EDTA-containing tubes (2 mg EDTA/ml) on ice, and 1 ml to clean tubes, on ice, for plasma and serum separation, respectively. While still under anaesthesia, animals from the control group received an i.p. injection of 3 mg Fe as iron dextran to replace Fe withdrawn in the blood. Animals from the Fe-loaded group received another i.p. injection of 200 mg Fe as iron dextran. A week later, on day 19, all animals were deprived of food for 8 h and then injected i.p. with 75 mg ascorbic acid in 1 ml saline. Blood (6 ml) was collected from each animal as on day 12.

**Analytical techniques**

All chemicals were from Sigma Chemical Co. (St Louis, MO, USA) except for thiobarbituric acid, which was from Kodak (Rochester, NY, USA). Iron dextran was from Fisons Corporation (Rochester, NY, USA). Bleomycin (Bleoxane<sup>®</sup>) was a gift of Bristol-Myers Squibb (Princeton, NJ, USA). Analyses in serum were performed on fresh samples. LDL preparation and analyses were completed within 4 d after blood collection on the same time schedule as for blood collected on days 12 and 19.

Serum was obtained by centrifugation of blood collected into clean tubes at 1000 g for 15 min at 4°C. Plasma was obtained by centrifugation of blood treated with EDTA, as described. LDL (d = 1.019–1.063 g/ml) was isolated by sequential ultracentrifugation of plasma. Briefly, 3 ml plasma of density adjusted to 1.019 g/ml with 84 µl NaBr, 1.470 g/ml, was made to 6.05 ml with NaBr, 1.019 g/ml, and centrifuged at 180 000 g for 1 h in a Beckman (Beckman-Coulter Inc, Fullerton, CA, USA) 50.3 rotor. The lower fraction (4 ml) was collected and dialysed in EDTA-containing tubes with fresh PBS. Protein content of LDL fractions, determined with the

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin E (mg/kg)</td>
<td>77.16</td>
</tr>
<tr>
<td>Iron (mg/kg)</td>
<td>235.92</td>
</tr>
<tr>
<td>Ash (g/kg)</td>
<td>68.00</td>
</tr>
<tr>
<td>Fibre (g/kg)</td>
<td>146.00</td>
</tr>
<tr>
<td>Linoleic acid (g/kg)</td>
<td>13.00</td>
</tr>
<tr>
<td>Fat (g/kg)</td>
<td>46.00</td>
</tr>
<tr>
<td>Protein (g/kg)</td>
<td>185.00</td>
</tr>
</tbody>
</table>

500 g on arrival, aged 3–5 months, were housed individually in suspended stainless-steel cages with wire-mesh bottoms in a humidity- and temperature-controlled room with a 12 h light–dark cycle. The animals had free access to distilled water and a commercial guinea-pig diet (Prolab Guinea Pig Formula; Agway Inc., Syracuse, NY, USA), ingredients: dehydrated alfalfa meal, wheat middlings, soyabean meal, ground corn, soyabean hulls, ground wheat, animal fat preserved with ethoxyquin, calcium phosphate, sodium chloride, ascorbic acid, magnesium oxide, iron sulfate, iron carbonate, manganous oxide, zinc oxide, copper oxide, calcium iodate, cobalt carbonate, vitamin A acetate, α-activated animal sterol, vitamin E supplement, choline chloride, niacin, riboflavin, menadione sodium bisulfite complex, pyridoxine hydrochloride, thiamine mononitrate, calcium pantothenate, folic acid, biotin, vitamin B<sub>12</sub> supplement.

### Table 1. Dietary information of the commercial guinea-pig diet that was offered to the animals throughout the study

<table>
<thead>
<tr>
<th>Nutrient</th>
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</tr>
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<tbody>
<tr>
<td>Protein (g/kg)</td>
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<td>235.92</td>
</tr>
<tr>
<td>Copper (mg/kg)</td>
<td>14.00</td>
</tr>
<tr>
<td>Ascorbic acid (mg/kg)</td>
<td>1984.00</td>
</tr>
<tr>
<td>Vitamin E (mg/kg)</td>
<td>77.16</td>
</tr>
</tbody>
</table>

* Prolab, Agway Inc., Syracuse, NY, USA. Ingredients: dehydrated alfalfa meal, wheat middlings, soyabean meal, ground corn, soyabean hulls, ground wheat, animal fat preserved with ethoxyquin, calcium phosphate, sodium chloride, ascorbic acid, magnesium oxide, iron sulfate, iron carbonate, manganous oxide, zinc oxide, copper oxide, calcium iodate, cobalt carbonate, vitamin A acetate, α-activated animal sterol, vitamin E supplement, choline chloride, niacin, riboflavin, menadione sodium bisulfite complex, pyridoxine hydrochloride, thiamine mononitrate, calcium pantothenate, folic acid, biotin, vitamin B<sub>12</sub> supplement.

### Table 2. Experimental design of the study

<table>
<thead>
<tr>
<th>Time-point (day)</th>
<th>Control</th>
<th>Iron-loaded</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Animals were allocated to groups</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>–</td>
<td>200 mg iron dextran were injected i.p.</td>
</tr>
<tr>
<td>5</td>
<td>–</td>
<td>200 mg iron dextran were injected i.p.</td>
</tr>
<tr>
<td>12</td>
<td>Food was withdrawn</td>
<td></td>
</tr>
<tr>
<td></td>
<td>After 8 h, 1 ml saline was injected i.p.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Blood (6 ml) was drawn 4 h later</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 mg iron dextran were injected i.p.</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Food was withdrawn</td>
<td></td>
</tr>
<tr>
<td></td>
<td>After 8 h, 1 ml AA was injected i.p.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Blood (6 ml) blood was drawn 4 h later</td>
<td></td>
</tr>
</tbody>
</table>

i.p., intraperitoneal; AA, ascorbic acid.

* All guinea pigs had free access to distilled water and a commercial guinea-pig diet throughout the study (see Table 1). For details of procedures see p. 681.
Peterson-Lowry method (Sigma Kit P-5656), was adjusted
to 75 µg/ml with PBS.

Total serum Fe and transferrin saturation were
determined (Sigma kit 565-B). In serum, bleomycin-reactive Fe
referred herein as ‘loosely-bound Fe’ was determined
according to Simpson et al. (1992) with the bleomycin
assay (Gutteridge & Hou, 1986; Gutteridge & Halliwell,
1987). The term ‘loosely-bound iron’ refers to ionic or
weakly chelated Fe capable of complexing with bleomycin,
an antitumour drug. The Fe–bleomycin complex is highly
reactive and cleaves DNA releasing thiobarbituric acid
reactive substances (TBARS). Briefly, 0·5 ml 1 mg/ml
DNA (calf thymus, treated with Chelex resin (0·3 g/ml), pH
7, 0·1 ml bleomycin sulfate (0·5 g/l), 0·1 ml 50 mM-
MgCl2, 0·1 ml 0·3 m-KH2PO4–KOH, pH 7·4 (treated
acid. To determine TBARS, 0·1 ml 0·1 M-EDTA, 1 ml
TBARS assay, 0·5 ml LDL containing 37·5
m
was expressed as the increase in concentration of TBARS
r
from correlation coefficient of the standard curve (
samples. The precision of the method may be evaluated
TCA (200 g/l) and treated as described for the serum

8 10 min at 100
thiobarbituric acid (10 g/l) and 1 ml 3 mM-HCl were added
to each sample and control. The mixture was heated for

1 ml LDL containing 75
m
ascorbic acid (treated with Chelex resin (0·4 g/ml)
and titrated with dichlorophenolindophenol) were mixed in
the order listed and incubated for 1 h at 37°C. Standards
were prepared from a ferric iron solution (atomic absorp-
tion standard) in 0·01 m-HCl (Chelex treated). The final
concentrations were 0·5 mg DNA/ml, 0·05 mg bleomycin/
ml, 5 mM-MgCl2, 30 mM-buffer and 0·75 mM-ascorbic
acid. To determine TBARS, 0·1 ml 0·1 M-EDTA, 1 ml
thiobarbituric acid (10 g/l) and 1 ml 3 mM-HCl were added
to each sample and control. The mixture was heated for
10 min at 100°C in a boiling-water bath. After the samples
cooled, they were centrifuged at 2000 g for 10 min and
the absorbance of the supernatant fraction was read at 532 nm.
The precision of the method may be evaluated from the
correlation coefficient of the standard curve (r 0·975) and
CV of duplicate measurements on serum samples (4-44 %)
(Miller & Miller, 1993).

Lipid peroxidation in serum was determined as TBARS.
Serum (0-1 ml) and PBS (0-4 ml), were mixed with 1 ml
thiobarbituric acid (6-7 g/l) and 1 ml TCA (200 g/l) and
heated at 100°C for 30 min. TBARS were extracted with
2 ml butanol and assayed fluorometrically with excitation
at 515 nm and emission at 553 nm. Standards
were prepared from tetraethoxypropane, which yields malon-
dialdehyde, in PBS. TBARS were expressed as ‘nmol
malondialdehyde equivalents’. Susceptibility of LDL to
in vitro oxidation was estimated in LDL samples incubated
at 37°C for 24 h in the presence of 5 µM-CuSO4. Oxidation
was expressed as the increase in concentration of TBARS
or conjugated dienes in a 24 h period. Briefly, for the
TBARS assay, 0·5 ml LDL containing 37·5 µg protein
were mixed with 1 ml thiobarbituric acid (6-7 g/l) and 1 ml
TCA (200 g/l) and treated as described for the serum
samples. The precision of the method may be evaluated from
correlation coefficient of the standard curve (r 0·997)
(Miller & Miller, 1993). For the conjugated diene assay,
1 ml LDL containing 75 µg protein was mixed with
0·5 mM-CuSO4 to give a final concentration of 5 µM-
CuSO4. Conjugated diene concentration was monitored at
234 nm (Steinbrecher et al. 1984). The precision of the
method may be evaluated from correlation coefficient of
the standard curve (r 0·994) (Miller & Miller, 1993).

Statistical analysis. Unpaired comparisons between the
control and the Fe-loaded group were done with the Mann-
Whitney test (rank sum test for two independent samples).

| Table 3. Serum iron and transferrin saturation in the control and in the iron-loaded groups throughout the study† |
|-------------|----------|----------|----------|
| Treatment group | Serum iron (µM) | Transferrin saturation |
| Control | Mean | SD | Mean | SD |
| Iron-loaded | Day 12 | 44·7 | 14·3 | 0·48 | 0·17 |
| Control | 60·5* | 9·3 | 0·78* | 0·15 |
| Iron-loaded | Day 19 | 35·1 | 5·9 | 0·50 | 0·12 |
| Control | 63·4* | 16·11 | 0·78* | 0·11 |

Ptalc means within a column were significantly different from other values (Mann-Whitney test for comparisons between the control and the iron-
loaded group and Wilcoxon sign-rank test for paired comparisons for data
collected before and after the ascorbic acid injections, *P < 0·05).
† All guinea pigs had free access to distilled water and a commercial guinea-
pig diet throughout the study (see Table 1). Animals from the iron-loaded
group were injected intraperitoneally with 200 mg iron dextran on days 1
and 5. All animals were injected intraperitoneally with ascorbic acid on day
19. For details of procedures, see p. 681.

Paired comparisons for data collected before and after
the ascorbic acid injections were performed with the Wilcoxon
sign-rank test. (Snedecor & Cochran, 1980). The CI was
95 %.

Results

Serum Fe concentration and transferrin saturation were
determined to assess Fe status (Table 3). Both indices were
higher for the Fe-loaded group compared with the control
throughout the experiment (P < 0·050). Treatments on day
12 did not produce any significant change in serum Fe or
transferrin saturation within the control or the Fe-loaded
group (P > 0·050). These results indicate that an Fe-loaded
state was induced successfully and that there was no further
change to the Fe status of either group throughout the
experimental period.

The body weights of the animals were recorded at
various time points throughout the experiment (Table 4).

| Table 4. Growth of animals in the control and the iron-loaded group
throughout the study† |
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Time-point of experiment (day)</td>
<td>Weight of animals (g)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Iron-loaded</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>0</td>
<td>497·2</td>
<td>23·4</td>
</tr>
<tr>
<td>7</td>
<td>535·2</td>
<td>30·8</td>
</tr>
<tr>
<td>11</td>
<td>569·3*</td>
<td>42·5</td>
</tr>
<tr>
<td>19</td>
<td>581·9*</td>
<td>46·5</td>
</tr>
</tbody>
</table>

Mean values in the control group were significantly different from values in the
iron-loaded group on a given day (unpaired comparisons, Mann-Whitney
test) and from values in the same group on day 0 (paired comparison within
the same group, Wilcoxon sign-rank test) (*P < 0·05).
† All guinea pigs had free access to distilled water and a commercial guinea-
pig diet throughout the study (see Table 1). Animals from the iron-loaded
group were injected intraperitoneally with 200 mg iron dextran on days 1
and 5. All animals were injected intraperitoneally with ascorbic acid on day
19. Blood (6 ml) was drawn for analysis on days 12 and 19. For details of
procedures, see p. 681.
Initially, and on day 7, there was no difference between the two groups ($P = 0.859$ and $P = 0.499$ respectively). On days 11 and 19, weights of the animals in the Fe-loaded group were lower than weight of the animals in the control group ($P = 0.018$ and $P = 0.028$ respectively). Overall (comparison between day 0 and day 19), the animals in the Fe-loaded group did not grow ($P = 0.398$) while the animals in the control did ($P = 0.012$). Some individuals seemed to be affected more than others and were examined by a veterinary surgeon. No signs of disease were identified in the examination. However, it appears that stunted growth in the Fe-loaded group is associated with Fe overloading.
Loosely-bound Fe (Fig. 1(a)) was not different between the two groups on day 12 ($P = 0.186$) but increased in the Fe-loaded group after the ascorbic acid injection compared with this group on day 12 ($P = 0.017$) or with the control group after the ascorbic acid injection ($P = 0.001$). No change was observed in the control group following ascorbic acid injection ($P = 0.498$). Lipid peroxidation in serum assessed in vitro as TBARS followed the same pattern (Fig. 1(b)), although statistical significance does not allow for equally confident conclusions since $P$ values were barely significant. In particular, TBARS appear no different in the two groups on day 12 ($P = 0.054$) but higher in the Fe-loaded group after the ascorbic acid injection, compared with the TBARS in the Fe-loaded group on day 12 ($P = 0.042$) or to the TBARS in the control group after the injection ($P = 0.023$). No change was observed in the control group following ascorbic acid injection ($P = 0.465$).

Susceptibility to in vitro oxidation of LDL from the control group was lower than that of LDL from the Fe-loaded group before the ascorbic acid injection. There was no difference in the susceptibility of LDL to in vitro oxidation between the two groups after the ascorbic acid injection. In particular, incubation of LDL at 37°C for 24 h in the presence of CuSO$_4$ produced higher levels of TBARS in the Fe-loaded group compared with the control group ($P = 0.005$), but only before the ascorbic acid injection. After the injection of ascorbic acid, levels of TBARS increased significantly in the control group ($P = 0.027$), but not in the loaded group ($P = 0.499$). There was no difference in TBARS between the control and the Fe-loaded group after the injections of ascorbic acid ($P = 0.560$) (Fig. 1(c)). Changes in conjugated diene concentration following LDL oxidation in vitro were very similar to those observed with the TBARS test (Fig. 1(d)). In particular, differences between the control and the Fe-loaded group in susceptibility of LDL to in vitro oxidation were observed before the ascorbic acid injection ($P = 0.004$) but not after ($P = 0.791$). Susceptibility of LDL to in vitro oxidation increased marginally after the injection of ascorbic acid in the control group ($P = 0.046$) but not in the Fe-loaded group ($P = 0.260$).

**Discussion**

Ascorbic acid, as well as other free radical scavengers, may inhibit lipid peroxidation in vivo. In fact, numerous studies suggest that ascorbic acid contributes to antioxidant defences in a plethora of systems in vitro (Frei et al. 1989; Dasgupta & Zdunek, 1992) as well as in vivo in animals (Kunert & Tappel, 1983; Dillard et al. 1984) and in human subjects (Rififi & Khachadurian, 1993). However, the behaviour of ascorbic acid in in vitro systems that contain high amounts of Fe may be complicated. Ascorbic acid may reduce Fe to the more reactive ferrous form. Moreover, it has been suggested that both ferrous and ferric Fe must be available to initiate lipid peroxidation (Minotti & Aust, 1992). Therefore, depending on the experimental conditions, ascorbic acid may act as a pro-oxidant in vitro and in vivo when Fe is present (O’Connel et al. 1985; Yamamoto & Niki, 1988; Minotti & Aust, 1992; Casalino et al. 1996). Observations on the pro-oxidant role of ascorbate in guinea pigs supplemented with high levels of ascorbic acid (Chen & Chang, 1978) have been reported, however, the mechanism by which ascorbic acid exerts its effect in these systems is not known. It has been suggested (Gutteridge et al. 1983) that, in in vitro model systems, ascorbic acid may reductively release Fe from biomolecules such as ferritin. This Fe may in turn catalyse lipid peroxidation.

In the present study we report a similar observation in the guinea pig. Our data suggest that ascorbic acid promotes the release of Fe from biomolecules where it is bound. Presumably, some of the released Fe takes the form of ionic Fe or weakly-chelated Fe compounds. This Fe, detectable with the bleomycin assay and referred herein as loosely-bound Fe, has been associated with oxidative processes. Thus, loosely-bound Fe appears to be Fe that is available to catalyse in vivo oxidation (Gutteridge & Halliwell, 1987). Ferritin is the likely source of this Fe (Gutteridge et al. 1983).

Lipid peroxidation measurements performed in vitro in serum suggest that, in the Fe-loaded group, Fe released by ascorbate promotes oxidative processes. Measurements on the susceptibility of LDL to in vitro oxidation, however, are not in full agreement with these results. In particular, oxidation of LDL from Fe-loaded animals was markedly higher than the control in the absence of injected ascorbic acid. When ascorbic acid was injected, oxidation increased in the control but not in the Fe-loaded group (Fig. 1(C) and (D)). This apparent discrepancy may be due to the fact that LDL oxidation had reached a maximum in the preparation from the Fe-loaded animals without the ascorbic acid injections and therefore did not respond to ascorbic acid injection. LDL from the control animals, on the other hand, was quite resistant to oxidation, leaving room for a response when ascorbic acid was injected. This discrepancy in results between serum and LDL may be at least partially explained by differences in the composition of serum and in vitro preparations of LDL. Serum was used untreated and is expected to contain various antioxidant compounds while the in vitro preparation of LDL was obtained through a procedure that involves removal of plasma fractions including, perhaps, antioxidants. It is therefore expected that serum and LDL may respond differently in in vitro tests. Differences in the methodology employed for testing in vitro oxidation are also important in interpreting these data. While oxidation in serum is assessed under mild in vitro conditions, susceptibility of LDL to in vitro oxidation is assessed under harsh conditions of prolonged heating in the presence of a catalyst. It is not surprising that under the challenge of these conditions, the behaviour of the samples was different (Esterbauer et al. 1992). It follows that if the challenge to oxidation had been done for a shorter time or under less severe conditions, ascorbate addition may have shown an enhancing effect at the Fe-loaded state or at both Fe levels. Literature suggests, however, that trends in susceptibility to in vitro oxidation are similar for long as well as for short periods of incubation under conditions similar to the conditions employed in this study (Parthasarathy et al. 1990; Reaven et al. 1991). Future time-course studies under various oxidation conditions may provide a further insight into the observed phenomena.
The effect of time on the treatments applied to the animals was not assessed in the current study. The experimental design employed, allows for paired and unpaired comparisons, however, it does not assess time effects since treatments of the animals and analyses of the samples were performed only on selected time points. Specifically, the effects of Fe (alone) and of ascorbate were studied only on days 12 and 19 respectively, and were not followed at various time points throughout the experiment. An alternative experimental design that allows tracking of the effects of treatment as well as of time may provide additional insights.

The animals in both groups appeared healthy throughout the experiment and a veterinary surgeon who examined the animals found no signs of disease. Nevertheless, differences in growth rates between the control and the Fe-loaded group suggest that the Fe loading did affect the health of the animals. The size and frequency of the iron dextran dosing were based on a study reported by Dillard et al. (1984). In that study, rats were given 1–2 g iron dextran/kg body weight injected i.p. There was no mention of toxic effects in the paper. Therefore, we did not expect a toxic reaction since we injected less iron: 0.8 g/kg body weight given as two injected doses of 0.4 g/kg. Iron dextran is rapidly cleared from the serum (Goldberg & Martin, 1960), therefore another dose of 0.4 g/kg body weight was given to the Fe-loaded group after the first week.

The dose of ascorbic acid as well as the pattern of the injection and blood drawing was based on a study reported by Kunert & Tappel (1983). The interpretation of the physiological relevance of this dose is related to the requirement for ascorbic acid of the guinea pig. Depending on diet composition, the dietary requirement is 5–50 mg/kg body weight, i.e. 2.5–25 mg for the average 500 g animal (Poole, 1987). Thus, the injected 75 mg dose was 3–30 times the requirement.

Our data suggest that ascorbic acid mobilises Fe in vivo and the mobilised Fe catalyses lipid peroxidation assessed in vitro. Although the in vitro tests may reflect in vivo reactions, we cannot conclusively infer that high doses of ascorbic acid lead to in vivo lipid oxidation. However, the present study provides additional support for the hypothesis that high intakes of Fe and/or ascorbic acid could increase in vivo lipid peroxidation of LDL and therefore could increase risk for atherosclerosis. (Sullivan, 1989). Serum ferritin concentration (Salonen et al. 1992), serum Fe concentration (Corti et al. 1997), and the serum transferrin receptor ratio: serum ferritin ratio (Tuomainen et al. 1998), as well as dietary Fe intakes (Tzonou et al. 1998), are associated with increased risk of myocardial infarction. However, in other studies this positive association between Fe status and atherosclerosis has not been demonstrated (Ascherio et al. 1994; Reunanen et al. 1995). In the First National Health and Nutrition Examination Survey (NHANES I), serum Fe and transferrin saturation were inversely associated with CHD (Liao et al. 1994).

In summary, the present study showed that in Fe-loaded guinea pigs, ascorbic acid promotes the release of Fe from biomolecules. Presumably, this loosely-bound Fe was responsible for the observed increase in peroxidation in the serum of Fe-loaded animals injected with ascorbic acid. The effect on oxidation of isolated LDL particles was different. Ascorbic acid injection increased LDL oxidation in the control but not in the Fe-loaded animals. Perhaps Fe loading caused maximal oxidation of isolated LDL so that the increase in loosely-bound Fe caused by ascorbic acid injection had no further effect. These data support the hypothesis that large doses of ascorbic acid mobilise Fe in vivo in Fe-loaded animals and this Fe acts as a catalyst for lipid peroxidation in the serum.

References


Aust SD (1987) Sources of iron for lipid peroxidation in biological systems. In Oxygen radicals and tissue injury, pp. 27–33 [B Halliwell, editor]. Bethesda, MD: Upjohn Co, by the FASEB.


Iron, ascorbic acid, and lipid peroxidation


