Original Paper

Journal of Biomedical Science

J Biomed Sci 2004;11:172–178
DOI: 10.1159/000076029

Received: June 30, 2003
Accepted: October 20, 2003

Short-Term Ascorbic Acid Deficiency Induced Oxidative Stress in the Retinas of Young Guinea Pigs

Yoshiji Ohta[a] Taku Okubo[c] Takafumi Niwa[b] Takashi Yamasaki[d] Masayuki Horiguchi[b]

Departments of [a]Chemistry and [b]Ophthalmology, School of Medicine, Fujita Health University, Toyoake, [c]Department of Ophthalmology, Shinshiro Municipal Hospital, Shinshiro, and [d]Yamasaki Eye Clinic, Kasugai, Japan

Key Words

Ascorbic acid · Reduced glutathione · Vitamin E · Lipid peroxide · Oxidative stress · Ascorbic acid deficiency (young guinea pigs) · Retina

Abstract

We examined whether short-term ascorbic acid deficiency induces oxidative stress in the retinas of young guinea pigs. Four-week-old guinea pigs were given a scorbutic diet (20 g/animal/day) with and without adequate ascorbic acid (400 mg/animal/day) in drinking water for 3 weeks. The serum concentrations of the reduced form of ascorbic acid and the oxidized form of ascorbic acid in the deficient group were 14.1 and 4.1%, respectively, of those in the adequate group. The retinal contents of the reduced form of ascorbic acid and the oxidized form of ascorbic acid in the deficient group were 6.4 and 27.3%, respectively, of those in the adequate group. The retinal content of thiobarbituric acid-reactive substances, an index of lipid peroxidation, was 1.9-fold higher in the deficient group than in the adequate group. Retinal reduced glutathione and vitamin E contents in the deficient group were 70.1 and 69.4%, respectively, of those in the adequate group. This ascorbic acid deficiency did not affect serum thiobarbituric acid-reactive substances and reduced glutathione concentrations but increased serum vitamin E concentration. These results indicate that short-term ascorbic acid deficiency induces oxidative stress in the retinas of young guinea pigs without disrupting systemic antioxidant status.

Introduction

Ascorbic acid (vitamin C) is an essential nutrient in humans, primates, and guinea pigs because these animals do not have the ability to synthesize the compound [4]. Although there is no information as to the content of ascorbic acid in whole human retinas, it is known that ascorbic acid is distributed in the avascular and vascular regions of premature human retinas and in the central and peripheral regions of mature human retinas [21]. The retinas of guinea pigs contain a high concentration of ascorbic acid (millimolar order) and the retinal ascorbic acid concentration of guinea pigs is equivalent to that of rats, rabbits, and cows which are capable of ascorbic acid synthesis [12]. In guinea pigs and baboons, the concentration of the reduced form of ascorbic acid is high in the neural retina, while the concentration of the oxidized form of ascorbic acid, i.e. dehydroascorbic acid, is high in the pig-

KARGER
Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1021–7770/04/0112–0172$21.00/0
Accessible online at:
www.karger.com/jbs

Yoshiji Ohta, PhD
Department of Chemistry
School of Medicine, Fujita Health University
Toyoake, Aichi 470-1192 (Japan)
Tel. +81 562 93 2573, Fax +81 562 93 4593/1193, E-Mail yohta@fujita-hu.ac.jp

ment epithelium-choroid [40, 49]. Ascorbic acid is known to scavenge reactive oxygen species (ROS) such as superoxide radicals, singlet oxygen, hydroxyl radicals, hydrogen peroxide, and peroxyl radicals by itself and exert an antioxidant action by interacting with reduced glutathione (GSH) or vitamin E [2, 3, 8, 30, 32, 35, 47]. Ascorbic acid is an important water-soluble antioxidant in the retina [7, 31]. Recent epidemiological studies have shown that a high intake of either ascorbic acid itself or a combination of ascorbic acid with β-carotene and vitamin E reduced the risk of age-related macular degeneration [15].

It has been shown in guinea pigs and baboons that the retinal level of the reduced form of ascorbic acid is reduced by mild photic damage [40, 49]. It has also been shown in scorbutic guinea pigs and monkeys that photic injury in the retina is enhanced by ascorbic acid deficiency [41, 48]. High contents of docosahexaenoic acid (22:6 n-3; DHA), a polyunsaturated fatty acid, which is highly susceptible to peroxidation, exist in retinal membrane phospholipids of guinea pigs [18–20] as well as in those of humans, rats, and cows [9]. Weisinger et al. [43–45] demonstrated that several visual functions assessed using the electroretinographic method were reduced in guinea pigs with DHA deficiency and that the reduction of visual functions in guinea pigs by DHA deficiency was recovered after the repletion of DHA. This suggests that DHA may play several functional and structural roles in the retina. Organisciak et al. [25, 26] reported that ascorbic acid supplementation protects the retinas of rats against light damage with the preservation of the rod outer segment DHA. They suggested that this vitamin inhibited oxidation of retinal membrane lipids during intense visible light possibly through its antioxidant action.

Oxidative stress is a state of imbalance between antioxidant defense systems and oxidative insult, which most likely results from decreased levels of antioxidants or increased ROS insult or both. Our recent report showed that when 4-week-old guinea pigs are maintained on marginal ascorbic acid deficiency for 6 months, an increase in lipid peroxidation and depletion of GSH and vitamin E occurred in the retinas, resulting in the induction of oxidative stress in the tissue [23]. Nakajima et al. [20] showed that feeding a scorbutic diet to young guinea pigs for 3 weeks did not increase lipid peroxide content but rather tended to reduce that content in the choroid-retina. However, it is still unclear whether short-term ascorbic acid deficiency induces oxidative stress in the retina of young guinea pigs.

In the present study, therefore, we examined the changes in retinal levels of antioxidants, i.e. ascorbic acid, GSH, and vitamin E, and thiobarbituric acid-reactive substances (TBARS), which is an index of lipid peroxidation, in young male guinea pigs with 3 weeks of ascorbic acid deficiency in order to clarify whether short-term ascorbic acid deficiency induces oxidative stress in the retina of young guinea pigs. The strain, age, and sex of guinea pigs used in the present study and the duration of ascorbic acid deficiency were the same as those described in the report by Nakajima et al. [20].

Materials and Methods

Materials

α-Tocopherol (α-Toc) was used as the vitamin E standard in the vitamin E assay and was purchased from Eisai Co. (Tokyo, Japan). *L*-Ascorbic acid (reduced form), α,α′-dipyridyl, 5,5′-dithiobis(2-nitrobenzoic acid) (Ellman's reagent), dithiothreitol (DTT), N-ethylmaleimide (NEM), ethylenediaminetetraacetic acid (EDTA), tetramethoxypropane, 2-thiobarbituric acid (TBA), trichloroacetic acid (TCA), and other chemicals were obtained from Wako Pure Chemical Ind. (Osaka, Japan). All chemicals used were of reagent grade and were not further purified.

Induction of Ascorbic Acid Deficiency and Sample Collection

Three-week-old male Hartley guinea pigs (n = 20), which were purchased from Japan SLC Co. (Hamamatsu, Japan), were housed in individual cages in a ventilated animal room with controlled temperature (23 ± 2°C) and relative humidity (55 ± 15%) and using a 12-hour light/dark cycle (light: 7:00–19:00). Ascorbic acid deficiency was induced in guinea pigs as follows: guinea pigs were initially fed a standard diet of ORC4 (Oriental Yeast, Tokyo, Japan) ad libitum for 1 week to adjust the ascorbic acid status in the guinea pigs used before starting the experiment of ascorbic acid deficiency. At this time, the animals were randomly divided into the following two groups. The ascorbic acid-adequate group (adequate group, n = 10) was given Oriental scorbutic diet (Oriental Yeast) in a fixed amount (20 g/animal/day) and *L*-ascorbic acid (400 mg/animal/day) in the drinking water. The ascorbic acid-deficient group (deficient group, n = 10) was given the same scorbutic diet (20 g/animal/day) and water not containing ascorbic acid. The complete intake of administered ascorbic acid in each animal in the adequate group was determined by checking the complete consumption of drinking water every day. The composition of the Oriental scorbutic diet was as follows (g/100 g diet): corn starch 29.5 g, milk casein 20 g, alfalfa meal 10 g, α-potato starch 10 g, cellulose powder 10 g, sucrose 10 g, soybean oil 6 g, AIN-76 mineral mixture 3.5 g, and AIN-76 vitamin mixture 1 g. The composition of the AIN-76 mineral mixture was as follows (g/100 g mixture adjusted with sucrose): $CaHPO_4$ 50 g, NaCl 7.4 g, $K_3C_6HO_7 \cdot H_2O$ 22 g, K_2SO_4 5.2 g, MgO 2.4 g, MnO_3 0.35 g, $FeC_6H_5O_7$ 0.6 g, $ZnCO_3$ 0.16 g, $CuCO_3Cu(OH)_2 \cdot H_2O$ 0.03 g, $Na_2SeO_3 \cdot 5\ H_2O$ 0.001 g, KIO_3 0.001 g, and $CrK(SO_4)_2 \cdot 12\ H_2O$ 0.55 g. The composition of the AIN-76 vitamin mixture was as follows (g/100 g mixture adjusted with sucrose): vitamin A acetate 40,000 IU, vitamin D_3 10,000 IU, vitamin E acetate 500 mg, vitamin

K_3 0.5 mg, vitamin B_1·HCl 60 mg, vitamin B_2 60 mg, vitamin B_6·HCl 70 mg, vitamin B_{12} 0.1 g, D-biotin 2 mg, folic acid 20 mg, pantothenic acid calcium salt 160 mg, nicotinic acid 300 mg, and choline bitartrate 20 g. Animals in each group were weighed and then humanely killed under ether anesthesia at 3 weeks after the beginning the new diet. They were killed between 9:00 and 10:00 a.m. At the time of the killing, blood was collected from the vena cava inferior. Serum was obtained from the collected blood by centrifugation. Just after being killed, both eyeballs were removed from the animals and then each retina was carefully isolated from the removed eyeballs under a stereoscope. In this isolation, as much choroid as possible was excluded from the isolated retina. Whole retinal tissue was collected from the isolated retina and weighed. The obtained serum and whole retinal tissue were stored under nitrogen atmosphere at −80 °C until use. All animals received humane care in compliance with the guidelines of the Animal Care and Use Committee at the Fujita Health University.

Sample Preparation and Assays for Retinal and Serum Components

Whole retinal tissue obtained from each animal was homogenized in 9 vol of ice-cold 0.15 *M* KCl containing 1 m*M* EDTA to prepare 10% homogenate. This homogenate was used for the determinations of ascorbic acid (total, reduced form, and oxidized form), GSH, and TBARS. The determinations of total ascorbic acid (reduced form plus oxidized form), the reduced form of ascorbic acid, and the oxidized form of ascorbic acid in serum and retina were done according to the methods of Zannoi et al. [51] and Okamura [24] as follows: for the determination of total ascorbic acid, 0.3 ml of serum or 10% retina homogenate was incubated with 0.1 ml of 10 m*M* DTT at 37 °C for 30 min to convert all ascorbic acid in an oxidized form in the serum or homogenate to its reduced form and then the excess DTT was removed with 0.1 ml of 0.5% NEM. An aliquot of the supernatant obtained after deproteinization with 0.5 ml of ice-cold 10% TCA was used for the assay of the resultant reduced form of ascorbic acid plus the original reduced form of ascorbic acid. For the determination of the reduced form of ascorbic acid, 0.3 ml of serum or 10% retina homogenate was mixed with 0.2 ml of a solution of 10 m*M* DTT-0.5% NEM. An aliquot of the supernatant obtained after deproteinization with 0.5 ml of ice-cold 10% TCA was used for the assay of the reduced form of ascorbic acid. The reduced form of ascorbic acid in each sample was measured using the α,α′-dipridyl method. The concentration of the reduced form of ascorbic acid was determined using the standard curve of authentic *L*-ascorbic acid in a reduced form. The concentration of the oxidized form of ascorbic acid in serum or retina was estimated from the difference between the concentrations of total ascorbic acid and the reduced form of ascorbic acid determined. GSH in serum and retina was measured using the method of Sedlak and Lindsay [33] with Ellman's reagent. TBARS in serum was measured using a commercial kit of Lipoperoxide Test Wako (Wako Pure Chemical Ind.) that was based on the TBA method of Yagi [50]. TBARS in retina was measured using the TBA method of Ohkawa et al. [22] except that 1 m*M* EDTA was added to the reaction medium. Tetramethoxypropane was used as an external standard in both TBARS assays. The amount of TBARS is expressed as that of malondialdehyde (MDA) equivalents. Vitamin E in serum and retina was measured using the method of Abe et al. [1] using high-performance liquid chromatography with fluorescence detection. The amount of vitamin E is expressed as that of α-Toc.

Table 1. Effect of ascorbic acid deficiency on body weight and retina weight in guinea pigs

	Ascorbic acid-adequate group (n = 10)	Ascorbic acid-deficient group (n = 10)
Body weight, g	360 ± 22	324 ± 24*
Retina weight, mg	64.8 ± 6.5	59.6 ± 15.1

Data are represented as means ± SD. The number of animals is shown in parentheses. * $p < 0.05$ significantly different from the ascorbic acid-adequate group.

Statistical Analysis

All results obtained are expressed as mean ± SD. The statistical analyses of the results were performed using a computerized statistical package (StatView). Each mean value was compared using one-way analysis of variance. Values of significance were set at $p < 0.05$.

Results

The body weight of young guinea pigs given a scorbutic diet at a fixed dose level for 3 weeks was significantly less than that of control guinea pigs with adequate ascorbic acid, while there was no difference in the weight of the retina between the two groups (table 1).

The serum concentrations of total ascorbic acid, the reduced form of ascorbic acid, the oxidized form of ascorbic acid, GSH, and vitamin E, and TBARS in young guinea pigs with and without 3 weeks of ascorbic acid deficiency are shown in table 2. The serum concentrations of total ascorbic acid, the reduced form of ascorbic acid, and the oxidized form of ascorbic acid in the deficient group were significantly lower than those in the adequate group. The total ascorbic acid, the reduced form of ascorbic acid, and the oxidized form of ascorbic acid in the serum of the deficient group were 11.8, 14.1 and 4.1%, respectively, of those in the serum of the adequate group. Mean serum GSH and TBARS concentrations in the deficient group were not significantly different from those in the adequate group. Mean serum vitamin E concentration in the deficient group was significantly higher than that in the adequate group; the deficient group had a 2.4-fold higher mean serum vitamin E concentration than the adequate group.

The retinal contents of total ascorbic acid, the reduced form of ascorbic acid, the oxidized form of ascorbic acid, GSH, and vitamin E, and TBARS in young guinea pigs

Table 2. Effect of ascorbic acid deficiency on serum concentrations of total ascorbic acid, the reduced form of ascorbic acid, the oxidized form of ascorbic acid, GSH, vitamin E, and TBARS in guinea pigs

	Ascorbic acid-adequate group (n = 10)	Ascorbic acid-deficient group (n = 10)
Total ascorbic acid, nmol/ml	99.6 ± 14.4	11.8 ± 1.9*
Reduced form of ascorbic acid nmol/ml	77.5 ± 12.9	10.9 ± 1.9*
Oxidized form of ascorbic acid nmol/ml	22.1 ± 6.5	0.9 ± 0.5*
GSH, nmol/ml	21.9 ± 2.6	22.1 ± 2.5
Vitamin E, μg α-Toc/ml	1.63 ± 0.61	3.83 ± 0.46*
TBARS, nmol MDA/ml	5.9 ± 0.7	6.8 ± 0.8

Data are represented as means ± SD. The number of animals is shown in parentheses. * $p < 0.05$ significantly different from the ascorbic acid-adequate group.

Table 3. Effect of ascorbic acid deficiency on retinal contents of total ascorbic acid, the reduced form of ascorbic acid, the oxidized form of ascorbic acid, GSH, vitamin E, and TBARS in guinea pigs

	Ascorbic acid-adequate group (n = 10)	Ascorbic acid-deficient group (n = 10)
Total ascorbic acid, μmol/g	2.77 ± 0.12	0.27 ± 0.05*
Reduced form of ascorbic acid μmol/g	2.33 ± 0.08	0.15 ± 0.03*
Oxidized form of ascorbic acid μmol/g	0.44 ± 0.12	0.12 ± 0.03*
GSH, μmol/g	1.27 ± 0.31	0.89 ± 0.08*
Vitamin E, μg α-Toc/g	7.75 ± 0.76	5.38 ± 0.88*
TBARS, nmol MDA/g	2.13 ± 0.31	4.01 ± 0.61*

Data are represented as means ± SD. The number of animals is shown in parentheses. * $p < 0.05$ significantly different from the ascorbic acid-adequate group.

with and without 3 weeks of ascorbic acid deficiency are shown in table 3. The retinal contents of total ascorbic acid, the reduced form of ascorbic acid, and the oxidized form of ascorbic acid in the deficient group were significantly lower than those in the adequate group. The retinal contents of total ascorbic acid, the reduced form of ascorbic acid, and the oxidized form of ascorbic acid in the deficient group were 9.7, 6.4 and 27.3%, respectively, of those in the adequate group. Retinal GSH and vitamin E contents in the deficient group were significantly lower than those in the adequate group. The retinas of the deficient group had 70.1% of GSH content and 69.4% of vitamin E content compared to the retinas of the adequate group. Retinal TBARS content in the deficient group was significantly higher than that in the adequate group. The deficient group had a 1.9-fold higher retinal TBARS content than the adequate group.

Discussion

It is known that a decrease in body weight gain occurs in guinea pigs with scurvy [19, 42]. In the present study, when 4-week-old guinea pigs were given a scorbutic diet at a fixed dose level (20 g/day/animal) for 3 weeks, the ascorbic acid-deficient guinea pigs had a lower body weight than the control guinea pigs with an adequate ascorbic acid intake. Accordingly, guinea pigs given a scorbutic diet at a fixed dose level for 3 weeks seem to have scurvy. However, there was no difference in the weight of the retina between the ascorbic acid-deficient and ascorbic acid-adequate guinea pigs. The ascorbic acid-deficient guinea pigs showed dramatic decreases in the serum concentrations of total ascorbic acid, the reduced form of ascorbic acid, and the oxidized form of ascorbic acid. Thus, 3 weeks of ascorbic acid deficiency caused a severe systemic ascorbic acid deficiency in young guinea pigs.

Ascorbic acid exerts an antioxidant action by scavenging ROS and by interacting with GSH or vitamin E [2, 3, 8, 30, 32, 35, 47], and this vitamin is an important antioxidant in the retina [7, 31]. We reported that when 4-week-old guinea pigs were given a scorbutic diet at a fixed dose level (20 g/day/animal) with marginal ascorbic acid in drinking water for 6 months, oxidative stress due to increased lipid peroxidation and depleted GSH and vitamin E occurred in the retina with approximately 50% ascorbic acid depletion [23]. In the present study, the retinal contents of total ascorbic acid and the reduced form of ascorbic acid in young guinea pigs given a scorbutic diet at a dose level of 20 g/day/animal for 3 weeks were less than 10% of those in the control guinea pigs with adequate ascorbic acid intake. However, the retinal content of the oxidized form of ascorbic acid in the ascorbic acid-deficient guinea pigs was approximately 30% of that in the ascorbic acid-adequate guinea pigs. Thus, 3 weeks of ascorbic acid deficiency caused a severe ascorbic acid

deficiency in the retinas of young guinea pigs. These findings suggest the possibility that short-term deficiency of ascorbic acid induces oxidative stress in the retinas of young guinea pigs.

Nakajima et al. [20] reported that the content of lipid peroxide (determined by the TBA method) in the choroid retinas of young guinea pigs given a scorbutic diet for 3 weeks tended to be lower than that of the control guinea pigs given the same amount of control diet composed of the scorbutic diet and ascorbic acid (150 mg/kg) for the same period. In the present study, when young guinea pigs were given a scorbutic diet at a fixed dose level (20 g/day/animal) for 3 weeks, the retinal content of TBARS, an index of lipid peroxidation, was approximately 2-fold higher than that of the control guinea pigs with adequate ascorbic acid intake. The results suggest that the antioxidant defense system was disrupted in the retinas of young guinea pigs with short-term ascorbic acid deficiency, resulting in an induction of oxidative stress in the tissue. In addition, the present results and the results shown by Nakajima et al. [20] may allow us to assume that, in young guinea pigs with short-term ascorbic acid deficiency, the antioxidant defense system is disrupted in the retina, while the antioxidant defense system is rather strengthened in the choroid.

GSH exerts an antioxidant action by working as a cosubstrate for GSH peroxidase, an enzyme to decompose hydroperoxides, and by scavenging ROS in a nonenzymatic manner [28]. GSH is present in high concentrations (millimolar order) in the retinas of guinea pigs [16, 23]. The retinal concentration of GSH in guinea pigs is similar to that in rats [46] and bovines [34]. Winkler et al. [47] showed that the conversion of the oxidized form of ascorbic acid to its reduced form by GSH occurs nonenzymatically in the cytosolic supernatant of rat retinas and that the level of the reduced form of ascorbic acid in cultured human retinal pigment epithelial cells exposed to oxidative stress is restored by the redox coupling between GSH and the oxidized form of ascorbic acid in a nonenzymatic manner. Keys and Zimmerman [17] reported that GSH exerted an antioxidant action in bovine photoreceptor cell membranes in vitro. The retinal Müller cell of guinea pigs is capable of GSH synthesis, a process that is critically dependent on the availability of extracellular glutamate and cystine [29]. Huster et al. [14] showed that oxidative stress induced by ischemia-reperfusion disrupted GSH synthesis in the retinal Müller cell of guinea pigs. In the present study, retinal GSH content in young guinea pigs given a scorbutic diet at a fixed dose level for 3 weeks was approximately 70% of that in control guinea pigs with adequate ascorbic acid intake. The GSH consumption may disrupt the retinal antioxidant defense system in the ascorbic acid-deficient guinea pigs. Thus, GSH consumption was found to occur in the retinas of young guinea pigs with short-term ascorbic acid deficiency. The lowered retinal GSH found in young guinea pigs given a scorbutic diet at a fixed dose level for 3 weeks may result from the increased GSH consumption, decreased GSH synthesis or both in the retina. There is also a possibility that GSH may directly react with ROS in the ascorbic acid-deficient retinas of young guinea pigs given a scorbutic diet for 3 weeks, leading to retinal GSH consumption.

Ascorbic acid functions as an antioxidant not only through its direct action to scavenge ROS in the aqueous phase of cells but also through its indirect action to regenerate an antioxidant form of vitamin E from its radical form in the hydrophobic membrane phase of cells [2, 3, 8, 30, 32, 35, 47]. Vitamin E exists in the retina of humans [10, 21], monkeys [6], and guinea pigs [20] as well as in the retina of rats, rabbits, and cats [36]. Stoyanovsky et al. [37] showed that endogenous ascorbic acid, but not endogenous GSH, regenerates an antioxidant form of vitamin E in the homogenate and rod outer segment of rat retinas. In the present study, the retinal content of vitamin E in young guinea pigs given a scorbutic diet at a fixed dose level for 3 weeks was approximately 70% of that of the control guinea pigs with adequate ascorbic acid intake. Thus, 3 weeks of ascorbic acid deficiency caused an apparent reduction in vitamin E content in the retinas of young guinea pigs. This result indicates that vitamin E consumption occurs in the retinas of young guinea pigs with short-term ascorbic acid deficiency, which may lead to disruption of the antioxidant defense system in the tissue. The lowered retinal vitamin E found in the young guinea pigs with 3 weeks of ascorbic acid deficiency could be a result of the lack of ascorbic acid to generate vitamin E from oxidized vitamin E. Retinal membrane phospholipids of guinea pigs have a high content of DHA, a polyunsaturated fatty acid, which is highly susceptible to peroxidation [43–45]. Terrasa et al. [38] report that vitamin E may act as an antioxidant in protecting rod outer segment membranes from deleterious effects using a selective mechanism that diminishes the loss of DHA from phosphatidylserine. Accordingly, the possibility can be assumed that an enhanced peroxidation of DHA occurs in the retinas of young pigs with short-term ascorbic acid deficiency.

In the present study, the changes in serum TBARS, GSH, and vitamin E concentrations in young guinea pigs

given a scorbutic diet at a fixed dose level (20 g/day/animal) for 3 weeks were further examined in order to clarify whether the changes described above in the retinal TBARS, GSH, and vitamin E contents in young guinea pigs with short-term ascorbic acid deficiency were associated with systemic changes in TBARS, GSH, and vitamin E concentrations. The guinea pigs with 3 weeks of ascorbic acid deficiency showed no changes in serum TBARS or GSH concentration. These results indicate that, in young guinea pigs with short-term ascorbic acid deficiency, an increase in retinal TBARS content and a decrease in retinal GSH content occurred without systemic changes in TBARS or GSH concentration. On the other hand, an apparent increase in serum vitamin E concentration occurred in young guinea pigs given a scorbutic diet at a fixed dose level for 3 weeks. However, the possibility seems to be unlikely that the changes in the retinal TBARS, GSH, and vitamin E contents found in young guinea pigs given a scorbutic diet for 3 weeks were associated with the systemic increase in vitamin E concentration, because the ascorbic acid-deficient guinea pigs had an increased retinal TBARS content and decreased retinal GSH and vitamin E contents despite an increase in vitamin E concentration in the serum. Accordingly, it can be thought that short-term ascorbic acid deficiency induced oxidative stress in the retinas of young guinea pigs without disrupting systemic antioxidant status.

Recently, Lykkesfeldt [18] reported that when young male guinea pigs (2 months old) were fed a diet containing low ascorbic acid (36 mg/kg) or normal ascorbic acid (1,036 mg/kg) ad libitum for 30–36 days, plasma ascorbic acid concentrations in the ascorbic acid-deficient and ascorbic acid-adequate guinea pigs were 0.3 and 123.4 μM (mean values), respectively. However, the plasma concentration of MDA, an index of lipid peroxidation, in the deficient guinea pigs was 4.4-fold higher than that in the adequate guinea pigs, although there was no significant difference in plasma α-Toc concentration between the groups. Furthermore, the same author showed that in mature male guinea pigs (9 months old) that were fed a diet containing low ascorbic acid (36 mg/kg) or normal ascorbic acid (1,036 mg/kg) ad libitum for 30–36 days, plasma ascorbic acid concentrations in the ascorbic acid-deficient and ascorbic acid-adequate guinea pigs were 1.2 and 75.0 μM (mean values), respectively. In addition, the plasma MDA concentration in the deficient guinea pigs was 1.65-fold higher than that in the adequate guinea pigs, although there was no significant difference in plasma α-Toc concentration between the groups [46]. Hill et al. [13] report that when weanling male guinea pigs were fed a scorbutic diet containing vitamin E acetate (200 mg/kg) or a normal diet containing ascorbic acid (842 mg/kg) and vitamin E acetate (200 mg/kg) ad libitum for 16 days, plasma ascorbic acid concentrations in the ascorbic acid-deficient and ascorbic acid-adequate guinea pigs were 0 and 59 μmol/l (mean values), respectively, while plasma α-Toc concentrations in the deficient and adequate guinea pigs were 1.7 and 2.5 μmol/l (mean values), respectively, although there was no significant difference in that concentration between the groups. In the present study, young male guinea pigs (4 weeks old) were fed the scorbutic diet containing vitamin E acetate (50 mg/kg) at a fixed dose level (20 g/day/animal) with and without adequate ascorbic acid (400 mg/day/animal) in drinking water for 3 weeks. Thus, plasma or serum vitamin E and lipid peroxide concentrations of guinea pigs under short-term ascorbic acid deficiency were affected by the differences in diet, age, feeding and duration. Therefore, it seems likely that the disruption of systemic antioxidant status in guinea pigs with short-term ascorbic acid deficiency contributes to the induction of oxidative stress in the retinas under experimental conditions which are different from the experimental conditions used in the present study.

An increase in serum cholesterol concentration has been reported in guinea pigs with acute ascorbic acid deficiency [5, 11]. The vitamin E status under physiological and pathological conditions has been assessed using the ratio of the level of serum vitamin E, which is concentrated in lipoproteins, to the level of serum lipids, especially cholesterol [27, 39]. We observed that the serum of young guinea pigs given a scorbutic diet at a fixed dose for 3 weeks contains 1.6-fold higher total cholesterol than that of the control guinea pigs with adequate ascorbic acid intake (unpubl. data). Therefore, the increase in serum vitamin E concentration found in the young guinea pigs with 3 weeks of ascorbic acid deficiency may be due to the systemic changes in lipid metabolism.

In conclusion, the results obtained in the present study indicate that short-term ascorbic acid deficiency induced oxidative stress in the retinas of young guinea pigs without disrupting the systemic antioxidant status. However, the exact mechanism by which oxidative stress was induced in the retina of young guinea pigs with short-term ascorbic acid deficiency is unknown at present. Therefore, further investigation is needed to clarify this.

References

1 Abe K, Yuguchi Y, Katsui G. Quantitative determination of tocopherol by high-speed liquid chromatography. J Nutr Sci Vitaminol 21: 183–188;1975.
2 Beyer RR. The role of ascorbate in antioxidant protection of biomembranes: Interaction with vitamin E and coenzyme Q. J Bioenerg Biomembr 26:349–358;1994.
3 Bodannes RS, Chan PC. Ascorbic acid as a scavenger of singlet oxygen. FEBS Lett 105: 195–196;1979.
4 Burns JJ. Biosynthesis of *L*-ascorbic acid; basic defect in scurvy. Am J Med 26:740–748;1959.
5 Chauhan VPS, Sarkar AK. Effect of acute ascorbic acid deficiency on the plasma lipids and postheparin lipolytic activity in guinea pigs. Ann NY Acad Sci 498:464–466;1987.
6 Crabtree DV, Adler AJ, Snodderly DM. Vitamin E, retinyl palmitate, and protein in rhesus monkey retina and retinal pigment epithelium-choroid. Invest Ophthalmol Vis Sci 37:47–60; 1996.
7 Delamere NA. Ascorbic acid and the eye. Subcell Biochem 25:313–329;1996.
8 Deutsch JC. Ascorbic acid oxidation by hydrogen peroxide. Arch Biochem Biophys 255:1–7; 1998.
9 Fliesler SJ, Anderson RE. Chemistry and metabolism of lipids in the vertebrate retina. Prog Lipid Res 22:79–131;1983.
10 Friedrichson T, Kalbach HL, Buck P, van Kuijk GM. Vitamin E in macular and peripheral tissues of human eye. Curr Eye Res 14:693–701;1995.
11 Ginter E. Cholesterol: Vitamin C controls its transformation to bile acids. Science 179:702–704;1973.
12 Heath H, Becj TC, Rutter AC. Biochemical changes in aphakia. Vision Res 1:274–286; 1962.
13 Hill KE, Montine TJ, Motley AK, Li X, May JM, Burk RF. Combined deficiency of vitamins E and C causes paralysis and death in guinea pigs. Am J Clin Nutr 77:1484–1488; 2003.
14 Huster D, Reichenbach A, Reichelt W. The glutathione content of retinal Müller (glial) cells: Effect of pathological conditions. Neurochem Int 36:461–469;2000.
15 Jacques PF. The potential preventive effects of vitamins for cataract and age-related macular degeneration. Int J Vitam Nutr Res 69:198–205;1999.
16 Kannan R, Tang D, Mackic JB, Zlokovic BV, Fernandez-Checa JC. A simple technique to determine glutathione (GSH) levels and synthesis in ocular tissues as GSH-bimane adduct: Application to normal and galactosemic guinea-pigs. Exp Eye Res 56:45–50;1993.
17 Keys SA, Zimmerman WF. Antioxidant activity of retinol, glutathione, and taurine in bovine photoreceptor cell membranes. Exp Eye Res 68:693–702;1999.
18 Lykkesfeldt J. Increased oxidative damage in vitamin C deficiency is accompanied by induction of ascorbic acid recycling capacity in young but not mature guinea pigs. Free Radic Res 36:567–574;2002.
19 Mårtensson J, Han J, Griffith OW, Meister A. Glutathione ester delays the onset of scurvy in ascorbic acid-deficient guinea pigs. Proc Natl Acad Sci USA 90:317–321;1993.
20 Nakajima H, Yagihashi O, Kashima Y, Ishikawa H, Kitano S, Kimoto I, Tomioka E, Mano M, Takeuchi S. Effects of ascorbic acid on trace element metabolism in the choroids-retina of streptozotocin-induced diabetic guinea pigs. Nippon Ganka Gakkai Zasshi 97:340–345; 1993.
21 Nielisen JC, Naash MI, Anderson RE. The regional distribution of vitamins E and C in mature and premature human retinas. Invest Ophthalmol Vis Sci 29:22–26;1987.
22 Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351–358; 1979.
23 Ohta Y, Okubo T, Niwa T, Yamasaki T, Horiguchi M. Prolonged marginal ascorbic acid deficiency induces oxidative stress in retina of guinea pigs. Int J Vitam Nutr Res 72:63–70;2002.
24 Okamura M. An improved method for determination of *L*-ascorbic acid and *L*-dehydroascorbic acid in blood plasma. Clin Chim Acta 103:259–268;1980.
25 Organisciak DT, Wang H-M, Li Z-Y, Tso MOM. The preventive effect of ascorbate in retinal light damage. Invest Ophthalmol Vis Sci 26:1580–1588;1985.
26 Organisciak DT, Wang H-M, Noell WK. Aspects of the ascorbate protective mechanism in retinal light damage of rats with normal and reduced ROS docosahexaenoic acid. Prog Clin Biol Res 247:455–468;1987.
27 Panemangalore M, Lee CJ. Evaluation of the indices of retinol and α-tocopherol status in free-living elderly. J Gerontol 47:B98–B104; 1992.
28 Reed DJ. Glutathione: Toxicological implications. Annu Rev Pharrmacol Toxicol 30:603–631;1990.
29 Reichelt W, Stabe-Burow J, Pannicke T, Weicher H, Heineman U. The glutathione level of retinal Müller glial cells is dependent on the high-affinity sodium-dependent uptake of glutathione. Neuroscience 77:1213–1224;1997.
30 Rose RC. Ascorbic acid metabolism in protection against free radicals: A radiation model. Biochem Biophys Res Commun 169:430–436; 1990.
31 Rose RC, Bode AM. Biology of free radical scavengers: An evaluation of ascorbate. FASEB J 7:1135–1142;1993.
32 Rose RC, Bode AM. Ocular ascorbate transport and metabolism. Comp Biochem Physiol 100A:273–285;1991.
33 Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 25:192–205;1968.
34 Saxena M, Singhal SD, Awasthi YC. A specific, sensitive, and rapid method for the determination of glutathione and its application in ocular tissues. Exp Eye Res 55:461–468;1992.
35 Som S, Raha C, Chaterjee JB. Ascorbic acid: A scavenger of superoxide radical. Acta Vitaminol Enzymol 5:243–250;1983.
36 Stephens RJ, Negi DS, Short SM, van Kuijk FJ, Dratz EA, Thomas DW. Vitamin E distribution in ocular tissues following long-term dietary depletion and supplementation as determined by microdissection and gas chromatography-mass spectrometry. Exp Eye Res 47: 237–245;1988.
37 Stoyanovsky DA, Goldman R, Darrow RM, Organisciak DT, Kagan VE. Endogenous ascorbate regenerates vitamin E in the retina directly and in combination with exogenous dihydrolipoic acid. Curr Eye Res 14:181–189; 1995.
38 Terrasa A, Guajardo M, Catalá TTT. Selective inhibition of the non-enzymatic lipid peroxidation of phosphatidylserine in rod outer segments by α-tocopherol. Mol Cell Biochem 211: 39–45;2000.
39 Thurnham DI, Davis JA, Crump BJ, Stinuayaka RD, Davis M. The use of different lipids to express serum tocopherol: Lipid ratios for the determination of vitamin E status. Ann Clin Biochem 23:514–520;1986.
40 Tso MOM, Woodford BJ, Lam K-W. Distribution of ascorbate in normal primate retina and after photic injury: A biochemical, morphological correlated study. Curr Eye Res 3:181–191; 1984.
41 Tso MOM. Retinal photic injury in normal and scorbutic monkeys. Trans Am Ophthalmol Soc 85:498–556;1987.
42 Veen-Baigent MJ, Ten Cate AR, Bright-See E, Rao AV. Effects of ascorbic acid on the health parameters in guinea pigs. Ann NY Acad Sci 258:339–354;1975.
43 Weisinger HS, Vingrys AJ, Bui BV, Sinclair AJ. Effects of dietary n–3 fatty acid deficiency and repletion in the guinea pig retina. Invest Ophthalmol Vis Sci 40:327–338;1996.
44 Weisinger HS, Vingrys AJ, Sinclair AJ. Effect of dietary n–3 deficiency on the electroretinogram in the guinea pig. Ann Nutr Metab 40: 91–98;1996.
45 Weisinger HS, Vingrys AJ, Sinclair AJ. The effect of docosahexaenoic acid on the electroretinogram of the guinea pig. Lipids 31:65–70; 1996.
46 Winkler BS, Giblin FJ. Glutathione oxidation in retina: Effects on biochemical and electrical activities. Exp Eye Res 36:287–297;1983.
47 Winkler BS, Orselli SM, Rex TS. The redox couple between glutathione and ascorbic acid: A chemical and physiological perspective. Free Radic Biol Med 17:333–349;1994.
48 Woodford BJ, Tso MOM. Exaggeration of photic injury in scorbutic guinea pig retinas. Invest Ophthalmol Vis Sci 25(suppl):90;1984.
49 Woodford BJ, Tso MOM, Lam K-W. Reduced and oxidized ascorbates in guinea pig retina under normal and light-exposed conditions. Invest Ophthalmol Vis Sci 24:862–867;1983.
50 Yagi K. A simple fluorometric assay for lipoperoxide in blood plasma. Biochem Med 15: 212–216;1976.
51 Zannoi V, Lynch M, Goldstein S, Sato P. A rapid micromethod for the determination of ascorbic acid in plasma and tissues. Biochem Med 11:41–48;1974.