Reduced and Oxidized Ascorbates in Guinea Pig Retina under Normal and Light-exposed Conditions

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Both reduced and oxidized ascorbates were measured in aqueous, neural retina, and pigment epithelium-choroid complex (PE-C) of pigmented guinea pigs. Normal values for total ascorbate of 16 mg/dl in aqueous, 22 mg/dl in neural retina, and 7 mg/dl in PE-C were found. After mild photic damage caused by varying lengths of exposure of 10,000 to 20,000 lux of fluorescent lighting, reduced ascorbate concentrations generally decreased in the neural retina, while oxidized ascorbate generally increased in PE-C. In both normal and light-exposed retinas, reduced ascorbate was predominant in the neural retina, and oxidized ascorbate was predominant in the PE-C. Histochemical localization of reduced ascorbate occurred in the Müller cell fibers and at the apices of the retinal pigment epithelium. Invest Ophthalmol Vis Sci 24:862–867, 1983

Low-level illumination has produced structural and functional damage to the retina in a variety of experimental conditions in rats, rabbits, and monkeys (for a review, see Noell1). In such photic injury of the retina, a mechanism has been proposed that involves the production of singlet oxygen or superoxide radical and subsequent lipid peroxidation of disc membranes of photoreceptor outer segments. The rod outer segments are particularly susceptible to such peroxidation because they are rich in polyunsaturated fatty acids. A number of agents have been suggested as natural protectants against peroxidation damage. Superoxide dismutase, catalase, peroxidase, α-tocopherol, glutathione, and ascorbic acid have been thought to play such a role in the eye.2

Ascorbate is found in high concentrations in the aqueous humor (3–28 mg/dl) and the lens (3.3–49.1 mg/dl) of many species, although plasma levels in most are less than 1 mg/dl.3 Ascorbate levels are also high in the retinas of the few species studied. The concentration of ascorbate in the retina of rats is 28 mg/dl, although aqueous and lens levels are 0–3 mg/dl.4 Heath et al5 reported that the concentration of ascorbate is 20 mg/dl in the retinas of rabbits, 22 mg/dl in cows, and 28 mg/dl in guinea pigs.

Several functions of the high level of ascorbate in the eye have been proposed. Friedenwald et al6 considered ascorbate vital in aqueous secretion; Cris-tiansson7 believed ascorbate to be critical in the polymerization of vitreal components; Varma et al8 implicated it in the protection of lens against oxidation; and recently, Nirankari et al9 established the importance of ascorbate in corneal lesion healing. Because of the strong evidence that ascorbate acts as an antioxidant in other systems, eg, brain,10 mast cells,11 and liver,12 we investigated its possible role as protectant against light damage in the retina of guinea pigs. The guinea pig was chosen because it is one of the few species other than primates, in which the scurvy state may be produced by dietary control.

We determined the level of reduced ascorbic acid (AA) and the oxidized form, dehydroascorbate (DHA), of the normal neural retina and pigment epithelium-choroid complex (PE-C) of the guinea pig, and localized histochemically the reduced ascorbate in the normal and scorbutic retina. We then established a light level that produced mild damage to the outer retina and retinal pigment epithelium (RPE) of the guinea pig, and compared the normal ascorbate values with those of the light-exposed neural retina and PE-C.

Materials and Methods

Twenty pigmented (Peruvian) and two albino guinea pigs (200–450 g) were housed under normal conditions of cyclic lighting (10 hours dark, 14 light) and fed a vitamin-C-enriched guinea pig diet...
(Purina®). Two albino guinea pigs were made scurvy with a vitamin-C-deficient diet (Purina®), supplemented with dry oatmeal. These animals were kept on the diet for at least two weeks and were judged scurvy by lack of weight gain, weakness, and/or skin lesions. Subsequent confirmation of the scurvy state was obtained by an analysis of aqueous, which contained less than 1 mg/dl ascorbate.

The normal room lighting consisted of a luminance at the floor of the cage of 3.2 to 15.8 Ft-L as measured with a Spectra Spotmeter® photometer. The illumination then was calculated to be about 10 Ft-C or 115 lux. Constant environmental lighting was obtained by placing Plexiglas® cages on a shelf with two 40-watt cool-white fluorescent bulbs, 8–9 inches from each side. White cardboards were placed under each cage and on the roof of the enclosure, 7 inches above the cages. The system provided a luminance at the floor of the cage of 10,000 to 20,000 lux or 800 to 1600 Ft-C. Sixteen guinea pigs were exposed for 6 (2 animals), 12 (2), 24 (2), or 48 (10) hours. The temperature of the enclosure was kept below 30°C which maintained the normal guinea pig body temperature of 37.8°C. Two animals were kept in each cage; throughout light exposure they were fed the same diet as when in normal cyclic lighting conditions. Eyes were enucleated immediately after exposure or, in five animals, after 7 days of normal cyclic lighting following 48 hours of light exposure. Normal and light-exposed eyes were processed for electron microscopy to determine morphologic damage caused by light exposure.

Guinea pigs were anesthetized with 25 mg/kg nembutal intraperitoneally. A drop of aqueous was aspirated from the anterior chamber with a No. 30 needle and frozen directly in a syringe covered with parafilm. The eyes were then rapidly removed, and the anterior portions of the eyes were cut away. The remaining eye cups were cleansed of vitreous, the neural retina was detached, and PE-C was dissected from sclera. Each sample was placed in a pre-weighted, pre-labeled capsule, which was again weighed and kept at –70°C until sent on dry ice for analysis.

Extracts of retinal tissues were obtained by homogenizing each tissue in 4% perchloric acid (one ml per 200 mg of wet tissue) and centrifuging. Aqueous was untreated. The ascorbate concentrations in aqueous humor and eye tissue extract were analyzed by high pressure liquid chromatography (HPLC), according to the method of Fox et al.13 Aqueous fluid or tissue extract was injected directly into the column, and the AA was measured by absorbance at 254 nm. Other aliquots of tissue extract were treated with dithioerythritol to reduce DHA. The total ascorbate concentration (AA and DHA) was then measured by HPLC. Aqueous was not treated further, since all ascorbate in aqueous was in the reduced form.13,15

Histochemical localization of AA was accomplished in the whole retinas of two normal and two scurvy albino guinea pigs by the silver method of Chinoy.14 Eye cups were fixed overnight at 3–5°C in a solution of 5 g AgNO₃, 34 ml H₂O₂, 66 ml absolute ethanol, and 5 ml glacial acetic acid, final pH 2.0 to 2.5, in lightproof containers. Eye cups were then washed two to three times in 70% alcoholic ammonia for 10–15 minutes, and then dehydrated and embedded in paraffin. Paraaffin sections were counterstained with cresyl violet. A brown precipitate of silver grains indicated sites of AA. Using the above conditions of incubation insured that other reducing agents such as glutathione, cystein, or reducing sugars were not involved and that oxidation of ascorbic acid did not occur.14 Albinos were used to decrease any reducing effect or masking of reaction product by melanin granules.

**Results**

The levels of AA and DHA of aqueous, neural retina, and PE-C of normal pigmented guinea pigs are shown in Fig. 2A. The form of ascorbate was predominantly AA in the neural retina and DHA in the PE-C.

Guinea pig retinas exposed to 10,000–20,000 lux for 48 hours showed evidence of morphologic damage, exhibiting vacuolation of the RPE and disruption, disorganization, and vacuolation of the outer and inner segments in focal areas of the retina (cf, Figs. 1A and B). The levels of AA and DHA of aqueous, neural retina, and PE-C of pigmented guinea pigs, light-exposed for 6, 12, 24, and 48 hours are shown in Figs. 2B–E. After 6 hours of exposure (Fig. 2B), ascorbate levels of neural retina lowered because of a decrease in AA that was statistically significant when compared with the normal ($P < 0.02$). Total ascorbate in PE-C was also less than that in the normal. When animals were exposed to successively longer periods (Figs. 2C–E) total retinal ascorbate levels gradually rose but still were below those of the normal retina. Total ascorbate of PE-C rose to that of the normal, but DHA was higher than in the normal, and AA remained low. Aqueous ascorbate after light exposure was slightly higher than that of the normal.

Ascorbate levels in eyes of animals allowed to recover for seven days after 48 hours of continuous fluorescent light are shown in Fig. 2F. In this recovery phase, there was a decrease of total ascorbate in the neural retina when compared with normal animals and with animals immediately after 48 hours of light.
exposure. However, in recovery, the percentage of AA in the neural retina remained about the same as in the normal or immediately after exposure, but in the PE-C the percentage of AA decreased further.

When the histochemical localization of reduced ascorbate was performed in the normal retina, silver grains were seen in Müller cell fibers, especially prominent in the inner plexiform layer, in focal areas of the outer plexiform layer, and around nuclei of the inner and outer nuclear layers. Silver grains also were
Fig. 2. Reduced (shaded area) and oxidized (clear area) aqueous (AQ), neural retina (R), and retinal pigment epithelium-choroid complex (PE-C). Values are expressed as mg/dl of aqueous fluid or mg/dl of wet tissue weight. The mean values of n eyes are represented along with the standard errors of the mean (I). The percent of total ascorbate which is in the reduced form (shaded area) is indicated above the bar. A. Normal light-adapted pigmented guinea pig. B. Immediately after 6 hours of fluorescent light exposure of 10,000–20,000 lux. C. Immediately after 12 hours of light exposure. D. Immediately after 24 hours of light exposure. E. Immediately after 48 hours of light exposure. F. One week after 48 hours of light exposure.

Discussion

In this study we have established concentrations of reduced and oxidized ascorbates in the neural retina and in the PE-C in the normal guinea pig. In a previous study, Heath et al. measured total ascorbate without differentiating reduced from the oxidized form in retinas of guinea pigs. Furthermore, it is not clear whether their reported value of 28.2 mg/dl included the RPE. Likewise, when Heath et al. reported on both reduced and oxidized forms of ascorbate in retinas of rats (total:24.5 mg/dl; 70–75% AA), it again was not clear whether their values included the RPE. In the normal guinea pigs in the present study, the predominance of AA in the neural retina and of DHA in the PE-C was evident. Because blood ascorbate is in the reduced form, it is reasonable to assume that the DHA in PE-C exists in the RPE and not in the stromal vessels.

Varma showed that the cation pump (as demonstrated by uptake of Rb+) of the in vitro lens was damaged by photochemical production of superoxide radicals. This damage was prevented by the addition of ascorbate to the medium. In more recent work, Varma has shown that lipid peroxidation, as measured by the formation of malonaldehyde, is caused by fluorescent light in in vitro rat lens. Such increases in malonaldehyde were prevented by addition of ascorbic acid. Hence, Varma suggested that the high level of reduced ascorbate in the aqueous and lens probably acts as an antioxidant to protect the lens from light damage. The presence of ascorbate in a similarly reduced form in the retina of the guinea pig and rat suggests that the ascorbate in the retina may likewise act as an antioxidant. 

The presence of DHA as the predominant form of ascorbate in the neural retina after light exposure further supports this hypothesis.
ascorbate in the PE may indicate the mechanism of transport of ascorbate into the retina. Like the aqueous, high concentrations of reduced ascorbate in the retina may indicate a process of active transport from the blood. Friedenwald reported that ascorbate was mainly in the oxidized form in the ciliary epithelium, although it was in the reduced form in blood, ciliary stroma, and aqueous. He suggested that the oxidation-reduction potential across the stromal-epithelial barrier is the driving force of aqueous secretion and of the active transport of ascorbate into the aqueous. After being secreted into the aqueous in the oxidized form, it is quickly reduced by an unknown mechanism. In a like manner, ascorbate as DHA might be actively transported from the blood and choroidal stroma through the RPE to the retina, where it exists in large concentrations.

This theory is supported further by our histochemical localization of reduced ascorbate in the apices of the RPE cells. In addition, the elevated levels of DHA in PE-C immediately after light exposure might indicate increased transport of ascorbate by the RPE to replenish decreased AA levels in the neural retina caused by photochemical oxidation.
Key words: retina ascorbate, guinea pig, aqueous, retinal pigment epithelium

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References