Low Vitamin E Level as a Subliminal Risk Factor in a Rat Model of Prednisolone-Induced Cataract

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PURPOSE. To investigate the relationship between vitamin E deficiency and prednisolone-induced cataract formation, long-term examination of lens changes was performed in rats under the condition of vitamin E deficiency or supplementation and administration of prednisolone.

METHODS. Rats were divided into six groups: normal chow (N), vitamin E-deficient chow (ED), normal chow with prednisolone instillation (NP), vitamin E-deficient chow with prednisolone instillation (EDP), NP treatment with vitamin E supplementation (NP+VE), and EDP treatment with vitamin E supplementation (EDP+VE). Prednisolone (1 mg/kg · d) and vitamin E (5%; 10 µL per administration per eye, 1 mg/kg · d) were applied in the cul-de-sac. Lens changes were documented and analyzed. Vitamin E status was confirmed by measuring peroxide-induced hemolysis.

RESULTS. After 15 months, 91.7% of the eyes in the EDP group showed development of anterior and posterior cortical cataracts. Supplementation with vitamin E significantly reduced cataract formation (to 38.9% of eyes). Neither a vitamin E-deficient diet nor prednisolone treatment alone significantly increased cataract formation. Hemolysis-susceptibility tests confirmed the expected vitamin E status of rats fed vitamin E-deficient chow and rats supplemented with eye drops containing vitamin E.

CONCLUSIONS. Vitamin E deficiency and long-term prednisolone treatment together cause cataracts. Singly, however, both conditions are subliminal cataractogenic risk factors. (Invest Ophthalmol Vis Sci. 2002;43:1116–1120)

Since Black et al.1 reported a high incidence of posterior subcapsular cataracts in 44 patients with rheumatoid arthritis in 1960, numerous studies concerning steroid-induced posterior subcapsular cataract have been performed. A review of corticosteroid-induced cataract was published by Urban and Cotlitter2 in 1986. The incidence of steroid cataract in patients receiving renal transplants varied from 6.5% to 96% because of differences in the duration of follow-up, patient age, and methods of steroid administration.3 However, there is no doubt that long-term steroid application is a risk factor for cataract formation.

There is no adequate therapy for steroid-induced cataract except cataract surgery. The search for treatments to prevent steroid-induced cataract is hampered by the lack of a suitable animal model. We have developed a prednisolone-induced cataract model using rats physically compromised by 2-Gy x-ray irradiation of the eye and subsequent long-term prednisolone administration.4,5 In these studies, either topical (eye drops) or systemic administration of prednisolone acetate induces morphologic changes in the rat lens similar to those found in human steroid-induced cataracts.6 Treatment of these animals with topical vitamin E ophthalmic solution prevents opacification.6 Based on these results, studies were initiated to determine whether cataracts could be induced in rats by combining a low-vitamin-E diet and long-term prednisolone administration. Cortical cataracts developed in animals treated in this manner. Application of topical vitamin E reduced the incidence of these cataracts.

MATERIALS AND METHODS

Animals were cared for and handled in accordance with the Guidelines for Animal Experiments in Kanazawa Medical University and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Seven-week-old male Brown-Norway rats were obtained from Sankyo Labo Service Co., Inc. (Toyama, Japan). The animals were maintained on normal rat chow (Labo MR stock; Sankyo Labo Service Co., Inc.) for 1 week before the start of the experiment. The anterior segments of all rats were examined using a slit lamp microscope (SL-5D; Topcon, Tokyo, Japan) and 53 rats with normal-appearing eyes were selected. Baseline values were recorded using an anterior segment analysis system (EAS-1000; Nidek, Gamagori, Japan) before starting the experiment.

Treatment Groups

The rats were divided into six groups. Six rats were fed a normal diet and served as the control (N group). The right eye of the control rats was treated with the vehicle used to prepare the vitamin E ophthalmic solution. Six rats were fed vitamin E-deficient chow (ED group). Both eyes of these animals were treated with the vehicle used to prepare the vitamin E ophthalmic solution. Twelve rats were fed normal chow and had 1% prednisolone acetate ophthalmic solution instilled in both eyes (NP group). Another 12 rats received the same treatment as the NP group and were fed vitamin E-deficient chow (EDP group). The fifth group (n = 8) received the same treatment as the NP group, but the rats were also treated with 5% vitamin E ophthalmic solution (VE) administered daily into both eyes (NP+VE). The final group (n = 9) received the same treatment as the EDP group, but with the daily administration of 5% vitamin E in both eyes (EDP+VE). The study was performed in a single-masked manner so that drug administration, documentation of lens conditions, and image analyses were conducted by three different examiners. Normal rat chow and vitamin E-deficient chow (AIN76) were provided by Sankyo Labo Service Co., Inc. Prednisolone acetate, vitamin E ophthalmic solution, and vitamin E vehicle were obtained from Santen Pharmaceutical Co., Ltd. (Osaka, Japan).

Drug Administration

Prednisolone acetate was dispersed in phosphate-buffered saline containing polysorbate 80. Ten microliters of a 1% solution of pred-
Vitamin E and Prednisolone-Induced Cataract

Evaluation of General Health and Vitamin E Status

The body weight of the rats was checked once a week as an index of general health condition. The appearance of their skin and hair and their activity were also checked weekly (not reported). Significant body weight differences were found during the following terms: N vs. ED (4 to 18 weeks and 29 to 41 weeks); ED vs. EDP (4 to 47 weeks); N vs. NP (4 to 75 weeks); ED vs. EDP + VE (9 to 18 weeks); EDP vs. EDP + VE (6 to 8 weeks and 63 to 75 weeks); N vs. NP + VE (8 to 12 weeks and 27 to 58 weeks); NP vs. NP + VE (5 to 7 weeks).

FIGURE 1. Mean values of body weight of the six experimental groups over the course of the experiment. N, 6 rats; ED, 6 rats; NP, 12 rats; EDP, 12 rats; NP + VE, 8 rats; EDP + VE, 8 rats. Significant body weight differences were found during the following terms: N vs. ED (4 to 18 weeks and 29 to 41 weeks); ED vs. EDP (4 to 47 weeks); N vs. NP (4 to 75 weeks); ED vs. EDP + VE (9 to 18 weeks); EDP vs. EDP + VE (6 to 8 weeks and 63 to 75 weeks); N vs. NP + VE (8 to 12 weeks and 27 to 58 weeks); NP vs. NP + VE (5 to 7 weeks).

Documentation of Changes in Lens Transparency and Cataract Formation

Lens changes were evaluated using a slit lamp microscope and were documented once a month with the analysis system (EAS-1000; Nidek) in rats under maximum mydriasis (1% Nitten; atropine sulfate ophthalmic solution; Nihon Tenganyaku Kenkyusho Co., Ltd., Nagoya, Japan). Changes in lens transparency were objectively evaluated using the quantitative retroillumination capabilities of the analysis system, as previously described.4–6

In brief, slit images were masked with a circle that excluded the cornea or iris. Reference images were obtained from each eye at the beginning of the experiment. Images obtained during the experiment were compared to these reference images. Changes in lens transparency were measured by determining the number of pixels in the image that showed increased opacity and compared to the reference image. Cataract diagnosis was performed using the slit lamp and from images generated using the analysis system. Human clinical criteria were applied in describing the location of opacities.

Statistical Analysis

Experimental data were evaluated using Student’s two-tailed t-test. P < 0.05 was considered significant.

RESULTS

Body Weight Changes

The body weights of the rats from each group were similar until 4 to 5 weeks after the start of the experiment. After that, the order of mean body weight of each group was: ED > EDP + VE > N > EDP > NP + VE > NP. The trend of the mean body weight order did not change throughout the experiment (Fig. 1).

Lens Changes Observed by Slit Lamp and Analysis System Documentation

Most of the rats in the normal diet groups (N, NP, NP + VE) and in two of the groups fed vitamin E-deficient chow (ED and EDP + VE) showed no observable lens opacities throughout the experiment. All groups showed an increase in eye size and slight increases in lens nuclear opacity. In contrast, most of the animals that were fed vitamin E-deficient chow and were treated with prednisolone (EDP) showed development of anterior and posterior cortical cataracts and an opacified subcapsular layer (Fig. 2).

A few rats from each group, except those fed normal chow and not treated with prednisolone (N), showed slight lens changes. Two eyes from two animals of the ED group (16.7%) had small anterior cortical cataracts that developed 8 months after the start of the experiment. These crescent-shaped opac-
ities elongated toward the equatorial region and had a filament-like extension by the end of the experiment. In four eyes of three animals (16.7%) from the NP group, crescent-shaped cataracts developed in the cortical layer at the equatorial region 10 months after the start of the experiment. Thereafter, these opacities elongated in the anterior and posterior directions. Rats from the NP/H11001VE group (four eyes from three animals, 25%) also had opacities that were similar to those just described.

In contrast to the occasional cataracts in the groups fed vitamin E-deficient chow or treated with prednisolone, 22 (91.7%) eyes of 12 animals of the EDP group had anterior cortical cataracts that developed 8 months after the start of the experiment. These opacities were originally similar to those seen in the ED group. By 15 months, however, animals in the EDP group had more severe anterior cortical cataracts and also had posterior cortical cataracts that included the subcapsular layer (Fig. 2).

When animals fed vitamin E-deficient chow and treated with prednisolone (EDP) were treated with topical vitamin E eye drops (EDP/VE), the incidence and severity of cataracts was significantly reduced (from 92% to 39%, P < 0.001; Fig. 2). It was also noted that the rats in the EDP group had a deeper anterior chamber than did the other groups (Figs. 2). Inspection of these eyes showed that the increase in anterior chamber depth was associated with thinning of the cornea, increased corneal curvature and, perhaps, a decrease in the size of the lens. Because this result was not seen in any other group, it must have been due to the combined effect of vitamin E deficiency and corticosteroid treatment.

**Comparison of Light-Scattering Areas in Each Group**

Figure 3 compares the mean area of increased light scattering in the treatment groups. The area of light scattering (as measured by the number of pixels with increased density in Scheimpflug slit images) increased steadily in all groups throughout the experiment. The light scattering in the EDP group, in particular, was significantly higher (from 92% to 39%, P < 0.001; Fig. 2).

A significant difference in light scattering between NP and NP/VE was seen at 4, 5, 8, 13, and 15 months (P < 0.05).

**Evaluation of Vitamin E Status**

Hemolysis induced by oxidative stress was used to evaluate the vitamin E status of each treatment group. All groups not fed a vitamin E-deficient diet showed low hemolysis (Fig. 4). Animals fed vitamin E-deficient chow and not treated with supplemental vitamin E (ED and EDP) showed high hemolysis rates. In contrast, when animals in the EDP group were treated with supplemental vitamin E, hemolysis was reduced to the range seen in animals fed a normal diet. These results show that the vitamin E eye drops used in this study restored animals deprived of dietary vitamin E to normal systemic vitamin E status.
It is known that drug penetration of the eye is more effective when the drug is instilled rather than administered to the whole body, because the eyeball becomes the drug’s target. This may explain why eye drop application of vitamin E supplementation showed positive effects.

The mechanisms of corticosteroid-induced cataract formation have been speculated to be inhibition of the Na,K-ATPase pump, binding of corticosteroids to lens proteins and the subsequent formation of lysine-keratoseptide adducts, inhibition of glucose-6-phosphate dehydrogenase, loss of adenosine triphosphate (ATP), and secondary oxidation of SH protein groups in lysine-keratoseptide adducts leading to the aggregation of crystallins. There are two ways of thinking about steroid-induced cataract mechanisms: The steroid itself directly affects the lens, or metabolite(s) of the steroid secondarily affect the lens. Circumstantial evidence for a direct effect of the steroid on the lens is that lenses cultured in the presence of prednisolone-containing medium show opacity in the outer lens cortex. This means that prednisolone can penetrate the lens and produce toxic effects directly in the lens. In our previous study, the application of eye drops resulted in a higher concentration of prednisolone in the lens than was obtained after retrobulbar or intramuscular injections. In addition, in vivo experimental results showed that the incidence of cataract formation was higher and the time course of development was faster with eye drop prednisolone application than in groups with systemic prednisolone application. These results indicate that prednisolone-induced cataract formation is related to the prednisolone concentration in the lens and that the prednisolone itself may have direct influence on opacification.

Nishigori et al. found that steroid injected into a chicken egg produced cataract in 15-day-old embryos. On the basis of these results they suggested that the effect of the steroid is indirect. In these studies, hydrocortisone treatment increased the content of glucose and lipid peroxide in the lens after 24 to 48 hours. Lenses from treated embryos also had decreased antioxidant protective systems, such as catalase, glutathione peroxidase, aniline hydroxylase, superoxide dismutase, and glutathione reductase. Because we did not measure any biochemical parameters in this experiment, we must perform further experiments to determine the indirect effect of prednisolone treatment in our model. We have only experience in measuring blood glucose in the model of 2-Gy x-ray and prednisolone administration. Although the groups treated with intravenous prednisolone (including the vitamin E-treated group) showed a higher blood glucose level from the 11th to the 30th weeks compared with the control group ($P < 0.05$), the difference was slight and not in the range of that in diabetes.

Vitamin E is well known to have functions including those of a chain-breaking antioxidant and membrane stabilizer. Possible mechanisms of prednisolone-induced cataract under

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Type of Opacity</th>
<th>Cataractous Eye Number/Eyes (%)</th>
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<tbody>
<tr>
<td>N (6)</td>
<td>No cataract</td>
<td>0</td>
</tr>
<tr>
<td>ED (6)</td>
<td>Ant. cortical</td>
<td>2/12 (16.7)</td>
</tr>
<tr>
<td>NP (12)</td>
<td>Ant./post. cortical</td>
<td>2/24 (16.7)</td>
</tr>
<tr>
<td>EDP (12)</td>
<td>Ant./post. cortical</td>
<td>22/24 (91.7)</td>
</tr>
<tr>
<td>NP+VE (8)</td>
<td>Ant./post. cortical</td>
<td>4/16 (25.0)</td>
</tr>
<tr>
<td>EDP+VE (9)</td>
<td>Ant./post. cortical</td>
<td>7/18 (38.9)</td>
</tr>
</tbody>
</table>

Ant. cortical, anterior cortical cataract; Ant./post. cortical, anterior and posterior cortical cataract.
the condition of vitamin E deficiency are as follows: A vitamin E-deficient condition is very sensitive to oxidation stress. An accumulation of prednisolone (or metabolites of prednisolone) in the lens can cause oxidation of the lens fiber, leading to cataract.

The mechanism of the effect of vitamin E against prednisolone acetate-induced cataract is probably its antioxidant effect and also its stabilization of the lens fiber membrane.

Although it is well known that steroids induce posterior subcapsular cataracts in humans, our rat prednisolone acetate-induced cataract model first showed anterior cortical cataract, with posterior subcapsular cataract developing several months later. In the future it will be important to clarify the reasons for the differences between human steroid cataracts and experimentally induced prednisolone acetate cataracts in rats.

General health conditions were monitored by changes in body weight. Our unexpected results showed that animals fed on the vitamin E-deficient chow had the highest mean body weight. This result may have been due to differences in the caloric content of the two diets or the amount of food consumed by animals fed the different formulations.

In this investigation we used a type of area densitometry of slit images to document changes in light scattering in the anterior segment. The accuracy and reproducibility of data obtained in this manner were satisfactory, even in small experimental animals. Tests of reproducibility had a coefficient of variation of 6%. Although image analysis of the lenses of young animals is always complicated by lens (and eye) growth, the methodology used in this study provides reliable measurements for lens transparency changes or opacification during most of the life of the animal.

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References


