Dietary α-Lipoic Acid Prevents UVB-Induced Corneal and Conjunctival Degeneration Through Multiple Effects

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PURPOSE. This study investigated the effects of dietary α-lipoic acid (α-LA) against ultraviolet B (UVB)-induced corneal and conjunctival degeneration in a mouse model.

METHODS. Female CBA mice were randomly divided into five study groups, including blank control, UVB without α-LA, and UVB with dietary α-LA at 1, 10, and 100 mg/kg body weight. Following UVB exposure, corneal surfaces were assessed along with immunohistochemistry for nuclear factor-kB (NF-κB), cyclooxygenase-2 (COX-2), malondialdehyde (MDA) accumulation, and P63+ basal cell distribution. Matrix metalloproteinase (MMP)-2 and MMP-9 activities were determined by gelatin zymography. ELISA assay was performed to confirm the findings of immunohistochemistry for NF-κB, COX-2, and MDA, along with the levels of TNF-α and IL-6. Tear production and goblet cell density were determined after tear strip assay and periodic acid Schiff staining, respectively.

RESULTS. The results showed that UVB irradiation caused corneal surface damage, polymorphonuclear leukocyte infiltration, and loss of P63+ basal cells. Dietary α-LA ameliorated the UVB-induced corneal damage while simultaneously reducing MDA accumulation and maintaining P63+ basal cell survival. NF-κB-p65, COX-2, TNF-α, IL-6, and MMP-9 activity were all reduced by dietary α-LA. In addition, α-LA helped to reverse aqueous tear reduction, conjunctival squamous epithelium metaplasia, and goblet cell loss after UVB exposure.

CONCLUSIONS. Dietary α-LA can prevent UVB-induced corneal damage and can be used as a prophylactic agent prior to excessive UVB exposure.

Keywords: α-lipoic acid, UVB, photokeratitis

Physiologically, the corneal surface serves to absorb ultraviolet B (UVB) with wavelengths ranging between 280 and 320 nm to protect the inner eye segments.1 Nevertheless, it is likely to be damaged if exposure to UVB becomes excessive, leading to the pathogenesis of photokeratitis.2–3 Excessive exposure to UV irradiation also causes conjunctival goblet cell degeneration, leading to decreased mucin secretion and formation of squamous metaplasia.3–6 Eventually, the corneal surface is likely to be desiccated with accelerated cell death in the corneal epithelium.

The molecular and cellular pathogenesis underlying photokeratitis has been extensively investigated in several animal models.7–9 Many molecules, such as interleukins, cytokines, matrix metalloproteinases (MMPs), and nuclear factor-kB (NF-κB), have been reported to be involved in the progression of this disease.7,8,10–13 Among these, NF-κB activation plays a key role through activation of cyclooxygenase-2 (COX-2).7,11,14–19 Excessive UVB exposure also elevates oxidative stresses, resulting in the accumulation of oxidative molecules such as malondialdehyde (MDA).20,21

α-Lipoic acid (α-LA) is known as an antioxidant and anti-inflammatory agent owing to its dual effects in both hydrophilic and lipophilic environments.22–24 Approximately 80% of the α-LA present in human tissues comes from the diet. Thus, insufficient intake of α-LA may lead to aggravation of diabetes-induced neuropathy.25 In experimental animal models, the beneficial effects of α-LA supplementation against diabetic retinopathy have been confirmed.26–28 The preventive effect of α-LA against UV-induced photokeratitis has been less well investigated. A previous report demonstrated that α-LA supplementation attenuated the damaging effects of UVA exposure in rabbit conjunctival and corneal tissues in vivo through reduction of MDA accumulation and elevation of glutathione peroxidase (GSH-PX) and superoxide dismutase (SOD) levels.29 Despite previous findings, the underlying mechanisms remain largely unknown, and whether dietary α-
LA supplementation can ameliorate UVB-induced photokeratitis and conjunctival disorders remains to be elucidated.

In this study, we used a mouse model to confirm that dietary intake of α-LA ameliorates the corneal damage caused by UVB irradiation and explored further the underlying mechanisms of this beneficial effect.

MATERIALS AND METHODS

Animals

Six-week-old female CBA mice were purchased from National Laboratory Animal Center, Taipei, Taiwan. The mice were examined with a slit lamp (model 99 BQ; Haag-Streit, Bern, Switzerland) prior to experiments. Only mice without anomalies of the anterior segment of the eye (cornea, anterior chamber, iris, or lens) were included in the experiments.

Study Groups, UVB Irradiation, and α-LA Treatment

The mice were randomly split into five groups, including (1) blank control (no UVB exposure, no α-LA treatment), (2) UVB (exposure to UVB, no α-LA treatment), (3) UVB/α-LA (1 mg/kg) (exposure to UVB with daily α-LA treatment at 1 mg/kg body weight), (4) UVB/α-LA (10 mg/kg) (exposure to UVB with daily α-LA treatment at 10 mg/kg body weight), and (5) UVB/α-LA (100 mg/kg) (exposure to UVB with daily α-LA treatment at 100 mg/kg body weight). To expose the corneas to UVB irradiation, the mice were anesthetized with intraperitoneal sodium pentobarbital injection (45 mg/kg body weight), with both of their eyes exposed to daily UVB light (CN-6; Vilber Lourmat, Germany) in a darkroom.10,30 Each daily UVB exposure was performed to reach a total amount of 0.72 J/cm² within 10 minutes, with the entire UVB exposure course completed in a consecutive 10-day period. The peak wavelength of UVB light was 312 nm. The UVB light was confirmed by using a UV detector (VXL-3W; Vilber Lourmat). For the groups with α-LA treatments, α-LA was supplemented in mouse chow, starting from day 0 (1 day before UVB exposure) and terminating on day 10. The UVB used in this study was a racemic mixture purchased from Sigma-Aldrich (cat. no. TS625-25G; St. Louis, MO). Care was taken to ensure that each mouse had taken the daily mouse chow containing the allocated dose of α-LA before any standard rodent chow could be further added. The mouse body weight was readily maintained between 25 and 27 g during the 10-day period of experiment. All mice were killed on day 11 for analysis. All experiment protocols were reviewed and approved by the Animal Care and Use Committee of Chung Shan Medical University and were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Scoring of Corneal Smoothness, Opacity, and Fluorescein Staining

All mice were anesthetized prior to analysis on day 11. One eye of each mouse was randomly selected for assessment of corneal smoothness. The other eye was then assessed for corneal opacity. The assessment procedures and criteria were applied in accordance with details published previously.10 Briefly, images of the corneal surface were taken with a stereoscopic zoom microscope equipped with ring illuminator (SMZ 1500; Nikon). Based on the digital images, the corneal smoothness scores were determined using a 5-point scale according to the number of distorted quadrants in the reflected ring: 0, no distortion; 1, distortion in one quadrant of the ring (3 clock hours); 2, distortion in two quadrants (6 clock hours); 3, distortion in three quadrants (9 clock hours); 4, distortion in all four quadrants (12 clock hours); and 5, severe distortion, in which no ring could be recognized. For corneal opacity scoring, the images were scored from 0 (normal) to 4 (severe).10 The criteria were as follows: 0, normal cornea; 0.5, mild haze seen only under dissection microscope; 1, mild haze; 2, moderate haze with visible iris; 3, severe haze with invisible iris; 4, severe haze with corneal ulceration. After corneal smoothness and opacity were scored, both corneas from each mouse were stained with 3 μL 1% fluorescein (Sigma-Aldrich). Images of fluorescein staining on the corneal surface were taken and scored according to a grading system based on areas of stain in the cornea.10 Briefly, the total area of punctuate staining was designated as grade 0; grade 1, less than 25% of cornea stained with scattered punctuate staining; grade 2, 25% to 50% of cornea stained with diffuse punctate staining; grade 3, 50% to 75% of cornea stained with punctuate staining and apparent epithelial defects; grade 4, more than 75% of cornea stained with abundant punctuate staining and large epithelial defects. All scorings were performed by two observers without prior knowledge of the UVB exposure and study groups.

Histopathologic Analysis and Immunohistochemistry

Following assessment of corneal damage, the mice were killed by cervical dislocation. One of the two eyes from each mouse was randomly selected and extracted. The extracted eyes, including the eyelids, were processed for hematoxylin–eosin (HE) stain following conventional procedures.10,31–35 For immunohistochemistry, the tissue sections were boiled in citrate buffer (pH 6.0) for 20 minutes for antigen retrieval and then incubated, respectively, with one of the following antibodies: mouse anti-β6 (1/50, cat. no. sc-8431; Santa Cruz Biotechnology, Santa Cruz, CA), rabbit anti-NF-κB-p65 (1/200, cat. no. E379; Epitomics, Cambridge, MA), rabbit anti-COX-2 (1/100, cat. no. ab15191; Abcam, Cambridge, MA), rabbit anti-malondialdehyde (MDA) antibody (1/100, cat. no. ab6465; Abcam), rabbit anti-MMP9 (1/100, cat. no. ab38898; Abcam), rabbit anti-MMP2 (1/100, cat. no. NB200-193; Novus Biologicals, Littleton, CO), or rabbit anti-lipidic acid (1/50, cat. no. ab58724; Abcam). The preparations were then incubated with a horseradish peroxidase-conjugated secondary antibody (1/200), either anti-mouse or anti-rabbit IgG (Jackson Immunoresearch Laboratories, Inc., West Grove, PA), followed by washes and incubation in diaminobenzidine tetrahydrochloride solution for color detection, and counterstain with hematoxylin.

ELISA and Zymography Assay

The expression levels of NF-κB, TNF-α, COX-2, MDA, and IL-6 in the cornea were measured by ELISA kits (for NF-κB, cat. no. KHO10271 [Invitrogen, Carlsbad, CA]; for TNF-α, cat. no. CMC0013 [Invitrogen]; for COX-2, cat. no. DYC4198-2 [R&D Systems, Inc., Minneapolis, MN]; for MDA, cat. no. STA-332 [Cell Biolabs, Inc., San Diego, CA]; for IL-6 [Koma Biotech, Inc., Seoul, South Korea]). A Tecan Sunrise ELISA reader (Tecan, Männedorf, Switzerland) was used. Zymography assay for MMP-2 and MMP-9 was performed following the procedures previously published by Di Girolamo et al.12

Aqueous Tear Production Test

Tear production was measured with a 1-mm-width strip cut off from pH test paper MR-BT (Advantech; Toyo Roshi Kaisha, Ltd., Tokyo, Japan) at days 0, 5, and 10. The animals were
anesthetized and rested for a standard time of 20 seconds. Then, a 1-mm-width strip was held with forceps under a dissection microscope and placed in the lateral canthus of the conjunctival fornix of the right eye for 20 seconds. The tear distance (in millimeters) was read under a microscope.

**Goblet Cell Density Assay**

Periodic acid Schiff (PAS) staining was performed to evaluate conjunctival epithelial morphology, and the number of goblet cells in the inferior conjunctiva was counted. The counting of goblet cells was performed under a microscope (ECLIPSE E100; Nikon, Melville, NY) with a ×20 objective. Five different sections were randomly selected for counting, and an average was calculated. All scorings were performed by two observers without prior knowledge of the UVB exposure and study groups.

**Statistical Analysis**

All data were obtained from triple repeats and are presented as the mean ± standard error of the means (SEM) and compared among groups. The corneal smoothness, opacity, and fluorescein staining scores were compared by the Kruskal-Wallis test.
The IL-6, TNF-α, NF-κB, COX-2, MDA, MMP-9 levels, P63+ cell density, goblet cell density, and tear secretion capability were analyzed by the Wilcoxon-Mann-Whitney test. All statistical analyses were performed using the SPSS program (SPSS, Inc., Chicago, IL).

RESULTS

Dietary α-LA Protects Against UVB-Induced Corneal Damage

The effects of UVB exposure on the cornea surface were first examined. Damage on the corneal smoothness was seen in the UVB group, but not in the blank control group. As an indication for damage to the corneal smoothness, the light circle seen on the corneal surface became irregular after exposure to UVB (Fig. 1A-b), in contrast to the regular circle seen in the control group (Fig. 1A-a). With dietary α-LA intake, the irregularity was significantly ameliorated, particularly in the UVB/α-LA (100 mg/kg) group (Fig. 1A-c). Quantitative analysis showed that the mean scores of corneal smoothness were significantly reduced in the UVB/α-LA (100 mg/kg) group ($P < 0.001$) and the UVB/α-LA (10 mg/kg) group ($P < 0.001$) as compared with the UVB group (Fig. 1B). Additionally, dietary α-LA intake helped to reduce the opacity in the cornea after UVB exposure (Fig. 1C). As shown in negative images, a broad area of opacity was routinely seen in the eyes from the UVB group (Fig. 1C-b), which was not found in the blank control group (Fig. 1C-a); and dietary α-LA helped to reduce cornea opacity after UVB exposure, particularly in the UVB/α-LA (100 mg/kg) group. The reduction of corneal opacity was also demonstrated by quantitative analysis (Fig. 1D).

Another way to characterize the UVB-induced damage on the corneal surface was to detect the devitalized epithelial areas after fluorescein staining. With UVB exposure, a large patch of green fluorescein stain could be seen, as represented by Figure 1E-b. In contrast, no green devitalized epithelial area was found in the eyes from the blank control group (Fig. 1E-a). Evident reduction of green fluorescein stain area was found after dietary α-LA intake, as indicated by Figures 1E-c, 1E-d, and 1E-e, particularly in their corresponding negative images. Quantitatively, significant reduction of green fluorescein stain areas was also demonstrated in the UVB/α-LA (10 mg/kg) and the UVB/α-LA (100 mg/kg) groups when compared with the UVB group (Fig. 1F).

To further characterize the protective effects of dietary α-LA against UVB-induced corneal damage, histologic analysis was performed. The results showed a significantly thinner corneal epithelial layer with UVB exposure (Fig. 1G-b) than without UVB exposure (Fig. 1G-a). The epithelial cells generally exhibited some vacuoles after UVB exposure, indicating the occurrence of cell death (Figs. 1G-b, 1G-c). Dietary α-LA helped to prevent the reduction of corneal epithelial thickness. When compared with those in the UVB group (Fig. 1G-b), the tissue sections from the eyes of the UVB/α-LA (1 mg/kg) (Fig. 1G-c), UVB/α-LA (10 mg/kg) (Fig. 1G-d), and UVB/α-LA (100 mg/kg) (Fig. 1G-e) groups were found to have a thicker epithelial layer in the central cornea.

Repletion of α-LA in the Corneal Epithelium Helps to Maintain P63+ Basal Cells With Inhibition of UVB-Induced MDA Accumulation and Proinflammatory Factors

Since different extents of protection against UVB-induced damage on the cornea surface were observed, the differential presence of α-LA in the cornea among study groups had to be confirmed as baseline data. Therefore, immunohistochemistry was performed to localize the distribution of α-LA in the corneas among study groups. In the blank corneas, α-LA was localized in the cornea epithelium, although only at a trace level (Supplementary Fig. S1A-a). After UVB exposure, α-LA was found to be invariably depleted (Supplementary Fig. S1A-b). Based on qualitative observation, repletion of α-LA in the cornea was found with different doses of dietary α-LA intake (Supplementary Fig. S1A-c, S1A-d, S1A-e).

To further understand the protective effects of dietary α-LA, we examined P63+ corneal basal cells to see whether the corneal repair capacity had been maintained. Decreased P63+ basal cell density was seen in the corneas exposed to UVB without dietary α-LA (Fig. 2A-b), in contrast to the normal distribution of P63+ basal cells in the blank control corneas (Fig. 2A-a). Interestingly, this scarcity of P63+ basal cells after UVB irradiation was not seen in the corneas from the UVB/α-LA (10 mg/kg) group (Fig. 2A-d) or from the UVB/α-LA (100 mg/kg) group (Fig. 2A-e). With dietary α-LA at 100 mg/kg body...
weight, the density of P63⁺ cells was close to that of the blank control group (Fig. 2B). Quantitatively, the mean P63⁺ cell number was significantly increased in the UVB/α-LA (100 mg/kg) group (P = 0.002) and in the UVB/α-LA (10 mg/kg) group (P = 0.002) as compared with the UVB group (Fig. 2B).

Since the thickness of epithelial layer in the central cornea was significantly reduced after UVB exposure, the epithelial cells must have been depleted beyond their regeneration capacity. A more direct analysis would be to analyze the dynamic changes of apoptotic and necrotic activities. However, as the UVB-exposed corneas were analyzed on day 10, it would not have been explanatory to examine the necrotic and apoptotic activities at this late stage. Therefore, we examined lipid peroxidation status, as represented by MDA accumulation, to serve as an alternative data to explain the protective effects of α-LA. Immunohistochemical staining showed that MDA was highly accumulated in the cornea after UVB irradiation (Fig. 2A-g) in contrast to the baseline status in the control group (Fig. 2A-k). MDA accumulation was depleted to a level close to that of the blank control group.

In addition, immunohistochemical staining was performed to examine the expression of two proinflammatory factors, NF-κB-p65 and COX-2, to further explain the protective effects of α-LA. A high level of NF-κB-p65 expression (Fig. 2A-i) and COX-2 expression (Fig. 2A-q) was seen in the corneas exposed to UVB without dietary α-LA, which was not observed in the blank group (Figs. 2A-k, 2A-p). Expression of NF-κB-p65 and COX-2 was attenuated in a dose-related manner with dietary α-LA intake (Figs. 2A-m, 2A-n, 2A-o, 2A-r, 2A-s, 2A-t, respectively). Particularly, high nuclear translocation of NF-κB-p65 was seen to correlate with the high level of NF-κB-p65 expression in the corneas exposed to UVB without dietary α-LA (Fig. 2A-i). As NF-κB-p65 expression was reduced in the corneas with dietary α-LA at 1 mg/kg (Fig. 2A-m) or 10 mg/kg (Fig. 2A-n) of body weight, more cytoplasmic localization of NF-κB-p65 was observed. When NF-κB-p65 expression was further reduced to approximately the same level (Fig. 2A-o) as that in the blank control (Fig. 2A-k), the localization of NF-κB-p65 became exclusive to the cytoplasm (Fig. 2A-o).

Since immunohistochemistry data allow only for qualitative comparison, we performed ELISA assay to further confirm our findings. The ELISA results showed that MDA accumulation was significantly decreased in the UVB/α-LA (100 mg/kg) group (P = 0.002) and in the UVB/α-LA (10 mg/kg) group (P = 0.004) as compared with the UVB group (Supplementary Fig. S2A). The ELISA results also showed that expression of NF-κB-p65 was significantly decreased in the UVB/α-LA (100 mg/kg) group (P = 0.01) as compared with the UVB group (Supplementary Fig. S2B). The expression of COX-2 was significantly decreased in the UVB/α-LA (100 mg/kg) group (P = 0.01) and in the UVB/α-LA (10 mg/kg) group (P = 0.025) as compared with the UVB group (Supplementary Fig. S2C).

Reduction of UVB-Induced Polymorphonuclear Leukocyte Infiltration and MMP-9 Expression by Dietary α-LA

After UVB irradiation, the corneal stroma was extensively infiltrated by polymorphonuclear (PMN) leukocytes (Figs. 3A-b, 3A-g). In contrast, significant reduction of PMN leukocyte...
infiltration was observed with dietary \( \alpha \)-LA intake at 10 or 100 mg/kg body weight (Figs. 3A-d, 3A-e, 3A-i, 3A-j). Quantitative analysis confirmed such reduction. The number of infiltrative PMN leukocytes was significantly reduced in the UVB/\( \alpha \)-LA (10 mg/kg) group (\( P < 0.001 \)) and the UVB/\( \alpha \)-LA (100 mg/kg) group (\( P < 0.001 \)) as compared with the UVB group (Fig. 3B).

Significant increase of MMP-9 activity in the corneas of the UVB group was detected as compared to that of the blank control group (Fig. 3C). The activity of MMP-9 in the cornea as reflected by zymography analysis was attenuated in a dose-related manner with dietary \( \alpha \)-LA intake (Fig. 3C). MMP-2 activity, however, was only barely detected after UVB irradiation (Fig. 3C). The inhibition of MMP-9 activity by dietary \( \alpha \)-LA was also reflected by the fold change of total MMP-9 expression as compared to that of the UVB group (\( P = 0.015 \) for 10 mg/kg and \( P = 0.004 \) for 100 mg/kg body weight) (Fig. 3D). Immunohistochemical detection for MMP-9 and MMP-2 was performed to confirm the results obtained by zymography analysis (Supplementary Fig. S1B). The results showed that MMP-9 expression was much more apparently increased after UVB irradiation than MMP-2. Dietary \( \alpha \)-LA supplementation, an evident decrease in MMP-9 expression was seen, while the change in MMP-2 expression was not easily detected since it remained at a basal level after UVB irradiation.

To further confirm the inhibitory effects of dietary \( \alpha \)-LA on PMN leukocyte infiltration after UVB exposure, we used ELISA assay to examine the expression of TNF-\( \alpha \) and IL-6, two common factors concurrent with PMN leukocyte infiltration. In agreement with the PMN leukocyte infiltration data, TNF-\( \alpha \) and IL-6 expression was highly induced by UVB exposure as compared to that in the blank control group, and dietary \( \alpha \)-LA at 10 or 100 mg/kg body weight was able to significantly reduce TNF-\( \alpha \) (Fig. 3E) and IL-6 expression (Fig. 3F). These data support a dose-related effect of dietary \( \alpha \)-LA to reduce PMN leukocyte infiltration, with simultaneous reduction of MMP-9 activity as well as TNF-\( \alpha \) and IL-6 expression in the cornea following UVB exposure.

**Dietary \( \alpha \)-LA Ameliorates UVB-Induced Photophobia and Aqueous Tear Reduction**

The mice without UVB exposure had widely opened eyes (Fig. 4A-a). After UVB irradiation, photophobia was reflected by a restriction of eye opening (Fig. 4A-b). Dietary \( \alpha \)-LA helped to ameliorate UVB-induced photophobia in the mice fed 10 mg/kg (Fig. 4A-d) or 100 mg/kg (Fig. 4A-e) body weight. The mice with \( \alpha \)-LA at 1 mg/kg, however, did not show obvious amelioration of photophobia (Fig. 4A-c).

The baseline level of aqueous tear secretion showed no significant difference among the four study groups. Significant decrease in aqueous tear secretion was found after 5 and 10 days of UVB exposure as compared to the value in the blank controls (Fig. 4B). The mean length of strip wetting of the control group (\( n = 10 \)) and the UVB group (\( n = 10 \)) was 2.58 ± 0.29 mm and 2.63 ± 0.35 mm and 1.23 ± 0.43 mm, respectively, on day 5 (\( P < 0.001 \)); and 2.55 ± 0.38 and 0.93 ± 0.40 mm on day 10 (\( P < 0.001 \)). Therefore, the mean length of strip wetting at day 10 in the UVB group was reduced to 37% of the baseline status at day 0 if no dietary \( \alpha \)-LA was given. With dietary \( \alpha \)-LA given at 10 mg/kg body weight (\( n = 10 \)), the mean length of strip wetting was 2.05 ± 0.31 mm on day 10, showing significant increase as compared with the value in the UVB group (\( P < 0.001 \)) (Fig. 4B-d). With dietary \( \alpha \)-LA given at 100 mg/kg body weight (\( n = 10 \)), significant increase of the mean length of strip wetting
exposed to UVB, the oxidative stresses will be induced to a phototoxic level, leading to activation of inflammatory factors such as NF-κB and TNF-α.19 Excessive UVB exposure also poses a risk to the conjunctival goblet cells. Therefore, shields to protect from UVB irradiation have to be used to minimize UVB exposure.

Apart from minimizing UVB exposure, interventions by drugs or natural products to prevent UVB-induced photokeratitis have been studied in animal models in recent years.37-41 Among these, α-LA has been demonstrated to exert beneficial effects on various ocular diseases, such as cataract,42 glaucoma,43 diabetic retinopathy,43-46 and macular degeneration.44-47

The potential beneficial effects of α-LA on photokeratitis have been less well investigated. Demir et al.29 reported that α-LA administration through intraperitoneal injection prevented eyes from damage caused by UVA irradiation in a rabbit model. However, whether oral α-LA administration has the same beneficial effects remains to be investigated.

In the present study, we showed that (1) dietary α-LA prevents UVB-induced corneal damage with simultaneous reduction of MDA accumulation and increase of P63 expression in the cornea; (2) multiple inflammatory factors, including NF-κB, p65, COX-2, IL-6, and TNF-α, can be inhibited by dietary α-LA, if sufficient dose is given; (3) dietary α-LA suppresses the activation of MMP-9 in the cornea after UVB irradiation; (4) aqueous tear secretion is reduced as a consequence of excessive UVB exposure, and the reduction can be prevented by dietary α-LA; and (5) conjunctival goblet cell density is also reduced due to excessive UVB exposure, and dietary α-LA helps to maintain conjunctival goblet cell density. Adding to previous findings, our results demonstrated that dietary α-LA attenuates UVB-induced corneal damage through multiple effects. The observed mechanisms provide new insights into the role of α-LA in the cornea. For example, whether all the beneficial effects can be attributed to inhibition of oxidative stresses remains to be determined. A recent report by Ying et al.45 confirmed that α-LA might inhibit NF-κB-dependent pathway without involvement of its antioxidant activity when human umbilical vein endothelial cells (HUVECs) were treated with TNF-α. In another report, it was shown that α-LA inhibits TNF-α-induced NF-κB activation in rheumatoid arthritis (RA) fibroblast-like synovial cells.46 A direct effect of α-LA was also found in the reduction of NF-κB activity and IL-4 synthesis in a mouse airway inflammation and hyperresponsiveness model of asthma.47 Such direct amelioration of

**DISCUSSION**

Ultraviolet B irradiation is the most critical factor in the pathogenesis of photokeratitis, since most of its energy is absorbed by the cornea.34-38 When eyes are excessively
inflammation by α-LA without involvement of its antioxidant activity was also reported in the aortic tissue and neurons, where a phosphatidylinositol 3-kinase/Akt-dependent mechanism was implicated. Moreover, α-LA was shown to directly interact with DNA and prevent nuclear translocation of NF-κB. With these previous reports taken into consideration, the multiple effects of dietary α-LA in the prevention of UVB-induced corneal and conjunctival degeneration likely result from more than just the antioxidant activity of α-LA.

Recent studies have suggested that corneal remodeling is mediated through sequential events involving activation of matrix metalloproteinases (MMPs), especially MMP-2 and MMP-9. Therefore, dysregulated activation of MMPs in the cornea might eventually contribute to decreased corneal surface smoothness or increased corneal opacity. In this study, we postulated that dietary α-LA may prevent UVB-induced corneal surface irregularity through alteration of MMPs. This postulate was supported by our results, showing elevation of MMP-9 activity in the cornea after UVB irradiation and the inhibitory effect on MMP-9 activity by dietary α-LA. Nevertheless, as to the inhibition of MMP-2 activity after UVB irradiation, our data did not show any apparent difference with dietary α-LA. This is likely due to the lack of apparent MMP-2 activity induction with our current UVB irradiation protocol. Our data are in agreement with a previous report by Kim et al. who showed the inhibitory effect of α-LA on MMP-9 through inhibition of NF-κB transcriptional activity in vascular smooth muscle cells. Ultraviolet B irradiation might have altered the activity of other MMPs in the mouse model, as reported by Di Girolamo et al., who showed UVB-induced MMP-1 expression in human ocular surface epithelial cells. Obviously, the ways in which UVB or other high-energy lights may alter the activities of MMPs, and how such alterations may interact with multiple inflammatory factors and affect the pathogenesis of photokeratitis, warrant further investigations.

After excessive UVB exposure, dietary α-LA reduces a number of proinflammatory factors, including NF-κB, COX-2, TNF-α, and IL-6, as demonstrated in this study. This will alleviate photokeratitis symptoms through at least two pathways. First, the activation of proinflammatory factors was shown to inhibit neurotransmitter release, leading to insufficient lacrimal gland stimulation. As a result, aqueous tear secretion would be reduced after excessive UVB exposure, adding to the damage caused by photokeratitis. Our data reflect this scenario by showing reduction of aqueous tear secretion following excessive UVB exposure and the preventive effects of dietary α-LA. Second, the persistent presence of proinflammatory factors in the aqueous tear is likely to cause conjunctival squamous metaplasia and loss of goblet cells in the conjunctival epithelium, which may subsequently lead to deterioration of tear quality and quantity. Our data also explain this scenario by showing the reduction of conjunctival goblet cell density and the reversal of effects by dietary α-LA.

If dietary α-LA is to be routinely used as a prophylactic agent against UVB-induced photokeratitis, the potential toxic effects have to be carefully considered. Obrosova and colleagues used daily DL-α-lipoic acid 100 mg/kg intraperitoneally for 6 weeks in a rat model without any toxicity observed. In the present study, the maximal daily dietary dose was 100 mg/kg for a 10-day period, and no toxic effect was found. α-lipoic acid is generally regarded as extremely safe in the amounts utilized clinically, and no serious adverse effects have been reported so far.

We conclude that dietary α-LA, given in a sufficient dose, is effective in protecting against UVB-induced photokeratitis and subsequent corneal and conjunctival degeneration, probably through multiple mechanisms other than antioxidative stresses. Given that α-LA has been used for years as a dietary supplement for other purposes, the extra beneficial effects against UVB-induced ocular surface damage may be considered.

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