Cornea

0.005% Preservative-Free Latanoprost Induces Dry Eye-Like Ocular Surface Damage via Promotion of Inflammation in Mice

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PURPOSE. To investigate the side effects of preservative-free 0.005% latanoprost on the murine ocular surface.

METHODS. We applied 0.005% latanoprost or vehicle in mice in two patterns for 14 to 28 days. Tear production was measured by phenol red cotton test, and corneal epithelial barrier function was assessed by Oregon-green-dextran (OGD) staining. Periodic acid-Schiff (PAS) staining was used to quantify conjunctival goblet cells (GCs). The expression of matrix metalloproteinase (MMP)-3 and -9, occludin-1 and zonula occludens (ZO)-1 in corneal epithelium was assessed by immunofluorescent staining and/or quantitative real-time PCR (qRT-PCR). Inflammation in conjunctiva was assessed by activation of P38 and NF-κB, infiltration of CD4+ T cells, and production inflammatory cytokines including TNF-α, IL-1β, IFN-γ, IL-17A, and IL-13. Apoptosis in ocular surface was assessed by TUNEL and immunofluorescent staining for activated caspase-3 and -8. Cell viability assay was performed in human corneal epithelial cells.

RESULTS. Topical latanoprost treatment decreased tear production, induced conjunctival GC loss, disrupted the corneal epithelial barrier, and promoted cell apoptosis in the ocular surface. Topical latanoprost treatment increased the expression of MMP-3 and -9, and decreased the expression of ZO-1 and occludin-1 in the corneal epithelium. Topical application of latanoprost promoted activation of P38 and NF-κB signaling and production of TNF-α and IL-1β in conjunctiva. Topical application of latanoprost increased CD4+ T cells infiltration, with increased production of IFN-γ and IL-17A and decreased production of IL-13 in conjunctiva.

CONCLUSION. 0.005% latanoprost induced dry eye-like ocular surface damage via promotion of inflammation in mice.

Keywords: latanoprost, dry eye, inflammation, ocular surface

Glaucoma is the second leading cause of irreversible blindness and affects nearly 70 million people worldwide.1 Increased pathologic intraocular pressure (IOP) is a major risk factor for glaucoma. The most widely prescribed therapies for glaucoma are topical ocular drops. Prostaglandin (PG) analog and beta-blockers are typically the initial medications. Some open angle glaucoma patients even need a lifetime medication to control IOP.

PG analog eye drops are often prescribed as first-line IOP-reducing agents for open angle glaucoma.2 Latanoprost, an ester prodrug of prostaglandinF2α (PGF2α), was the first of the currently available topical PG analogs to be launched for glaucoma or ocular hypertension and still accounts for the majority of prescriptions.3 However, it is proven to have deleterious effects on the ocular surface. A growing body of clinical and experimental studies revealed that long-term use of latanoprost eye drops may lead to tear film instability; epithelial apoptosis; corneal epithelial barrier disruption; conjunctival inflammation; and ocular discomfort including burning, stinging, pain, and foreign body sensation.4–7

Latanoprost eye drops usually contain a preservative, which is generally considered to be the major factor responsible for ocular surface side effects. Preservatives used in latanoprost eye drops, such as benzalkonium chloride (BAC), may cause or aggravate dry eye disease through various mechanisms such as its toxic and proinflammatory effects as well as detergent properties, which have been well demonstrated in numerous experimental and clinical investigations.8 Several clinical studies have shown that switching from a preserved latanoprost eye drops to preservative-free formulation significantly alleviated ocular surface damage and ocular symptoms.9,10
The contribution of latanoprost itself to the adverse effects on the ocular surface remain controversial. A clinical trial showed that patients who used latanoprost eye drops containing 0.02% BAC exhibited a higher expression of ocular surface inflammation marker HLA-DR in conjunctiva than patients who used unpreserved substitute tears eye drops with the same concentration of BAC. Suggesting that latanoprost itself may induce ocular surface inflammation. On the other hand, an experiment performed in human cultivated corneal epithelial sheets indicated that preservative-free latanoprost eye drops destroyed corneal epithelial barrier function after 6 hours of exposure. By contrast, several latanoprost eye drops destroyed corneal epithelial cytotoxic effect than BAC alone. Further studies are necessary to explore the effects of latanoprost itself on the ocular surface.

In this study, we focused on the toxic effect of 0.005% preservative-free latanoprost eye drops on murine ocular surface and investigated the potential mechanism underlying it. The significance of the findings was further discussed.

Materials and Methods

Animals

Female C57/BL6J mice (aged 8–11 weeks) were used in this study (Shanghai SLAC Laboratory Animal Center, Shanghai, China). The protocol of this research was approved by the Experimental Animal Ethics Committee of Xiamen University and it conformed to the standards in the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Topical Administration of 0.005% Latanoprost

Nonpreserved Latanoprost (0.005%, L1167-5MG; Sigma-Aldrich Corp., St. Louis, MO, USA) was dissolved in PBS containing 0.01% dimethyl sulfoxide (DMSO). The mice were randomly assigned to three groups: untreated control mice (UT) that did not receive any administration of eye drops, vehicle eye drops–treated mice (V), and 0.005% latanoprost eye drops–treated mice (L). Topical application of the eye drops was performed four times daily for 7 to 14 days. In another different experimental procedure, mice were divided into the same groups and the same concentration of latanoprost was topically administered, but only once daily for 28 days.

Measurement of Tear Production

Tear production (20 eyes/10 mice/group) was measured with phenol red cotton threads (Zone-Quick, Yokota, Tokyo, Japan) at the same time point (8 AM). The thread was placed on the lower conjunctival fornix at approximately one-third of the lower eyelid distance from the lateral canthus for 15 seconds. The length of the wet red thread was measured in millimeters using the scale on the cotton thread.

Corneal Permeability

Corneal permeability (10 eyes/5 mice/group) to Oregon green dextran (OGD; 70,000 MW, Anionic, Lytine Fixable, Catalog no. D7173; Invitrogen, Eugene, OR, USA) was assessed as previously described. Briefly, 0.5 µL OGD (50 mg/mL) was instilled onto the ocular surface 60 seconds before euthanasia. Corneas were then rinsed with 5 mL saline (1 mL/time, 5 times), and photographed with a digital camera (AZ100; Nikon, Tokyo, Japan) under fluorescence excitation at 470 nm. A 3-mm diameter circle was placed on the central cornea in the digital images to measure the OGD intensity using the analysis software (NIS Elements, version 4.1; Nikon, Melville, NY, USA).

Histology

The left eyes and ocular adnexa of mice from each group were surgically excised, embedded in optimal cutting temperature (OCT) compound (Catalog No. 4583; SAKURA Tissue-Tek, Torrance, CA, USA). Sagittal 5-µm sections were cut using a cryostat (CM1850 UV; Leica Microsystems, Wetzlar, Germany) at the center of the eye. The frozen sections were stored at −80°C, and used for immunostaining. The right eyes and ocular adnexa of mice from each group were excised, fixed in 4% formaldehyde for 24 hours, dehydrated with an automatic tissue hydroextractor (ASP 2005; Leica Microsystems) and infiltrated with paraffin (Catalog No. P3683; Sigma-Aldrich Corp.) at 60°C. The paraffin sections were used for PAS staining and TUNEL.

Conjunctival Goblet Cell Count

Goblet cells (GCs) in the conjunctiva were stained using a PAS staining kit (Catalog No. 395B-1KT; Sigma-Aldrich Corp.). Digital images of representative areas of the conjunctiva were captured with a light microscope (Eclipse 50i; Nikon). The number of GCs in the whole conjunctiva was counted using analysis software (Nikon).

Immunofluorescent Staining

Immunofluorescent staining was performed with polyclonal antibodies to immunolocalize matrix metalloproteinase (MMP)-3, MMP-9, activated (AC)-caspase-3 and AC-caspase-8. Cryosections were first fixed with cold acetone at −20°C for 10 minutes, and then permeabilized with PBS containing 0.2% Triton X-100 for 20 minutes. Samples were incubated with primary antibodies (goat anti-MMP-3 antibody, 1:50, Catalog No. sc-6839; Santa Cruz Biotechnology, Dallas, TX, USA; Rabbit anti-MMP-9 antibody, 1:250, catalog no. ab38898; Abcam, Inc., Cambridge, MA, USA; Rabbit anti-AC-caspase-3 antibody, 1:250, catalog no. ab52181; Abcam, Inc.; or rabbit anti-AC-caspase-8 antibody, 1:250, catalog no. ab25901; Abcam, Inc.) at 4°C overnight. Negative controls were incubated with PBS excluded any primary antibody at the same time. The next day, after three rinses with PBS, secondary antibody, appropriate Alexa Fluor 488–conjugated donkey anti-goat (1:500; catalog no. A11055; Invitrogen), or anti-rabbit IgG (1:300; catalog no. A21206; Invitrogen) was applied and incubated in a dark chamber for 1 hour at room temperature, followed by counterstaining with 4,6-diamidino-2-phenylindole (DAPI; catalog no. H-1200; Vector Laboratories, Inc., Burlingame, CA, USA). Digital images of representative areas were captured with an upright microscope (DM2500; Leica Microsystems), and the fluorescent intensity was measured using analysis software (Nikon).

Whole Mounted Cornea Staining

Whole freshly harvested murine corneas were fixed in cold acetone at −20°C for 5 minutes, then washed three times with PBS containing 1% Triton X-100 and 1% DMSO; each was lasted 10 minutes. After being blocked with 2% bovine albumin (BSA) for 1 hour at room temperature to reduce nonspecific labeling, samples were incubated with polyclonal rabbit anti-ZO-1 (1:100, catalog no. 617300; Invitrogen) overnight at 4°C. Samples without primary antibody were used as negative controls. Alexa Fluor 488–conjugated donkey anti-rabbit IgG.
Latanoprost and Dry Eye

(1:300, catalog no. A21206; Invitrogen) was performed in the dark for 5 hours at 4°C, followed by counterstaining with Hoechst. Digital images were captured with a laser-scanning confocal microscope (FV10MPE-B; Olympus Corp., Tokyo, Japan).

**Immunohistochemistry**

Frozen sections were performed to detect the infiltration of CD4 positive cells in conjunctiva. After being blocked with 20% goat serum for 30 minutes, samples were stained with rat anti-mouse CD4 antibody (1:50, catalog no. 553647; BD Pharmingen, San Jose, CA, USA) for 60 minutes, biotinylated goat anti-rat secondary antibody (1:25, catalog no. 559286; BD Pharmingen) for 30 minutes, and a detection kit (Vectastain Elite ABC using NovoRed reagents, catalog no. PK-6100; Vector Laboratories, Inc.). The CD4 positive staining cells in conjunctiva were counted using image analysis software (Nikon). The data was expressed as the total number of positively cells in conjunctiva.

**TUNEL Assay**

TUNEL assay was performed on paraffin sections with a commercially available kit (DeadEnd Fluorometric TUNEL System, catalog no. G2350; Promega, Madison, WI, USA) according to manufacturer’s instructions. Digital images (512 pixels) of representative areas of the corneal epithelium and tarsal conjunctiva were captured with an upright microscope (DM2500; Leica Microsystems), and the TUNEL-positive cells in the corneal epithelium and tarsal conjunctiva in 200-μm length in the sagittal sections were counted.

**Total RNA Extraction, Reverse Transcription, and Quantitative Real-Time PCR**

Pooled samples were collected from the corneal epithelium or conjunctiva of both eyes of each mouse. Total RNA was isolated using an RNA isolation kit (ARCTURUS PicoPure, catalog no. KIT0204; Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer’s instructions. We measured the RNA concentration by its absorption at 260 nm to determine the RNA volume to transcription. First-strand cDNA was synthesized using a reverse transcription kit (catalog no. RR047A; TaKaRa, Shiga, Japan). Real-time PCR was performed on a real-time PCR system (LightCycler 96, catalog no. 05815916001; Roche, Basel, Switzerland) using a master mix (no Rox; Hieff qPCR SYBR Green, catalog no. 11201ES08; Yeasen, Shanghai, China). The primer sequences are summarized in the Table. The amplification program included an initial denaturation step at 95°C for 600 seconds, followed by 45 cycles of 95°C for 10 seconds, 60°C for 10 seconds and 72°C for 20 seconds, after which a melt curve analysis was conducted to verify amplification specificity. Results were analyzed using a comparative threshold cycle (CT) method and normalized with β-actin as an endogenous reference.

**ELISA Analysis**

Conjunctivas proteins were minced and lysed in cold RIPA buffer (Sigma-Aldrich Corp.). The protein concentration was measured with a BCA protein assay kit (catalog no. 23225; Thermo Fisher Scientific), and balanced at a same concentration in all groups. ELISA kits were used to detect the protein concentrations of IL-17A (catalog no. BMS6001; eBioscience, San Diego, CA, USA), IL-13 (catalog no. BMS6015; eBioscience), IFN-γ (catalog no. BMS6066; eBioscience), IL-1β (catalog no. BMS6075; eBioscience), and TNF-α (catalog no. BMS6002; eBioscience) according to the manufacturer’s instructions. The optical absorbance was measured at 450 nm with a microplate reader (Bio-Tek ELx800; Bio-Tek Instruments, Winooski, VT, USA), and the concentration of each sample was measured according to the instruction manual.

**Western Blot**

Conjunctival proteins were extracted with cold RIPA buffer. Following the protein concentration balanced to a same level, the supernatant was heated to 100°C for 10 minutes to denature the protein. Equal protein content was loaded to electrophoresis on 10% Tricine gels, and then electronically transferred to polyvinylidene fluoride (PVDF) membranes (catalog no. IPVH00010; Millipore, Billerica, MA, USA). The membranes were blocked in 2% BSA at 1 hour at room temperature, and then incubated overnight at 4°C with primary antibodies including phosphorylated (p)-NF-κB (1:500; catalog no. 3033S; Cell Signaling Technology, MA, USA), NF-κB (1:500; catalog no. 8242S; Cell Signaling Technology), p-P38 (1:1000, catalog no. 9216S; Cell Signaling Technology) or P38 (1:500, catalog no. 9212L; Cell Signaling Technology). β-actin (1:10,000; Sigma-Aldrich Corp.) was used as a loading control. Next day, after three washes with Tris-buffered saline containing 0.05% Tween 20 (TBST) for 10 minutes, the membranes were incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (1:10,000; catalog no. a0545; Sigma-Aldrich Corp.) or goat anti-mouse IgG (1:10,000; Sigma-Aldrich Corp.) for 1h at room temperature. The specific bands were visualized by an enhanced chemiluminescence reagent (catalog no. ECL-500; ECL, Lulong, Inc., Xiamen, China), and the image intensity was calculated with the transilluminator (ChemiDoc XRS System; BioRad Laboratories, Inc., Philadelphia, PA, USA).

**Cell Culture and Cell Viability by CCK-8 Assay**

Human corneal epithelial (HCE) cells, obtained from RIKEN BioResource Research Center (Tokyo, Japan), was cultured in Dulbecco’s modified eagle medium: Nutrient mixture F-12 (DMEM-F12; Invitrogen, Carlsbad, CA, USA) supplemented with 6% heat-inactivated fetal bovine serum (FBS) and 1% penicillin/streptomycin.

The viability of the cell was measured with a cell counting kit (Cell Counting Kit-8 [CCK-8], C0038; Beyotime, Beijing, China). HCE cells were separately seeded into 96-well plates (1 × 10⁴ cells per well) with 100 μL DMEM-F12. After 24 hours incubation with 5% CO₂ atmosphere at 37°C, the culture medium was removed and replaced with 100 μL untreated DMEM-F12 medium (UT). DMEM-F12 medium containing 0.005% preservative-free latanoprost (0.05% latanoprost), and DMEM-F12 medium containing 0.01% DMSO (vehicle control) respectively (n = 6), and the cells were further incubated for 2 hours, 6 hours, 12 hours, and 24 hours. A 10-μL CCK-8 reagent was then added into each well for 2 hours’ incubation in the dark; the optical absorbance was measured at 450 nm with a microplate reader (Bio-Tek Instruments).

**Statistical Analysis**

Data was represented as mean ± SD. Statistical significance was evaluated by 2-way ANOVA with Bonferroni’s post hoc test or Mann-Whitney test using graphing software (GraphPad Prism 5.0; GraphPad Software, San Diego, CA, USA). A value of P ≤ 0.05 was considered statistically significant.
RESULTS

Topical Application of Latanoprost Eye Drops Decreased Tear Production

Compared to the vehicle control mice, the latanoprost eye drop–treated mice showed significantly decreased tear production since day 7 (Fig. 1A; vehicle control versus latanoprost, 3.8 ± 1.4 mm vs. 2.6 ± 0.9 mm at day 7, *P* < 0.01; 3.7 ± 0.6 vs. 2.4 ± 0.8 at day 14, *P* < 0.05). Meanwhile, it also was demonstrated that once daily application of latanoprost eye drops significantly decreased tear production eventually (Supplementary Fig. S1A).

Topical Application of Latanoprost Eye Drops Resulting in Conjunctival GC Loss

Topical application of latanoprost eye drops resulted in a decreased number of conjunctival GCs since day 7 (Figs. 1B, 1C; vehicle control versus latanoprost, 107 ± 18 vs. 77 ± 16, *P* < 0.05 at day 7; 103 ± 8 vs. 39 ± 8 at day 14, *P* < 0.01). In addition, it was revealed that once daily application of latanoprost eye drops also significantly decreased the number of conjunctival GCs eventually (Supplementary Fig. S1B).

Topical Application of Latanoprost Eye Drops Disrupted Corneal Epithelial Barrier Function

The corneal epithelial barrier function can be assessed by measuring the corneal permeability to OGD. The mean intensity of corneal OGD staining in the latanoprost eye drop–treated group was significantly higher than that in the vehicle control group at day 7 (Figs. 2A, 2B, vehicle control versus latanoprost, 7.2 ± 0.3 vs. 8.2 ± 1.1, *P* < 0.05).

Meanwhile, once daily application of latanoprost eye drops also significantly increased the mean intensity of corneal OGD staining eventually (Supplementary Fig. S2).

Taken together, it was demonstrated that topical application of latanoprost in both two different patterns of administration eventually induced reduction of tear production, loss of conjunctival GCs and damage of corneal epithelium.

Next we investigated the underlying mechanism of the damage of corneal epithelium induced by latanoprost. ZO-1 and occludin-1, as markers of corneal epithelial tight junction,
are expressed in the superficial cell layer of the corneal epithelium. In this study, immunofluorescent staining showed topical latanoprost eye drops treatment resulted in loss of ZO-1 in the superficial corneal epithelial cellular border (Fig. 2C), and qRT-PCR showed that topical latanoprost eye drops treatment significantly decreased the mRNA levels of ZO-1 and occludin-1 in corneal epithelium at day 7 (Figs. 2D, 2E). MMP-3 and -9 are key factors in corneal epithelial barrier disruption in ocular surface disease. This study investigated the expression of MMP-3 and -9 in corneal epithelium using immunofluorescent staining and qRT-PCR, and found that topical application of latanoprost eye drops significantly promoted the expression of MMP-3 and -9 in corneal epithelium at day 7 (Fig. 3). These results, taken together, suggested that topical application of latanoprost could disrupt the corneal epithelial barrier function.

Topical Latanoprost Treatment Activated P38-NF-κB Signaling Pathway in Ocular Surface

Latanoprost binds with high affinity to the PGF2α receptor. The PGF2α receptor is a member of the G protein-coupled receptor family, and can stimulate various inflammatory signaling pathways such as P38 and NF-κB. This study examined whether latanoprost could affect the phosphorylation of P38 and NF-κB in conjunctiva. As shown in Figures 4A through 4D, topical latanoprost eye drops treatment promoted activation of P38 and NF-κB in conjunctiva. Considering that P38-NF-κB signaling refers to inflammation and production of inflammatory cytokines, we investigated the effect of topical latanoprost treatment on production of inflammatory cytokines, and found that topical latanoprost eye drops treatment significantly promoted the production of TNF-α and IL-1β in conjunctiva at day 7 (Figs. 4E-H).

Topical Application of Latanoprost Eye Drops Induced CD4+ T Cell–Mediated Pathogenesis in Ocular Surface

Topical application of latanoprost eye drops significantly promoted the infiltration of CD4+ T cells in conjunctiva at day 7 (Figs. 5A, B). qRT-PCR and ELISA assay showed that topical latanoprost eye drops treatment significantly increased the expression of CD3, CD4, and CD8 in conjunctiva at day 7 (Figs. 5C, D). Furthermore, topical latanoprost eye drops treatment significantly promoted the infiltration of CD4+ T cells in conjunctiva at day 7 (Figs. 5E, F). These results suggest that topical latanoprost eye drops treatment significantly increased the infiltration of CD4+ T cells in conjunctiva at day 7.
the production of IL-17A and IFN-γ and decreased the production of IL-13 in conjunctiva at day 7 (Figs. 5C, 5D).

Topical Latanoprost Treatment Promoted Apoptosis in Ocular Surface

Topical latanoprost eye drops treatment significantly increased the immunoreactivity of AC-caspase-3 and -8 in conjunctiva at day 7 (Figs. 6A–D). TUNEL assay showed that topical latanoprost eye drops treatment significantly increased the number of apoptotic cells in ocular surface at day 7 (Figs. 5E–G).

Latanoprost Decreased Cell Viability of HCE Cells

We also conducted the cell culture experiment to determine if latanoprost has toxic effects on the HCE cells by measurement of cell viability with CCK-8 assay. It was demonstrated that latanoprost at 0.005% induced the reduction of cell viability of HCE cells after treatment of 2, 6, 12, and 24 hours (Fig. S3). On the other hand, since latanoprost was dissolved in DMSO, we examined the effects of the concentration of DMSO used in cultured HCE cells. It showed that DMSO at 0.01% only did not induce any significant changes of cell viability of HCE cells after incubated for 2, 6, 12, and 24 hours (Fig. S3).

DISCUSSION

The findings of the present study demonstrated that topical application of 0.005% latanoprost eye drops induced dry eye-like ocular surface damage in mice. For many years, dry eye has been shown to be a common side effect of PG analog eye drops. The side effects caused by long-term use of PG analogs...
The effects of topical latanoprost eye drops treatment on activation of P38-NF-κB signaling in conjunctiva. P38 and NF-κB activation was evaluated by Western blot, with β-actin as a loading control. The level of phosphorylated-NF-κB (p-NF-κB) and total-NF-κB in conjunctiva (A). The ratio of the intensity of p-NF-κB to total NF-κB in conjunctiva (B). The level of phosphorylated-P38 (p-P38) and total-P38 in conjunctiva (C). The ratio of the intensity of p-P38 to total P38 in conjunctiva (D). The mRNA levels of IL-1β (E) and TNF-α (F) in conjunctiva. The protein levels of IL-1β (G) and TNF-α (H) in conjunctiva detected by ELISA assay. Data shown as mean ± SD; *P < 0.05.

Eye drops are typically considered to be related to preservative, such as BAC. The present study demonstrated that topical application of preservative-free 0.005% latanoprost also decreased tear production, induced conjunctival GCs loss, disrupted corneal epithelial barrier, and promoted cell apoptosis and inflammation in ocular surface in mice. These changes are all considered to be main manifestation of clinical dry eye. This evidence suggests that dry eye induced by latanoprost eye drops are not only due to the preservative but also related to latanoprost itself.

The present study showed that topical application of latanoprost eye drops induced ocular surface inflammation resembling to that in dry eye. Dry eye is a chronic inflammatory ocular surface disease. The inflammatory response in dry eye is an increscent process that involves activation of inflammatory pathways such as p38 and NF-κB in the ocular surface; Release of proinflammatory cytokines and chemokines including IL-1β, TNF-α, and MMP-9; upregulation of major histocompatibility complex class II and activation of antigen presenting cells in ocular surface; recruitment of T helper (Th1) and Th17 cells to the inflammatory site; and amplification of immune response and forming of a vicious circle. Latanoprost can bind with high affinity to the PGF2α receptor that is abundant in the conjunctiva. The PGF2α receptor is a member of the G protein–coupled receptor family, and plays an active role in transcriptional regulation that triggers various signaling pathways including P38 and NF-κB. NF-κB is a key transcriptional activator of multiple proinflammatory cytokines, such as MMPs, IL-1β, and TNF-α. As previously reported, latanoprost can activate P38-NF-κB signaling pathways in human Tenon’s capsule fibroblasts, which lead to increased production of inflammatory cytokines and chemokines. Similar to the previous report, the present study showed that topical application of 0.005% latanoprost activated P38-NF-κB signaling pathways with increased production of inflammatory cytokines including TNF-α, IL-1β, MMP-3, and MMP-9 in conjunctiva. In addition, the present study showed that topical application of latanoprost increased CD4+ T cells infiltration with increased production of IFN-γ and IL-17A and decreased production of IL-13 in conjunctiva, which may be due to the consequence of activation of P38-NF-κB signaling and increased production of inflammatory cytokines in ocular surface. Taken together, the evidence of this study suggested that latanoprost, binding to its receptors, may induce dry eye–like ocular surface inflammation, including activation of P38-NF-κB pathway, production of inflammatory cytokines, and CD4+ T cell infiltration in ocular surface.

Corneal epithelial barrier dysfunction is a key feature of dry eye. Several lines of evidence suggested that inflammatory cytokines are responsible for corneal epithelial barrier defect in ocular surface disease. IL-17A, a cytokine of the Th17 response, has been reported to induce corneal epithelial barrier dysfunction via promoting production of MMP-3 and -9 in dry eye. TNF-α, IL-1β, and IFN-γ have been shown to induce corneal epithelial barrier dysfunction through downregulation of the corneal epithelial cell adhesion molecules of tight junctions, such as ZO-1 and occludin-1.

Table 1. Primers Used in This Study

<table>
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<th>Gene</th>
<th>Sense Primer</th>
<th>Anti-Sense Primer</th>
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<tr>
<td>MMP-3</td>
<td>CTTTGTATGGCCCTGGAAC</td>
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<tr>
<td>MMP-9</td>
<td>CACTCTTGCAAGTGTTGAG</td>
<td>AGTDAAGGAGGGGCCCTGTTA</td>
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<td>ZO-1</td>
<td>GTTTCGCTTGAAGCAAGGCCGTC</td>
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<td>Ocludin-1</td>
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<td>IL-17A</td>
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<td>IFN-γ</td>
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<td>IL-13</td>
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<td>β-actin</td>
<td>CCTAAGGCGACCGTGAAAG</td>
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FIGURE 4. The effects of topical latanoprost eye drops treatment on activation of P38-NF-κB signaling in conjunctiva. P38 and NF-κB activation was evaluated by Western blot, with β-actin as a loading control. The level of phosphorylated-NF-κB (p-NF-κB) and total-NF-κB in conjunctiva (A). The ratio of the intensity of p-NF-κB to total NF-κB in conjunctiva (B). The level of phosphorylated-P38 (p-P38) and total-P38 in conjunctiva (C). The ratio of the intensity of p-P38 to total P38 in conjunctiva (D). The mRNA levels of IL-1β (E) and TNF-α (F) in conjunctiva. The protein levels of IL-1β (G) and TNF-α (H) in conjunctiva detected by ELISA assay. Data shown as mean ± SD; *P < 0.05.
study, topical application of latanoprost increased the corneal OGD staining, decreased the expression of ZO-1 and occludin-1 in corneal epithelium, and promoted the production of inflammatory cytokines including MMP-9, MMP-3, IL-17A, TNF-α, IFN-γ, and IL-1β in ocular surface, indicating that topical application of latanoprost may induce corneal barrier dysfunction via promoting the production inflammatory cytokines.

APOPTOSIS has been suggested to contribute to the pathogenesis of dry eye. Several cytokines, such as IFN-γ, IL-1β, and TNF-α, have been shown to trigger cell apoptosis. TNF-α has been reported to promote conjunctival epithelial apoptosis through caspase-8-mediated extrinsic apoptotic pathways in mice under desiccating stress. Accordingly, we speculate that, in the present study, the ocular surface cell apoptosis induced by topical application of latanoprost may be related to the increased production of inflammatory cytokines.

Conjunctival GCs play an important role in maintaining the ocular surface homeostasis. It has been reported that decreased IL-13/IFN-γ ratio contributes to conjunctival GC loss in dry eye. IL-13, the predominant Th2 cytokine, has been shown to have homeostatic effects in promoting GC differentiation, whereas IFN-γ, the Th1 cytokine, has been shown to promote GCs apoptosis. In the present study, topical latanoprost notably decreased the number of conjunctival GCs, and this is accompanied by decreased production of IL-13 and increased production of IFN-γ. These findings suggested that topical application of latanoprost induces conjunctival GCs loss through breaking the balance of IL-13 and IFN-γ.

This present study, consistent with the previous report, demonstrates that latanoprost can significantly decreased the cellular viability of HCE cells.

Generally, most glaucoma clinicians have prescribed latanoprost to patients one time daily. In the present study, we also performed an experiment to explore the toxic effects of one

![Figure 5](image-url)
time daily of 0.005% latanoprost for 4 weeks in mice. It demonstrated that this design of application of latanoprost also eventually induced identical toxic effects on ocular surface, which were consistent with that of our design (4 times daily for 1–2 weeks). Based on our limited information, no long-term data demonstrated that latanoprost itself could cause dry eye-like ocular surface damage in glaucoma patients, which requires further investigation; however, our results provide experimental evidence that latanoprost itself does have ocular surface toxicity and can cause dry eye-like ocular surface changes in mice. The findings could be a reference for clinical medication.

In summary, this study showed that topical application of 0.005% latanoprost could induce dry eye-like ocular surface damage via promotion of inflammation in mice. The mechanism by which latanoprost reduces IOP is mainly due to expansion of the intermuscular spaces in the ciliary body through extracellular matrix remodeling by MMPs. Multiple studies have elucidated that latanoprost can stimulate human ciliary smooth muscle cells to secret MMPs such as MMP-1, MMP-3, and MMP-9. However, the findings of this study showed that the inflammation induced by latanoprost is harmful to ocular surface in mice. Therefore, glaucoma clinicians need to consider the possible ocular surface toxicity of latanoprost after long-term usage when they prescribe it to glaucoma patients, especially to those with severe ocular surface damage or severe tear film instability.

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References