Protective Effect of Uridine on Cornea in a Rabbit Dry Eye Model

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PURPOSE. To investigate the effect of uridine on cultured human corneal epithelial cells and keratocytes in vitro and to evaluate whether the application of uridine-containing eye drops could improve ocular surface health in an in vivo dry eye model.

METHODS. Uridine was added to cultivated epithelial cells (3 × 104 cells/well) and keratocytes (1 × 104 cells/well) at various concentrations (0.5–50 μM). Cytotoxicity was tested with the use of MTT assay, and the cells were assessed for apoptosis with the use of flow cytometry. Expressions of hyaluronic acid (HA), glycosaminoglycan (GAG), nitric oxide (NO), and matrix metalloproteinase (MMP)-9 were measured. In vivo, the degree of rec epithelialization was assessed after topical application of uridine (100 μM) in a rabbit corneal wound model. Changes in tear production and conjunctival goblet cell counts were investigated after instillation of various concentrations of uridine-containing eye drops in a rabbit dry eye model.

RESULTS. In vitro, uridine showed no cellular toxicity. It increased the biosynthesis of HA and GAG and reduced MMP-9 levels in cultured corneal epithelial cells and keratocytes. In vivo, uridine enhanced corneal wound healing and significantly increased the number of conjunctival goblet cells in rabbits.

CONCLUSIONS. Uridine can restore the health of the ocular surface in a rabbit corneal wound and dry eye model. (Invest Ophthalmol Vis Sci. 2007;48:1102–1109) DOI:10.1167/iovs.06-08089

Dry eye syndrome is a common disorder that affects approximately 10% to 20% of the adult population worldwide.1,2 Dry eye is associated with a decrease in tear aqueous production and abnormalities of the lipid, protein, and mucin profiles. These changes cause desiccation of the ocular surface, leading to epithelial damage as a result of inflammation on the surface. Although dry eye develops from multiple etiologies, it is most often treated with artificial tear supplementation, which is palliative in nature and provides relief for only a short period. Nevertheless, only two pharmacologically active agents are available to treat this condition—cyclosporine A (Restasis; Allergan Inc., Irvine, CA), which reduces inflammation, and hyaluronic acid (HA), which promotes epithelial healing.3,4 Uridine is transformed to uridine 5’-triphosphate (UTP) by uridine kinase in vivo. UTP, as a full agonist at the P2Y2 receptor,5 is known to stimulate mucin secretion in various tissues such as conjunctiva,6 nasal epithelial cells,7 and tracheal gland cells.8 One report suggests that elevated uridine 5’-diphosphate (UDP)-glucose contributes to an increase in the synthesis of intrinsic HA,9 which promotes cell migration, stabilizes the ocular surface epithelial barrier, and controls localized inflammation in patients with keratoconjunctivitis sicca.10–12

In the present study, we investigated the effect of uridine on cultured human corneal epithelial cells and keratocytes in vitro and evaluated whether the application of uridine-containing eye drops could improve ocular surface health in an in vivo dry eye model.

MATERIALS AND METHODS

This study was approved by the Institutional Review Board and the Institutional Animal Care and Use Committee of the Seoul National University Hospital and was performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Human Corneal Epithelial Cells and Keratocytes Culture

In accordance with the tenets of the Declaration of Helsinki and with proper informed consent, 20 human corneoscleral rims were obtained 5 days after harvest from the Northwest Lions Eye Bank within 8 hours of penetrating keratoplasty. Each tissue sample was treated with 0.05% trypsin and 0.01% EDTA at 37°C and then was collected four times at 20-minute intervals. NIH/3T3 (ATCC, Manassas, VA) cell lines were used as a feeder layer and were treated with 3 µg/mL mitomycin C (Sigma, St. Louis) at 37°C for 2 hours and were plated to 80% confluence for coculture with epithelial cells. Suspended cells seeded in 1.5 × 105 cells/cm2 on the 3T3-preconditioned plates were primarily cultured with the SFEM medium at 37°C in a carbon dioxide incubator for 2 weeks. Culture medium was a mixture of Dulbecco modified Eagle medium (DMEM) and Ham F12 medium (1:1 mixture) and included fetal bovine serum (FBS; 10%), insulin (5 mg/mL), cholera toxin (0.1 nM), epidermal growth factor (10 ng/mL), and penicillin-streptomycin (PS; 50 IU/mL). For the keratocyte culture, the remaining human limbal tissues were treated with 1.2 U/mL dispase II for 16 hours at 4°C. Next, 200 U/mL type I collagenase (5 mL) was applied to the neutral protease for coculture with epithelial cells. Suspended cells seeded in 1.5 × 105 cells/cm2 on the 3T3-preconditioned plates were primarily cultured with the SFEM medium at 37°C in a carbon dioxide incubator for 2 weeks. Culture medium was a mixture of Dulbecco modified Eagle medium (DMEM) and Ham F12 medium (1:1 mixture) and included fetal bovine serum (FBS; 10%), insulin (5 mg/mL), cholera toxin (0.1 nM), epidermal growth factor (10 ng/mL), and penicillin-streptomycin (PS; 50 IU/mL). For the keratocyte culture, the remaining human limbal tissues were treated with 1.2 U/mL dispase II for 16 hours at 4°C. Next, 200 U/mL type I collagenase (5 mL) was applied to the neutral protease (Dispase)-treated tissues at 37°C, and keratocytes were cultured every 30 minutes after shaking three times. Collected cells were cultured in DMEM/F12 (1:1) with 10% FBS and 1% PS at 37°C in a carbon dioxide incubator for 2 weeks.

Assessment of the Effect of Uridine on Cultured Corneal Epithelial Cells and Keratocytes In Vitro

After 2 weeks of cultivation, epithelial cells (3 × 104 cells/well) and keratocytes (1 × 104 cells/well) were harvested and plated on collagen...
The percentage of apoptotic cells was calculated in the sub-G0/G1 peak. Cells were permeabilized and stained with propidium iodide (PI; 0.1 M) for oxidative stress, and the Griess reagent (100 µM, 4 ng/mL) was used for colorimetric determination of NO2− as a measure of total plasma NO.

**Cytotoxicity Test.** We added 10 µL uridine to cultured epithelial cells and keratocytes at various concentrations (0.1–100 µM). After 48 hours, the viable cells were quantitated by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide; Roche, Mannheim, Germany) assay according to a protocol recommended by the manufacturer.

**Apoptosis Assay.** Epithelial cells (4 × 105 cells) and keratocytes (1 × 105 cells), treated with 50 µM and 100 µM uridine for 48 hours, were permeabilized and stained with propidium iodide (PI; 0.1 µg/mL) and analyzed by flow cytometry (FACSCalibur; BD Biosciences, Oxford, UK). Apoptotic cells showed low DNA stainability resulting in a distinct, quantifiable region below the G0/G1 peak, and then the percentage of apoptotic cells was calculated in the sub-G0/G1 peak region.

**In Vitro HA Biosynthesis Assay.** Various concentrations of uridine (0.5–50 µM) were added once to cultured epithelial cells and keratocytes with uridine, was added to the sample (100 µL) for oxidative stress, and the Griess reagent (100 µM, 4 ng/mL) was used for colorimetric determination of NO2− as a measure of total plasma NO.

**In Vitro Nitric Oxide Assay.** H2O2 (100 µM, 10 µL), including cultured epithelial cells and keratocytes with uridine, was added to the sample (100 µL) for oxidative stress, and the Griess reagent (100 µM, 4 ng/mL) was used for colorimetric determination of NO2− as a measure of total plasma NO.

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FIGURE 1. Cytotoxicity test using MTT assay at various concentrations of uridine (0.1–100 µM) in cultured corneal epithelial cells (A) and keratocytes (B). Uridine had no cellular toxicity at any concentrations examine. Data are expressed as mean ± SD. *P < 0.05 (Student’s t test).

IV (5 µg/mL)-coated 96-well and plain 96-well plates, respectively, and were stabilized for 1 day before additional experiments.

**In Vitro Matrix Metalloproteinase-9 Assay.** Total RNA was isolated from corneal epithelial cells with uridine by acid guanidinium thiocyanate-phenol-chloroform extraction according to a previously described method.13 PCR primers for matrix metalloproteinase (MMP)-9 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were designed from published human gene sequences (552 bp; sense, ATC CAG TTT GTC GCG AGC; antisense, GAA GGG GAA GAC GCA CAG CT). Reverse transcription–polymerase chain reaction (RT-PCR) was performed with a RT-PCR kit (TaKaRa; Takara, Shiga, Japan) to evaluate the expression of these MMP-9 genes by corneal epithelial cells with a housekeeping gene, GAPDH, as the internal control.14 Expression levels of...
MMP-9 transcripts were semiquantified with an image analyzer and densitometry (Tina 2.0; Raytest, Straubenhardt, Germany).

Assessment of the Effect of Uridine on Corneal Wound Repair in a Rabbit by Topical Eye Drops Application

Establishment of Corneal Epithelial Wound Model in a Rabbit Using Mechanical Scraping. Twenty-four adult New Zealand White rabbits (male, 2–3 kg) were used. The cornea was marked with 7.0-mm trephine, and the epithelium was mechanically scraped with a cotton-tip applicator after application of 90% ethyl alcohol for 20 seconds.

Assessment of Reepithelialization. Uridine (100 µM) and PBS solution were administered four times per day in the study group and in controls, respectively (n = 12 in each group). Photographs were taken 1, 2, 3, 4, and 5 days after epithelium removal, and the ratio of the epithelial defect area to the total corneal area was calculated with an image analyzer (Image Pro Plus; Media Cybernetics, Silver Spring, MD).

Assessment of the Effect of Uridine in a Rabbit Dry Eye Model by Topical Eye Drops Application

Establishment of a Short-term Rabbit Dry Eye Model. Twenty-three adult New Zealand White rabbits (male, 2–3 kg) were injected with 0.5 mL concanavalin A (Con A; 10 mg/mL; Sigma). Con A was injected twice, with a 3-day interval, through the fornix into the lacrimal gland of 13 rabbits15; 10 other rabbits were used for controls. Tear production was measured by the Schirmer test each day, and impression cytology was performed on the superior conjunctiva on day 3 and day 10 after the first Con A injection. Lacrimal glands of three rabbits in the Con A group were acquired and sectioned 3 days after injection and were evaluated histologically with hematoxylin and eosin (H&E) staining.

Application of Uridine-Containing Eye Drops. Sixty adult New Zealand White rabbits (male, 2–3 kg) were used. Uridine-containing eye drops were formulated in normal saline at concentrations of 1%, 5%, and 10%. Each of the concentrations of topical uridine and (0.1% HA; Hyalene; Santen, Osaka, Japan) was topically applied randomly to one eye beginning 3 days after the first injection of Con A and consecutively for 7 days thereafter (n = 5 in the 1% and 10% uridine groups; n = 10 in the hyaluronic acid group; n = 20 in the 5% uridine group). In the group used as controls, no eye drops were administered in another 20 eyes from 20 rabbits after Con A injection.

Measurement of Aqueous Tear Production. Tear production was measured with the Schirmer test in each group at day 3 and day 10 after the first Con A injection, respectively (i.e., just before eye drop application and 7 days after onset of eye drop application).

Measurement of Conjunctival Goblet Cell Density. Impression cytology16 was performed in each group at day 3 and day 10 after the first Con A injection, respectively (i.e., just before eye drop application and 7 days after the onset of eye drop application). Cytology specimens were taken from the superior conjunctiva of each eye, and goblet cells were counted in 10 consecutive high-power fields (HPFs) after periodic acid-Schiff (PAS) reagent staining.

GAG assay (epithelial cells)

<table>
<thead>
<tr>
<th>Concentration of uridine (µM)</th>
<th>0</th>
<th>0.5</th>
<th>1</th>
<th>5</th>
<th>10</th>
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<td>HA assay (epithelial cells)</td>
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GAG assay (keratocytes)

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<thead>
<tr>
<th>Concentration of uridine (µM)</th>
<th>0</th>
<th>0.5</th>
<th>1</th>
<th>5</th>
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<th>50</th>
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<tr>
<td>HA assay (keratocytes)</td>
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FIGURE 4. Glycosaminoglycan (GAG) biosynthesis assay using an enzyme-linked immunosorbent assay at various concentrations of uridine (0.5–50.0 µM) in cultured epithelial cells (A) and keratocytes (B). GAG increased significantly in uridine-added epithelial cells and keratocytes at all the concentrations examined. Data are expressed as mean ± SD. *P < 0.05 (Student’s t test).
Within each group, temporal changes in tear amount and goblet cell counts were tested with the Student’s t test. The difference between pretreatment and posttreatment measurements in tear amount and goblet cell counts was obtained by subtracting measurements at day 3 from measurements at day 10 in each group; mean change was compared between the groups using a Kruskal-Wallis test.

\[ P < 0.05 \] was considered statistically significant.

**RESULTS**

**Effect of Uridine on Cultured Corneal Epithelial Cells and Keratocytes**

MTT assay revealed cell proliferation rather than cytotoxicity at all concentrations of uridine (0.1–100 \( \mu M \); Figs. 1A, 1B). No differences in apoptosis rates were observed between uridine-treated cells and untreated controls through flow cytometry of epithelial cells and keratocytes (Figs. 2A, 2B).

HA increased significantly in uridine-added epithelial cells and keratocytes. Concentrations of HA were 0.97 ng/mL in the control without uridine and increased to 2.55, 2.69, 2.87, and 1.92 ng/mL in cultured epithelial cells at 1.0 \( \mu M \), 5.0 \( \mu M \), 10.0 \( \mu M \), and 50.0 \( \mu M \), respectively. The increase in HA was not dose dependent (Fig. 3A). In cultured keratocytes, the concentrations of HA at baseline were 4.29 ng/mL but increased to 8.56, 8.84, 9.82, and 11.64 ng/mL at 1.0 \( \mu M \), 5.0 \( \mu M \), 10.0 \( \mu M \), and 50.0 \( \mu M \), respectively (Fig. 3B). HA increase was dose dependent.

GAG concentration also increased significantly with uridine. It was 1.38 \( \mu M \) in the control without uridine and increased to 4.76 \( \mu M \), 6.45 \( \mu M \), and 6.45 \( \mu M \) in epithelial cells with 0.5 \( \mu M \), 5.0 \( \mu M \), and 50.0 \( \mu M \) uridine, respectively (Fig. 4A). In keratocytes, the concentrations of GAG were 3.92 \( \mu M \), 4.76 \( \mu M \), and 6.45 \( \mu M \) when 0.5 \( \mu M \), 5.0 \( \mu M \), and 50.0 \( \mu M \) of uridine was added respectively, whereas it was 1.38 \( \mu M \) when uridine was not added. GAG increase was dose dependent (Fig. 4B).

The application of uridine reduced NO production significantly at 50 \( \mu M \), but only in keratocytes (Fig. 5). Uridine also decreased the expression of MMP-9 at all studied concentrations (Fig. 6). NO assay (Fig. 5A) and MMP-9 assay (Fig. 6A) showed significant decrease at 50 \( \mu M \) uridine in keratocytes, whereas the decrease in NO was not significant in epithelial cells. Data are expressed as mean ± SD.

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**Statistical Methods**

Within each group, temporal changes in tear amount and goblet cell counts were tested with the Student’s t test. The difference between pretreatment and posttreatment measurements in tear amount and goblet cell counts was obtained by subtracting measurements at day 3 from measurements at day 10 in each group; mean change was compared between the groups using a Kruskal-Wallis test. \( P < 0.05 \) was considered statistically significant.

**FIGURE 5.** Nitric oxide (NO) assay using an enzyme-linked immunosorbent assay at various concentrations of uridine (0.5–50.0 \( \mu M \)) in cultured epithelial cells (A) and keratocytes (B). NO decreased significantly at 50 \( \mu M \) uridine in keratocytes, whereas the decrease in NO was not significant in epithelial cells. Data are expressed as mean ± SD. \( * P < 0.05 \) (Student’s t test).

**FIGURE 6.** Reverse transcription-polymerase chain reaction analysis for MMP-9 mRNA in cultured epithelial cells (A). When quantified with densitometry, MMP-9 decreased at all applied concentrations of uridine in cultured epithelial cells, showing the highest reduction at 50.0 \( \mu M \) (B). Data are expressed as mean ± SD *\( P < 0.05 \) (Student’s t test).

**FIGURE 7.** Assessment of reepithelialization in a rabbit corneal wound model with topical application of uridine (100 \( \mu M \)). The ratio of epithelial defect area to total corneal area was calculated by an image analyzer. Corneal wound healing was more rapid in the eyes with uridine, compared with those in control group, though it was not statistically significant. Data are expressed as mean ± SD.
between treated eyes and controls before and after treatment. No significant differences in tear secretion were observed compared with the values at day 3 before treatment. Among all groups, the increase in the number of goblet cells at day 10 from the values at day 3 was significant in 5% and 10% uridine–treated eyes compared with those in control eyes ($P < 0.001$; Kruskal-Wallis test; Figs. 11, 12A, 12B).

**Discussion**

HA is a linear polymer of glucuronic acid N-acetylgalcosamine disaccharide molecules. It was originally discovered in the vitreous body of the eye but subsequently was found in most parts of the body, including the skin and the synovial fluid of joints. It has been demonstrated that an HA receptor, CD44, is expressed in corneal and conjunctival cells.\(^{17}\) HA promotes cell migration in vitro and can stabilize the ocular surface epithelial barrier.\(^{10,11}\) suggesting that it may be directly involved in the process of epithelial repair by means of the activation of CD44.\(^{18}\) Many reports have attested to the effects of exogenous HA in producing a beneficial wound-healing outcome. In animal experiments, topically applied HA has been shown to accelerate skin wound healing in rats\(^{19,20}\) and hamsters.\(^{21}\) Corneal epithelial wound healing is also reported to be stimulated by exogenously applied HA.\(^{22}\) It has been proposed that HA may have a role in controlling localized inflammation often present in patients with keratoconjunctivitis sicca\(^{23}\); the reported benefits of exogenously applied HA in tissue repair suggest that HA might be helpful on the ocular surface in patients with dry eye. The half-life of HA in keratocyes in the epidermis, however, has been reported to be less than 1 day, whereas in the chondrocytes of cartilages it has been reported to be 2 to 3 weeks. Moreover, approximately one third of HA in the body is metabolized within 1 day, suggesting that exogenous HA may play a limited role in vivo. Uridine is transformed to UTP by uridine kinase, and UTP is used in the synthesis of HA in vivo. Elevated UDP-glucose contributes to an increase in the synthesis of HA.\(^{24}\) Therefore, exogenous UTP might possibly result in an increase of endogenous biosynthesis of HA. Furthermore, UTP is a P2Y\(_2\) receptor agonist, and P2Y\(_2\) receptor agonists such as UTP and ATP\(^{5}\) stimulate mucin secretion in vitro and in vivo in various tissues, such as conjunctiva,\(^{6}\) nasal epithelial cells,\(^{7}\) and tracheal gland cells.\(^{8}\) Mucin stimulants may be good candidates for dry eye treatment because mucin increases tear film stability and protects against desiccation of the ocular surface. We performed this study to evaluate the effect of uridine on the ocular surface of dry eye and to assess the possibility of uridine as a treatment for dry eye syndrome.

In this study, uridine had no cellular toxicity, and it increased the biosynthesis of HA and GAG. In corneal epithelial cells, HA increased even at a concentration as low as 1.0 $\mu$M.
Figure 10. Histopathologic analysis of the lacrimal glands (H&E; original magnification, ×100) at preinjection baseline (A) and 3 days after Con A injection (B). Marked inflammation was observed in the lacrimal gland after Con A injection.

uridine; however, the increase of HA was not dose dependent at a high concentration of 50.0 μM. This might have been attributed to receptor anergy,26 suggesting that a high concentration of uridine is not necessary as a drug for dry eye and that pulse therapy using uridine might be more useful as a treatment regimen rather than as a continuous application. In this study, uridine also reduced MMP-9 in cultured corneal epithelial cells and kerocytes. MMP-9, as an activator of inflammatory cytokines such as TGF-β1 and IL-1β, is known to be present at significantly higher levels in the tear fluid of patients with keratoconjunctivitis sicca. MMP-9 itself is a degradative enzyme and subsequently impedes the reepithelialization of the cornea. This uridine-induced decrease in MMP-9 might have been the reason for rapid wound healing of the rabbit cornea in our in vivo study. Alternatively, in the in vivo dry eye animal model—or epithelial defect model—may represent the roles of HA have been contradictory in reports on inflammation and moderation of keratocytes and fibroblasts. However, the wound tissue, in the early inflammatory phase of wound repair, is rich in HA, probably a reflection of increased synthesis.23 HA can act as a promoter of early inflammation that is important in the wound healing process. In a murine air pouch model of carrageenan/IL-1–induced inflammation, HA was shown to enhance cellular infiltration.25 Kobayashi and Terao26 have shown a dose-dependent increase of the proinflammatory cytokines TNF-α, IL-1β, and IL-8 production by human uterine fibroblasts through a CD44-mediated mechanism. HA also has an anti-inflammatory role by which it acts to decrease MMP in fibroblasts. One report shows that HA suppresses the production of MMP-1 by rheumatoid synovial fibroblasts stimulated by the proinflammatory cytokines TNF-α and IL-1β through intercellular adhesion molecule-1 (ICAM-1) and through the downregulation of NF-κB and p38.27,28 HA may participate in the negative feedback loop of inflammatory activation. TNF-α, an important cytokine generated in inflammation, stimulates the expression of TSG-6 in fibroblasts and inflammatory cells. TSG-6, a hyaluronan-binding protein, also forms a stable complex with the serum proteinase inhibitor Iα with a synergistic effect on the latter plasmin-inhibitory activity. Plasmin is involved in activation of the proteolytic cascade of MMP and other proteinases leading to inflammatory tissue damage. Therefore, the action of the TSG-6/Iα complex, which may be additionally organized by binding to HA in the extracellular matrix, may serve as a potent negative feedback loop to moderate inflammation and to stabilize the granulation tissue as healing progresses.29 Similarly, endothelial cells, in response to inflammatory cytokines such as TNF-α and IL-1β, also synthesize HA.30 Each of the roles of HA, as an initiator or a modulator of inflammation, appears to be involved in different stages of wound healing. HA also has important functions in epithelial cells.18 The primary function of HA in the epidermis is to maintain the extracellular space and to provide an open, hydrated structure for the passage of nutrients. It is also implicated in the control of keratinocyte proliferation, which is essential in normal epidermal function and in reepithelialization during tissue repair. In healing skin wounds, HA is expressed at the wound margin in the connective tissue matrix and in collocating with CD44 expression.34 In renal proximal tubular epithelial cells, HA attenuates TGF-β1–mediated signal...

Table 1. Schirmer Test Results before and at Various Time Points after Topical Uridine Application in a Rabbit Dry Eye Model

<table>
<thead>
<tr>
<th>Treatment</th>
<th>5% Uridine (n = 20)</th>
<th>1% Uridine (n = 5)</th>
<th>Hyaluronan (n = 10)</th>
<th>Control</th>
<th>Base Day 3 Day 10</th>
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<tr>
<td>5% Uridine</td>
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<tr>
<td>Before</td>
<td>11.6 ± 2.1</td>
<td>12.0 ± 2.9</td>
<td>12.6 ± 1.3</td>
<td>11.6 ± 1.7</td>
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<td>3 days after</td>
<td>6.6 ± 1.6</td>
<td>7.0 ± 1.8</td>
<td>6.9 ± 0.5</td>
<td>7.2 ± 0.7</td>
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<td>10 days after</td>
<td>12.8 ± 1.8</td>
<td>13.2 ± 1.5</td>
<td>13.4 ± 1.8</td>
<td>12.6 ± 1.8</td>
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<td>1% Uridine</td>
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<td>Before</td>
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<td>3 days after</td>
<td>7.0 ± 1.8</td>
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<td>10 days after</td>
<td>13.2 ± 1.5</td>
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<td>Hyaluronan</td>
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<td>3 days after</td>
<td>6.9 ± 0.5</td>
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<tr>
<td>10 days after</td>
<td>13.4 ± 1.8</td>
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<tr>
<td>Control</td>
<td>11.6 ± 1.7</td>
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As a control, no eye drops were administered in 20 rabbits. No significant changes occurred in tear secretion between the groups. Data are expressed as mean (millimeters) ± SD.

Figure 11. Conjunctival goblet cell counts before and at various time points after topical application in a rabbit dry eye model (n = 20 in the 5% uridine group, n = 5 in the 1% and 10% uridine groups, n = 10 in the hyaluronic acid group, and n = 20 in the control group). Statistically significant differences were noticed in the study groups compared with the values before treatment. The greatest increase in goblet cell density was observed in the 5% uridine group. Among all the groups, the degree of increase in goblet cell counts at day 10 from the values at day 3 was significant in the 5% and 10% uridine-treated groups compared with those in the control group (P < 0.001). Data are expressed as mean ± SD. *P = 0.038; †P = 0.003; ‡P = 0.012; §P = 0.022.
In corneal epithelial cells, it promotes migration in vitro. In our study, the expression of MMP was decreased in corneal epithelial cells when uridine was added, possibly because of the anti-inflammatory nature of HA. Given that MMP-9 level alone could not represent the anti-inflammatory action of uridine, further study is required to evaluate inflammatory cytokines or inflammatory cells for anti-inflammatory action of uridine on the cornea. In addition, we cannot yet explain the exact mechanism by which MMP is decreased by HA in epithelial cells. It may be possible through the inhibition of ICAM-1 interaction or through changes in cytokines, such as IL-8 and IL-10. More study is needed to investigate this mechanism.

In a short-term dry eye model using the rabbit, we found that topical application of uridine increased the number of conjunctival goblet cells, and 5% uridine eye drops demonstrated the best effect on increasing the goblet cell counts. This effect of uridine on goblet cell counts suggests that uridine may possibly work as a P2Y2 receptor agonist, leading to increased mucin secretion. Another possibility is that uridine may rapidly restore the health of the ocular surface by increasing internal HA synthesis or by reducing inflammation as in the in vivo eye model. Schirmer test results in this study favored the latter hypothesis.

In conclusion, the results of this study demonstrated that uridine could restore the health of the ocular surface rapidly, possibly because of an increase in internal HA synthesis, reduction in the amount of degradative enzyme such as MMP-9, or enhancement of the number of goblet cells. We are now investigating the action of uridine as a P2Y2 receptor agonist and as a hyaluronate promoter.

References


