Secreted Mucins on the Ocular Surface

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Mucins, which play important roles on the ocular surface in wettability, lubrication, and barrier function, are classified into two categories: secreted mucins and membrane-associated mucins. The most important secreted mucin on the ocular surface is MUC5AC, which is secreted by the conjunctival goblet cells. In the human conjunctiva, goblet cells are present in higher concentrations in the fornix, inferior nasal bulbar, and the lid wiper on the lid margin. The number of conjunctival goblet cells and MUC5AC expression/secretion are decreased in a patient with dry eye. In Japan, drugs that stimulate mucin secretion or increase the number of conjunctival goblet cells are commercially available. A P2Y2 receptor, diquafosol, stimulates tear fluid secretion from conjunctival epithelial cells and promotes mucin secretion from conjunctival goblet cells. Rebamipide was marketed originally as an oral therapeutic drug to treat gastritis in Japan. Topical rebamipide increases numbers of goblet cells in the bulbar conjunctiva and the lid wiper area of palpebral conjunctiva. Many researchers have reported decreases in the ocular surface mucin expression including MUC5AC secreted by goblet cells in patients with dry eye. However, it is unknown whether changes in mucin expression on the ocular surface cause or result from dry eye. Further study is needed to determine the true mechanism of dry eye disease.

Keywords: MUC5AC, goblet cell, Osaka study, diquafosol, rebamipide

The conjunctival goblet cells that secrete MUC5AC are scattered on the conjunctival epithelium (Figs. 1A, 1B). Eyelid movement during blinking evenly spreads MUC5AC secreted from the goblet cells into the lacrimal fluid over the entire ocular surface to maintain its wettability and lubrication (Fig. 2). The distribution of goblet cell varies depending on the anatomic location. In the human conjunctiva, goblet cells are present in higher concentrations in the fornix, and in particular, in the inferior nasal fornix near the exit of the tear drainage system. Knop et al. recently reported that goblet cells are abundant in the lid wiper portion of the lid margins to facilitate lubrication of the ocular surface during blinking. Kase et al. also found significantly more goblet cells in the lid wiper than the palpebral conjunctiva in a study of 30 eyelid tissues surgically removed due to involutional entropion. Goblet cell expression likely increases in the area subjected to more friction from blinking between the eyelid and cornea and between the eyelid and bulbar conjunctiva. As such, goblet cells are abundant on the ocular surface, and the MUC5AC secreted from them contribute to maintaining the homeostasis of the ocular surface.

The conjunctiva is innervated by sympathetic and parasympathetic nerves, the nerve endings of which surround the goblet cells at the level of the secretory granules. Goblet cells have muscarinic receptors of M3 and M2 subtypes for neurotransmitters released from parasympathetic nerves including acetylcholine (Ach) and vasoactive intestinal peptide (VIP). The parasympathetic nerves release Ach and VIP, which stimulate goblet cell secretion through M3 and M2 muscarinic receptors.

Goblet cells are associated with epidermal growth factor receptor (EGFR). Several studies have reported that the EGFR-signaling pathway serves an important role in goblet cell proliferation and mucin secretion in rat conjunctiva.
The functions of MUC5AC on the ocular surface.

Thereby preventing dry eye.32 Further studies should elucidate cells, lacrimal secretion increased in a compensatory manner, reported that, although those mice lacked conjunctival goblet et al. conducted an experiment with SPDEF-knockout mice and pointed domain-containing ETS transcription factor (SPDEF) promotes differentiation of conjunctival goblet cells.32 Marko et al. reported that Muc5ac expression increased in a compensatory mechanism in the Muc5ac-knockout mice, and no findings indicated signs of dry eye, such as inflammatory change, keratoconjunctival epithelial disorder, or decreased lacrimal fluid in the Muc5ac-knockout mice.31 The SAM pointed domain-containing ETS transcription factor (SPDEF) promotes differentiation of conjunctival goblet cells.2,32 Marko et al. conducted an experiment with SPDEF-knockout mice and reported that, although those mice lacked conjunctival goblet cells, lacrimal secretion increased in a compensatory manner, thereby preventing dry eye.32 Further studies should elucidate the functions of MUC5AC on the ocular surface.

**Changes in Numbers of Goblet Cells and MUC5AC Expression in Ocular Surface Disease**

Several studies have reported decreased MUC5AC secretion by goblet cells in patients with dry eye.33,34 Argueso et al. reported that MUC5AC expression was significantly lower in the tear fluid and conjunctival epithelial cells in patients with Sjögrens syndrome compared with normal individuals. Shimazaki-Den et al. also reported that MUC5AC expression levels in the conjunctival epithelium were significantly lower in patients with aqueous deficiency-type dry eye and dry eye with a short tear film breakup time.

In a murine experiment, Muc5ac expression increased in response to interleukin 13, a Th2 cytokine, and decreased in response to IFN-γ, a Th1 cytokine.35,36 Plügfelder et al. further compared patients with aqueous tear deficiency or meibomian gland dysfunction and a control group and discovered that IFN-γ; goblet cell density, and MUC5AC expression decreased significantly in the conjunctiva of patients with aqueous tear deficiency.37 The authors concluded that decreased tear fluid on the ocular surface increased IFN-γ expression, which consequently reduced the goblet cell count and MUC5AC expression.37

Changes in MUC5AC expression also have been reported in allergic eye diseases. Kunert et al. reported a significant decrease in the conjunctival goblet cell count and Muc5ac expression in the conjunctiva in mouse allergic conjunctivitis models.38 Dogru et al. reported that the MUC16 and MUC5AC expression levels decreased significantly in the conjunctival epithelial cells in patients with atopic keratoconjunctivitis.

As described previously, conjunctival goblet cells and mucin expression decrease in ocular surface diseases, particularly in dry eye, which emphasizes the need to develop drugs that increase mucin expression and secretion.

**Dry Eye Therapies and Their Effect on Mucin Production**

To date, several drugs or agents have been reported to induce mucin expression or secretion by the ocular surface epithelium. These are good treatments for dry eye, especially for the mucin deficiency type of dry eye.

The 2007 International Dry Eye Workshop report, “Tear stimulation: Secretagogues,” which stimulate aqueous or mucous secretion or both, described the drugs as future potential topical pharmacologic agents for treating dry eye, including diquafosol, rebamipide, gefarnate, ecbacet sodium (mucous secretion stimulants), and 15(S)-HETE (an MUC1 stimulant).40 In 2017, the Tear Film & Ocular Surface Society launched the Dry Eye Workshop II, which reported several topical pharmacologic agents that stimulate aqueous, mucin, and/or lipid secretion as secretagogues, including diquafosol, lacritin, rebamipide, galectin-3, mycophenolate mofetil, trefoil factors, and nerve growth factor.51 In Japan, drugs are commercially available that stimulate mucin secretion or normalize the ocular surface mucosa to increase the number of conjunctival goblet cells and used as the first-choice treatments for dry eye, including diquafosol and rebamipide.

**Recent Progress in Japan Regarding Secreted Mucins: Secreted Mucin Study in Epidemiologic Survey in Japan**

To investigate the prevalence of dry eye in patients who use visual display terminals (VDTs), a large epidemiologic survey.
was performed at a company in Osaka, Japan (Osaka Study, see section on epidemiology).52 Among many published manuscripts associated with the Osaka Study, two papers have reported alterations in tear MUC5AC levels in office workers who use VDTs.43,44 Uchino et al. reported that office workers with prolonged daily use of VDTs (>7 hours) had significantly low MUC5AC concentrations in their tears.43 They also reported in the other manuscript that cigarette smoking in office workers decreased goblet cell density and tear MUC5AC concentrations significantly.44

NEW THERAPEUTIC APPROACH FOR TREATMENT OF DRY EYE

Regarding secretory mucins, other areas of interest and progress in research in Japan are in the mucin production therapies (i.e., the secretagogues, which stimulate mucous secretion) for treating dry eye.46 In Japan, two new secretagogue eyedrops were launched in December 2010 and January 2012, respectively, to treat dry eye: diquafosol and rebamipide. Both drugs induce expression of MUC5AC secreted from conjunctival goblet cells. Several investigators have reported the effects of these drugs in basic and clinical research.

Diquafosol (Diquas Ophthalmic Solution 3%; Santen Pharmaceutical, Co., Ltd., Osaka, Japan) is a potent, purinergic P2Y2 receptor agonist. Generally speaking, P2Y2 receptor agonists act on P2Y2 receptors in cellular membranes and activate phospholipase C via G proteins to produce inositol triphosphate. Thus, calcium ion (Ca2+) release is induced from the cell endoplasmic reticulum, which elevates intracellular Ca2+ concentrations and induces various physiologic responses.45 P2Y2 receptors have been found at a number of ocular sites (i.e., the palpebral and bulbar conjunctival epithelium, conjunctival goblet cells, corneal epithelium, and meibomian glands).45,46 Regarding the mechanism of action, after binding to P2Y2 receptors, diquafosol stimulates tear fluid secretion from conjunctival epithelial cells and promotes mucin secretion from conjunctival goblet cells.47 Diquafosol decreased the periodic acid Schiff (PAS)-positive staining area in a dose-dependent manner immediately after instillation.48 When diquafosol was applied at concentrations exceeding 0.1%, the PAS-positive staining area was maximally decreased and reached nearly 40%.45 These results suggested that diquafosol stimulated mucin secretion from the conjunctival goblet cells. A similar stimulatory effect on mucin secretion was observed in evaluations of the PAS-positive staining areas using conjunctival histopathologic samples from normal rats.45 Hori et al. reported that the MUC5AC level in rabbit tears increased 15 minutes after instillation of 3% diquafosol tetrasodium eyedrops.49 In addition to MUC5AC, Takaoka-Shichijo and Nakamura reported that diquafosol increased the mRNA expression of membrane-associated mucins, MUC1, MUC4, and MUC16, after a 3-hour incubation with 100 μM diquafosol in cultured human corneal epithelial cells.51

To date, many studies have reported the effectiveness of 3% diquafosol ophthalmic solution for treating many types of dry eye, including the aqueous deficient type,52-55 short tear film breakup time,54-56 tear film instability, and keratoconjunctivitis.57,58 Diquafosol also stimulates tear fluid secretion from conjunctival goblet cells and can be used as a therapeutic drug for treating dry eye.50,51,59

gastric mucosal disorders and gastritis. To date, rebamipide for gastritis has been approved in several countries in Asia, including Japan, South Korea, China, Russia, and India. The agent improves the quality of gastric ulcer healing and reduces the recurrence rate of ulcers.60 The major properties of rebamipide include stimulation of prostaglandin and mucous glycoprotein synthesis and inhibition of reactive oxygen species, inflammatory cytokines, chemokines, and neutrophil activation.60 Urashima et al. reported that rebamipide increased the number of conjunctival goblet cells detected in normal rabbits and the presence of mucin-like substance contents on the ocular surface in the N-acetylcysteine-treated rabbit model.62 Biologically, goblet cells are associated with the EGFR signaling pathway, the activation of which leads to goblet cell metaplasia and mucin secretion from the cells.28 Rios et al.29 reported that rebamipide activated the EGFR-signaling pathway and induced proliferation of cultured rat conjunctival goblet cells. Ohguchi et al.30 showed that rebamipide increased the expression of muc5 mRNA on the ocular surface using superoxide dismutase-1 knockout mice.

In human conjunctiva, Kase et al.64 reported that topical rebamipide administration for 3 months increased markedly the number of goblet cells histologically in patients with conjunctival hyperemia and that topical rebamipide induced the number of EGFR-positive cells and goblet cells in the lid of human conjunctiva and bulbar conjunctiva.24 These data indicated that topical rebamipide might decrease the friction during blinking by increasing the mucin levels on the ocular surface.

In addition to increasing the number of conjunctival goblet cells and MUC5AC expression, rebamipide affects the human corneal epithelial cells. RT-PCR and Western blot analysis showed that rebamipide increased membrane-associated mucins in mRNA and protein levels in the cultivated human corneal epithelial cells.65,66 Uchino et al.67 reported that rebamipide increased MUC16 protein biosynthesis in the cultured human corneal epithelial, but not MUC1, MUC4, or MUC20. Two Japanese groups reported that rebamipide increased transmembrane electric resistance and protected TNF-α-induced disruption of the barrier function in cultured human corneal cells.67,68

Generally speaking, the goblet cell density decreased significantly after ocular surgery and/or application of the postoperative eyedrops, such as the topical nonsteroidal anti-inflammatory drug diclofenac. In a clinical study, Kato et al.69 reported that rebamipide significantly increased the number of conjunctival goblet cells 14 days after vitrectomy. They also reported the results of a randomized clinical trial that compared the goblet cell density after cataract surgery with diclofenac versus diclofenac and rebamipide.70 The reduction of the goblet cell density was significantly suppressed by the concomitant use of topical rebamipide.70

COMPARISON OF DIQUAFOSOL AND REBAMIPIDE

As secretagogues, both diquafosol and rebamipide are useful for treating dry eye in Japan (see section on TFOT and TFOD). To clarify the difference between diquafosol and rebamipide, we compared the short-term changes in tear volume after instilling these eyedrops in normal rabbits.71 The tear meniscus area increased significantly up to 30 minutes after instillation of diquafosol compared with rebamipide.71 We also compared the short-term effects on the MUC5AC level in rabbit tear fluid and conjunctival goblet cells after instilling both eyedrops. Only diquafosol increased the MUC5AC level 15 minutes after one instillation.70 These results suggested that diquafosol
promotes rapid secretion of MUC5AC from conjunctival goblet cells into the tear film after instillation. We hypothesized that diquafosol might improve tear fluid stability in the short term with its stimulatory effect on the tear fluid and mucin secretion via the P2Y2 receptor; on the other hand, rebamipide might improve the mucosal epithelia and increase the goblet cell numbers, which cure the ocular surface in patients with dry eye. Further studies should be performed to clarify the difference between these two drugs.

**FUTURE DIRECTIONS**

Many researchers have investigated the structure, function, or regulation of secreted mucins on the ocular surface worldwide. Traditionally, the tear film was thought to have three distinct layers: a 0.1- to 0.2-mm-thick superficial lipid layer, a 7- to 8-mm-thick aqueous layer, and a mucin layer up to 30 μm thick, which contains the secreted mucin MUC5AC and the membrane-associated mucins. However, to date, that hypothesis has changed completely, and now there are thought to be two layers: a superficial lipid layer and an aqueous layer that contains secreted mucin MUC5AC dispersed within it. In the future, an optical system capable of detecting the distribution of secreted mucins in the tear film should be established.

Mucins play important roles on the ocular surface by contributing to the wettability, lubrication, and barrier function on the ocular surface. In dry eye, because the expression of both secreted and membrane-associated mucins is decreased, those functions are disrupted on the ocular surface. However, it remains unknown whether the alteration of mucin expression is a cause of or results in dry eye. Further studies are needed to elucidate the true mechanism of dry eye disease.

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