The effects of 3% diquafosol sodium application on the tear functions and ocular surface of the Cu,Zn-superoxide dismutase-1 (Sod1)–knockout mice

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Purpose: To investigate the role of a water and mucin secretagogue (3% diquafosol sodium eye drops) on the tear function and conjunctival ocular surface changes in Sod1−/− in comparison to the wild-type (WT) mice.

Methods: Fourteen eyes of 7 Sod1−/− male mice with C57BL/ background and 14 eyes of 7 C57BL6 strain wild-type male mice were examined at 40 weeks in this study. All mice had application of 3% diquafosol ophthalmic solution six times a day for 2 weeks. Tear film stability and corneal epithelial damage was evaluated by fluorescein and Rose Bengal stainings. Anterior segment photography was performed before and after eye drop instillations. Aqueous tear quantity was measured with phenol red–impregnated cotton threads without anesthesia. Animals were sacrificed at 42 weeks after diquafosol treatment and the whole globe specimens were subjected to periodic acid Schiff staining. Goblet cell density was quantified by J Image software. Quantitative real-time PCR for conjunctival muc 5AC messenger RNA expression was also performed.

Results: Sod1−/− mice had significantly higher fluorescein staining scores compared to the WT mice before eye drop instillation. The mean tear film breakup time, Rose Bengal staining scores, and muc5 messenger RNA expression improved significantly with diquafosol treatment in both the WT and the knockout mice. The mean fluorescein staining score and aqueous tear quantity significantly improved in the Sod1−/− mice with treatment. A notable and consistent increase in goblet cells and decrease in inflammatory cell infiltrates could be confirmed in all specimens after 2 weeks of diquafosol eye drop application.

Conclusions: Three percent diquafosol ophthalmic solution appears to be effective in the treatment of ocular surface disease in this age-related dry eye disease mouse model.

According to the 2000 general population census statistics of the Japanese Ministry of Health, Labour and Welfare website, there were approximately 22.7 million elderly people above 65 years of age at this time, corresponding to 17.3% of the overall population in Japan. In comparison, approximately 34.8 million people in the United States (12.4% of the overall population) were seniors aged 65 years or older [1].

Aging brings about inflammation, age-related chronic disease, and disability. In the field of ophthalmology, age-related diseases include cataracts, age-related macular degeneration, glaucoma, diabetes-related retinal disease, and dry eye. Dry eye symptoms are common in the elderly population. In particular, the short tear film break-up time (BUT) type of dry eyes has been reported in 70% of the Japanese elderly [1-8]. Population-based dry eye studies in the elderly have shown that dry eye symptoms are common in White, Asian, Hispanic, and Danish people [9-14].

An imbalance between the generation of free radicals and radical scavenging antioxidant systems results in oxidative stress, a condition that has been associated with inflammatory cell injury observed in many age-related diseases and is also considered a major factor in the process of senescence [15]. One of the well-known antioxidant defense systems is superoxide dismutase 1, which is widely distributed in the tissues and represents 90% of the total SOD activity [16,17].

It has been reported that the Sod1−/− mice had oxidative stress–related damage and inflammation in the lacrimal glands with a decrease in tear secretion ability [18]. Recently, Kojima et al. reported signs of increasing oxidative stress and conjunctival inflammatory cell infiltrates from 10 to 40 weeks in a Sod1−/− mouse model had (unpublished data, presented at 2010 Gordon Conference, March 7–12, Ventura). Currently available treatment modalities for dry eye disease...
include artificial tears substitutes, gels/ointments, moisture chamber spectacles, anti-inflammatory agents (topical CsA and corticosteroids, omega-3 fatty acids), tetracyclines, punctual plugs, secretagogues, autologous serum drops, contact lenses, systemic immunosuppressives, and surgery [19]. There has been a commensurate increase in the knowledge regarding the pathophysiology of dry eye in recent years. This has led to a paradigm shift in dry eye management from simply lubricating and hydrating the ocular surface with artificial tears to strategies that stimulate natural production of tear constituents through the administration of secretagogues, maintain ocular surface epithelial health and barrier function, and inhibit the inflammatory factors that adversely impact the ability of ocular surface and glandular epithelia to produce tears [19].

Diquafosol is a uridine triphosphate–related compound, an agonist of the purinergic P2Y2 receptor, which contributes to water transfer and mucin secretion and is expressed in several ocular (including the conjunctival epithelium, meibomian glands, and goblet cells) and pulmonary tissues [20-22]. In this study, we investigated tear functions, muc5AC messenger RNA (mRNA) expression, and conjunctival histopathological alterations in Sod1−/− mice, comparing the results with wild-type (WT) mice and the changes in the results associated with 2 weeks of diquafosol sodium eye drop instillation.

METHODS

Animals: Fourteen eyes of seven Sod1−/− male mice with C57BL/background and 14 eyes of seven C57BL6 strain WT male mice were examined at 40 weeks in this study. The Sod1−/− mice were received from the Department of Advanced Aging Medicine, Chiba University Graduate School of Medicine (Chiba, Japan) and the WT C57BL/6 mice were purchased from Japan Clea (Osaka, Japan). All mice were subject to application of 3% diquafosol ophthalmic eye drops for 2 weeks, six times a day. The diquafosol eye drops used in this study contained benzalkonium chloride, potassium chloride, sodium chloride, and dibasic sodium phosphate. On day 15, tear functions were reevaluated and mice were sacrificed for histopathological examinations 14 h after termination of eye drop use. All studies were performed in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

Ocular surface epithelial damage and tear film breakup time assessment: Corneal fluorescein staining was evaluated with slit-lamp biomicroscopy using cobalt blue light after instillation of 2 μl of 0.5% sodium fluorescein. Excess of fluorescein was wiped from the lateral tear meniscus. The cornea was examined with a handheld slit lamp 2 min after fluorescein instillation. The tear film breakup time was initially evaluated by waiting for the natural blink response of the mouse, which was recorded three times; the mean of the measurements was then calculated. Punctuate staining was recorded using a grading system of 0–3 points for superior, central, and inferior corneal areas after breakup time examination. The fluorescein staining scores ranged from a minimum of 0 to a maximum of 9 points. Fluorescein staining was followed by introduction of 2 μl of 1% Rose Bengal solution by a micropipette. The Rose Bengal staining scores ranged from a minimum of 0 to maximum of 9 points. Corneal statuses before and after staining were recorded through photographs for each mouse on days 1 and 15 before and at the end of topical diquafosol applications. After Rose Bengal staining, the mouse corneas were washed with 2 μl of distilled water. Tear quantity measurements were performed 3 h after Rose Bengal staining.

Tear quantity measurements: Aqueous tear quantity was measured with phenol red–impregnated cotton threads (Zone-Quick, Showa Yakuhin Kako co., Ltd., Tokyo, Japan) without anesthesia. The threads were held with jeweler forceps and immersed into the tear meniscus in the lateral canthus for 60 s. The length of wetting of the thread was measured in millimeters. Aqueous tear production was weight adjusted by dividing the amount of total aqueous tear produced over 60 s by the animal’s weight.

Conjunctival specimen collections and histopathological assessment of specimens: Animals were anesthetized intraperitoneally for conjunctival biopsies measuring 2×2 mm from the inferior temporal conjunctiva at 40 weeks. The same animals were sacrificed using a combination of 6 mg/ml of ketamine and 4 mg/ml of xylazine at 42 weeks after 2 weeks of 3% diquafosol application. The whole globes were rapidly removed after sacrifice. Samples were immediately fixed in 4% buffered paraformaldehyde at 4 degrees for 24 h, embedded in paraffin wax, sliced in 4 μm thick paraffin sections, and processed according to conventional histological techniques for periodic acid Schiff staining.

Periodic acid Schiff staining and goblet cell density evaluations: Samples were deparaffinized in xylene for 10 min, hydrated in descending grades of alcohol (100%, 90%, 80%, 70%; 3 min in each solution), treated with periodic acid for 10 min, and washed in distilled water three times (5 min each wash). The specimens were then immersed in Schiff reagent for 30 min, washed with distilled water for 5 min, stained with hematoxylin for 2 min, and rewashed with distilled water for another 5 min. The specimens were dehydrated in
Data were processed using Graph Pad software (InStat, San Diego, CA). The Wilcoxon matched pairs test was used for the analyses of nonparametric values. A probability level less than 1% was considered statistically significant.

**RESULTS**

Changes in tear film breakup time and corneal epithelial staining scores: The mean tear film breakup time values were significantly higher in both the Sod1−/− mice and the WT mice after diquafosol eye drop application (5±0.25 s and 4.75±0.25 s, respectively) compared to preinstillation values (2.5±0.5 s and 3.0±0.25 s, respectively; p<0.01; Figure 1). The anterior segment photographs showed improvement of fluorescein and Rose Bengal stainings both in the Sod1−/− (Figure 2) and the WT mice (Figure 3) after 2 weeks of diquafosol eye drop instillation. The mean fluorescein scores showed a significant decrease after 2 weeks of diquafosol eye drop instillation in the Sod1−/− mice (postinstillation fluorescein score: 2.0±0.5 points; preinstillation fluorescein score: 7.0±0.5 points; p<0.01; Figure 4). The mean Rose Bengal scores showed a significant decrease after 2 weeks of diquafosol eye drop instillation both in the Sod1−/− and the WT mice (0.5±0.25 points and 0.5±0.25 points, respectively) compared to the preinstillation scores (6.5±0.5 points and 3.5±0.25 points, respectively; p<0.01; Figure 5).

Changes in tear quantity: We measured aqueous tear production using the cotton thread test and divided the values by the respective mouse weights. The mean weight-adjusted aqueous tear production was slightly but insignificantly lower in the Sod1−/− mice compared with the age- and sex-matched WT mice before administration of diquafosol eye drops, as shown in Figure 6. A significant increase in tear quantity after 2 weeks of topical diquafosol application was observed in both the Sod1−/− and the WT mice (0.23±0.04 mm/g and 0.25±0.01 mm/g, respectively) compared to preinstillation values (0.12±0.04 mm/g and 0.15±0.03 mm/g, respectively; Figure 6).

Conjunctival histopathological alterations: Periodic acid Schiff staining showed an apparently normal conjunctival architecture in the WT mice before and after diquafosol eye drop instillation (data not shown). Conjunctival specimens in the Sod1−/− mice consistently showed lack of goblet cells and infiltration with inflammatory cells before diquafosol eye drop instillation (Figure 7A). A notable and consistent increase in goblet cells and lack of inflammatory cell infiltrates could be confirmed in all specimens after 2 weeks of diquafosol treatment (Figure 7B). The mean goblet cell counts in conjunctival specimens of the Sod1−/− and WT mice were 61±5 cells/mm² and 181±10 cells/mm², respectively, before diquafosol instillation. The mean goblet cell counts increased to 1090±50 cells/mm² and 545±70 cells/mm², respectively, after diquafosol treatment. These increases in goblet cell densities were significant (p<0.01).
Figure 1. Tear film breakup time changes with 3% topical diquafosol application in the Sod1⁻/⁻ mice and wild type (WT) mice. Note the significant improvement in tear stability with 2 weeks of diquafosol sodium treatment. * represents p < 0.01.

Wilcoxon matched pairs test  *p<0.01

Figure 2. Anterior segment photographs showing changes in vital staining with 3% topical diquafosol application in the Sod1⁻/⁻ mice. Upper inserts: Note the improvement in fluorescein staining after diquafosol sodium application. Lower inserts: Note the improvement in Rose Bengal staining after diquafosol sodium application.

**Changes of corneal fluorescein staining with 3% diquafosol application**

*Sod1⁻/⁻* Before eye drops  
*Sod1⁻/⁻* After eye drops

**Changes of corneal Rose Bengal staining with 3% diquafosol application**

*Sod1⁻/⁻* Before eye drops  
*Sod1⁻/⁻* After eye drops
Figure 3. Anterior segment photographs showing changes in vital stainings with 3% topical diquafosol application in the wild-type (WT) mice. Upper inserts: Note the improvement in fluorescein staining after diquafosol sodium application. Lower inserts: Note the improvement in Rose Bengal staining after diquafosol sodium application.

Figure 4. Changes of fluorescein staining scores with 3% topical diquafosol application in the Sod1−/− mice and wild type (WT) mice. Note the significant improvement in the fluorescein staining score in the Sod1−/− mice with 2 weeks of diquafosol sodium treatment. * represents p < 0.01.
Figure 5. Changes in Rose Bengal staining scores with 3% topical diquafosol application in the Sod1−/− mice and wild type (WT) mice. Note the significant improvement in the Rose Bengal staining score in the Sod1−/− mice with 2 weeks of diquafosol sodium treatment. * represents p < 0.01.

Wilcoxon matched pairs test  *p<0.01

Figure 6. Weight-adjusted tear quantity changes with two weeks of 3% topical diquafosol application in the Sod1−/− mice and wild type (WT) mice. Note the significant improvement in tear quantity in both the Sod1−/− and WT mice with 2 weeks of diquafosol sodium treatment. * represents p < 0.01.

Wilcoxon matched pairs test  *p<0.01
Conjunctival PAS staining before 3% DQS application  40 week Sod1<sup>−/−</sup> mouse

Conjunctival PAS staining after 3% DQS application  42 week Sod1<sup>−/−</sup> mouse

Figure 7. Periodic acid Schiff stainings of conjunctival specimens showing changes in the Sod1<sup>−/−</sup> mice before and after 3% topical diquafosol (DQS) application. **A:** Note the extensive inflammatory cell infiltration and lack of goblet cells in the conjunctival epithelium. **B:** Note the marked decrease in inflammatory cells and the presence of numerous goblet cells.

%RNA GAPDH

![Graph](image)

Wilcoxon matched pairs test  *p<0.01

Figure 8. Real time reverse transcriptase polymerase chain reaction mucin 5AC glycoprotein messenger RNA expression changes with diquafosol application in the Sod1<sup>−/−</sup> mice and wild type (WT) mice. Note the significant increase in muc 5Ac mRNA expression in both WT and the Sod1<sup>−/−</sup> mice with 2 weeks of diquafosol sodium treatment. * represents p < 0.01.
Conjunctival muc5AC messenger RNA expression alterations: The expression of conjunctival muc5AC mRNA was significantly lower in the Sod1<sup>−/−</sup> mice than the WT mice before eye drop instillations (p<0.01), as shown in Figure 8. The expression of conjunctival muc5AC mRNA significantly increased both in the Sod1<sup>−/−</sup> and the WT mice after diqua-fosol instillations (p<0.01; Figure 8).

**DISCUSSION**

Oxygen free radicals and antioxidant systems have been demonstrated to be potentially important in the pathogenesis of ocular diseases such as cataracts, uveitis, retinopathy of prematurity, age-related macular degeneration, keratitis, keratoconus, and bullous keratopathy [18]. The role of oxidative stress in the pathogenesis of dry eye disease and its relationship with this disease have not previously been investigated in detail in an aging animal model or in humans. Because the amount and activity of Sod1 are the highest among the three isozymes in humans, it seemed reasonable to hypothesize that the lack of Sod1 would accelerate oxidative stress and age-related pathological changes in the lacrimal glands of the Sod1<sup>−/−</sup> mice [18]. We previously demonstrated that the lack of Sod1 led to increased oxidative lipid and DNA damage, increased CD4<sup>+</sup> T-cell inflammation, and epithelial-mesenchymal transition in the lacrimal glands of the current mouse model. This interfered with glandular secretory functions, resulting in dry eyes; moreover, this translated into an ocular surface disease [18]. In addition, we also reported that the Sod1<sup>−/−</sup> mouse model had signs of increasing oxidative stress and conjunctival inflammatory cell infiltrates from 10 to 40 weeks (unpublished data, presented at 2010 Gordon Conference, March 7–12, Ventura).

In this study, we investigated the effect of 3% diqua-fosol ophthalmic solution treatment for 2 weeks on the tear functions and conjunctival epithelial status in the Sod1<sup>−/−</sup>. First, we noted that the 40-week-old Sod1<sup>−/−</sup> mice had tear instability and ocular surface epithelial damage, as evidenced by increased fluorescein and Rose Bengal staining scores, as well as a loss of goblet cells in the conjunctiva. These observations were consistent with our previous findings on age- and sex-matched Sod1<sup>−/−</sup> mice, in which we revealed certain pathological alterations in the conjunctiva, including increased apoptosis of the conjunctival epithelium, decrease in goblet cells, and increase of subconjunctival inflammatory cell infiltration (unpublished data, reported at 2010 Gordon Conference March 7–12, Ventura, CA). To further investigate whether these changes translated into disturbances of the most abundant ocular surface mucin expression (the investigation of which might have explained the tear instability, since muc5AC is a well-known ocular surface mucin contributing to tear stability [23,24]), we performed real-time RT-PCR for muc5AC mRNA expression. These efforts revealed that the expression of conjunctival muc5AC mRNA was significantly lower in the Sod1<sup>−/−</sup> mice than in the WT mice before eye drop instillation. The decrease in goblet cells and muc5AC expression and increased inflammatory cell density in the conjunctiva of the Sod1<sup>−/−</sup> mice may all be held responsible for the tear instability and ocular surface disease. To clarify whether the diseased ocular surface could be salvaged by a tear and mucin secretagogue, we used 3% diqua-fosol sodium eye drops in both the Sod1<sup>−/−</sup> and the WT mice.

Recently, diqua-fosol has been reported to be an agonist of the purinergic P<sub>2</sub>Y<sub>2</sub> receptor, which is expressed in several ocular tissues, including the conjunctival epithelium and goblet cells. At the cellular level, the P<sub>2</sub>Y<sub>2</sub> receptor is known to contribute to water transfer and mucin secretion [20–22]. In animal studies involving rabbits, diqua-fosol has been shown to stimulate both water secretion from conjunctival epithelial cells and mucin secretion from conjunctival goblet cells via the P<sub>2</sub>Y<sub>2</sub> receptors [25]. Diquafosol has also been shown to prevent corneal epithelial damage in a rabbit dry eye model [26,27]. In a rat model of dry eye disease, diqua-fosol was demonstrated to improve tear secretion and restore the corneal epithelial barrier function [27].

Interestingly, we observed that tear stability, Rose Bengal staining (which is also known to indicate mucin-secreting cells and assess disorders of mucin secretion [28]) scores, and muc5 mRNA expression improved significantly with diqua-fosol treatment in both the WT and our KO mice, which was consistent with the observations in other animal models. We presume that the improvements in Rose Bengal scores were due to an increase of mucin expressions on the ocular surface. Fluorescein staining and aqueous tear quantity significantly improved in the Sod1<sup>−/−</sup> mice with diqua-fosol treatment. The increase in tear quantity may have occurred due to stimulation of tear secretion from the lacrimal glands or increased water secretion from the conjunctiva, the mechanisms of which need to be clarified in future studies. We also suggest that the improvement in tear stability may have resulted from an increase in goblet cell density or increased mucin expression.

Future investigation into the relationships between the changes in mucin expression, goblet cell differentiation, role and type of inflammatory pathway involvement, and P<sub>2</sub>Y<sub>2</sub> receptor stimulation should provide very interesting information. Studies looking into P<sub>2</sub>Y<sub>2</sub> receptor changes in this age-related dry eye disease mouse model and the possibility of upregulation with diqua-fosol should also provide
invaluable knowledge for the literature. In conclusion, this study revealed that 3% diquafosol ophthalmic solution was effective in the treatment of ocular surface disease in this KO mouse model; the mechanisms of action need to be clarified in future studies.

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REFERENCES

1. Uchino M, Dogru M, Yagi Y, Goto E, Tomita M, Kon T, Saiki M, Matsumoto Y, Uchino Y, Yokoi N, Kinoshita S, Tsubota K. The features of dry eye disease in a Japanese elderly population. Optom Vis Sci 2006; 83:797-802. [PMID: 17106406].
2. Ying W, Xiong ZG. Oxidative stress and NAD+ in ischemic brain injury: current advances and future perspectives. Curr Med Chem 2010; 17:2152-8. [PMID: 20423305].
3. Spector A. Oxidative stress-induced cataract: mechanism of action. FASEB J 1995; 9:1173-82. [PMID: 7672510].
4. Gritz DC, Montes C, Atalla LR, Wu GS, Sevanian A, Rao NA. Histochemical localization of superoxide production in experimental autoimmune uveitis. Curr Eye Res 1991; 10:927-31. [PMID: 1659971].
5. Niesman MR, Johnson KA, Penn JS. Therapeutic effect of liposomal superoxide dismutase in an animal model of retinopathy of prematurity. Neurochem Res 1997; 22:597-605. [PMID: 9131639].
6. Winkler BS, Boulton ME, Gottsch JD, Sternberg P. Oxidative damage and age-related macular degeneration. Mol Vis 1999; 5:32. <http://www.molvis.org/molvis/v5/a32/>
7. Alió JL, Artola A, Serra A, Ayala MJ, Mulet ME. Effect of topical antioxidant therapy on experimental infectious keratitis. Cornea 1995; 14:175-9. [PMID: 7743801].
8. Behndig A, Karlsson K, Johansson BO, Brannstrom T, Marklund SL. Superoxide dismutase isoenzymes in the normal and diseased human cornea. Invest Ophthalmol Vis Sci 2001; 42:2293-6. [PMID: 11527942].
9. Chia EM, Mitchell P, Rochtchina E, Lee AJ, Maroun R, Wang JJ. Prevalence and associations of dry eye syndrome in an older population: the Blue Mountains Eye Study. Clin Experiment Ophthalmol 2003; 31:229-32. [PMID: 12786773].
10. Schaumberg DA, Buring JE, Sullivan DA, Dana MR. Hormone replacement therapy and dry eye syndrome. JAMA 2001; 286:2114-9. [PMID: 11694152].
11. Lin PY, Tsai SY, Cheng CY, Liu JH, Chou P, Hsu WM. Prevalence of dry eye among an elderly Chinese population in Taiwan: the Shihpai Eye Study. Ophthalmology 2003; 110:1096-101. [PMID: 12799232].
12. Hom M, De Land P. Prevalence and severity of symptomatic dry eyes in Hispanics. Optom Vis Sci 2005; 82:206-8. [PMID: 15767875].
13. Bjerrum KB. Keratoconjunctivitis sicca and primary Sjögren’s syndrome in a Danish population aged 30–60 years. Acta Ophthalmol Scand 1997; 75:281-6. [PMID: 9253975].
14. Hikichi T, Yoshida A, Fukui Y, Hamano T, Ri M, Araki K, Horimoto K, Takamura E, Kitagawa K, Oyama M, Danjo Y, Kondo S, Fujishima H, Toda I, Tsubota K. Prevalence of dry eye in Japanese eye centers. Graefes Arch Clin Exp Ophthalmol 1995; 233:555-8. [PMID: 8543205].
15. Dröge W. Free radicals in the physiological control of cell function. Physiol Rev 2002; 82:47-95. [PMID: 11773609].
16. Crape JD, Oury T, Rabouille C, Slot JW, Chang LY. Copper, zinc superoxide dismutase is primarily a cytosolic protein in human cells. Proc Natl Acad Sci USA 1992; 89:10405-9. [PMID: 1332049].
17. Fridovich I. Superoxide anion radical (O2-), superoxide dismutases, and related matters. J Biol Chem 1997; 272:18515-7. [PMID: 9228001].
18. Kojima T, Wakamatsu TH, Dogru M, Ogawa Y, Igarashi A, Ibrahim OM, Inaba T, Shimizu T, Noda S, Ohata H, Nakamura S, Wakamatsu A, Shirasawa T, Shimazaki J, Negishi K, Tsubota K. Age-related dysfunction of the lacrimal gland and oxidative stress: evidence from the cu.zn-superoxide dismutase-1 (sod1) knockout mice. Am J Pathol 2012; 180:1879-96. [PMID: 22440255].
19. Geerling G, Tauber J, Baudouin C, Goto E, Matsumoto Y, O’Brien T, Rolando M, Tsubota K, Nichols KK. The international workshop on meibomian gland dysfunction: report of the subcommittee on management and treatment of meibomian gland dysfunction. Invest Ophthalmol Vis Sci 2011; 52:2050-64. [PMID: 21450919].
20. Hosoya K, Ueda H, Kim KJ, Lee VHL. Nucleotide stimulation of Cl- secretion in the pigmented rabbit conjunctiva. J Pharmacol Exp Ther 1999; 291:53-9. [PMID: 10490886].
21. Li Y, Kuang K, Yerxa B, Yerxa B, Wen Q, Rosskothen H, Fischbarg J. Rabbit conjunctival epithelium transports fluid, and P2Y22 receptor agonists stimulate Cl- and fluid
secretion. Am J Physiol Cell Physiol 2001; 281:C595-602. [PMID: 11443059].

22. Jumblatt JE, Jumblatt MM. Regulation of ocular mucin secretion by P2Y2 nucleotide receptors in rabbit and human conjunctiva. Exp Eye Res 1998; 67:341-6. [PMID: 9778415].

23. Dilly PN. Structure and function of the tear film. Adv Exp Med Biol 1994; 350:239-47. [PMID: 8030483].

24. Danjo Y, Watanabe H, Tisdale AS, George M, Tsumura T, Abelson MB, Gipson IK. Alteration of mucin in human conjunctival epithelia in dry eye. Invest Ophthalmol Vis Sci 1998; 39:2602-9. [PMID: 9856770].

25. Murakami T, Fujihara T, Horibe Y, Nakamura M. Diquafosol elicits increases in net Cl- transport through P2Y2 receptor stimulation in rabbit conjunctiva. Ophthalmic Res 2004; 36:89-93. [PMID: 15017104].

26. Fujihara T, Murakami T, Nagano T, Nakamura M, Nakata K. INS365 suppresses loss of corneal epithelial integrity by secretion of mucin-like glycoprotein in a rabbit short-term dry eye model. J Ocul Pharmacol Ther 2002; 18:363-70. [PMID: 12222766].

27. Fujihara T, Murakami T, Fujita H, Nakamura M, Nakata K. Improvement of corneal barrier function by the P2Y2 agonist INS365 in a rat dry eye model. Invest Ophthalmol Vis Sci 2001; 42:96-100. [PMID: 11133853].

28. Argüeso P, Tisdale A, Spurr-Michaud S, Sumiyoshi M, Gipson IK. Mucin characteristics of human corneal-limbal epithelial cells that exclude the rose bengal anionic dye. Invest Ophthalmol Vis Sci 2006; 47:113-9. [PMID: 16384952].