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REVIEW

$P2Y_2$ receptor agonists for the treatment of dry eye disease: a review

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Abstract: Recent advances in the understanding of dry eye disease (DED) have revealed previously unexplored targets for drug therapy. One of these drugs is diquafosol, a uridine nucleotide analog that is an agonist of the $P2Y_2$ receptor. Several randomized controlled trials have demonstrated that the application of topical diquafosol significantly improves objective markers of DED such as corneal and conjunctival fluorescein staining and, in some studies, tear film break-up time and Schirmer test scores. However, this has been accompanied by only partial improvement in patient symptoms. Although evidence from the literature is still relatively limited, early studies have suggested that diquafosol has a role in the management of DED. Additional studies would be helpful to delineate how different subgroups of DED respond to diquafosol. The therapeutic combination of diquafosol with other topical agents also warrants further investigation.

Keywords: dry eye disease, meibomian gland disease, aqueous tear deficiency, diquafosol, $P2Y_2$ agonists

Introduction

Dry eye disease (DED) is a complex clinical entity characterized by symptoms of discomfort and visual disturbance. It is associated with tear film instability, increased tear film osmolarity, and ocular surface inflammation.[1] It has a high prevalence that, depending on the population studied, varies from 5% to 35%.[2,3] DED commonly causes symptoms including foreign body sensation, dryness, irritation, itching, and light sensitivity.[4] It impacts patients' daily life and function. Utility assessment tools have shown that it influences quality of life as significantly as systemic morbidities such as angina and dialysis for chronic renal impairment.[5,6]

Broadly, DED results from either decreased aqueous tear production (aqueous tear-deficient dry eye [ADDE]) or increased tear evaporation (evaporative dry eye [EDE]). Both conditions may occur together.[1] The former may be associated with Sjögren's syndrome or other systemic inflammatory conditions. The latter is predominantly associated with meibomian gland dysfunction.

With the rapid increase in information regarding the pathogenesis of DED, recent insights have provided a rational basis for the development of new pharmacologic agents. Among these is diquafosol tetrasodium ("diquafosol"), a uridine nucleotide analog that is an agonist of the $P2Y_2$ receptor. Diquafosol has been approved by regulatory bodies in Japan and Korea and is available under the trade name Diquas™ Ophthalmic Solution 3% (Santen Pharmaceutical Co, Ltd, Osaka, Japan).

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This paper reviews the current evidence for the use of topical diquafosol for the treatment of DED. It will first provide a brief outline of the basic and clinical science underlying the use of this medication in dry eye treatment, followed by a review of published human clinical trials. A previous review has comprehensively evaluated the mechanism of diquafosol and human clinical data from Japanese studies;[7] this present report includes clinical studies conducted elsewhere.

The $P2Y_2$ receptor and its role in ocular surface physiology

The $P2Y_2$ receptor belongs to the family of purinergic receptors,[8] which have been classified into P1 receptors and P2 receptors on the basis of their native agonism by purine nucleosides and purine and pyrimidine nucleotides, respectively.[9] P2 receptors are further distinguished physiologically into two types. P2X receptors are fast ion channels, whereas P2Y receptors are metabotropic guanosine triphosphate binding protein (G-protein) coupled receptors that mediate diverse biological effects through a cascade of downstream intracellular processes. The eight members of the P2Y receptor subfamily are divided into two groups on the basis of their G-protein coupling. Specifically, the $P2Y_2$ receptor couples to G_q to activate phospholipase C-β.[10]

Physiologically, P2Y receptors are activated by a range of purine and pyrimidine extracellular mononucleotides, such as adenosine-5′-triphosphate and uridine-5′-triphosphate,[11] and dinucleotides, such as diadenosine polyphosphates.[9,12] Although these mono- and dinucleotides are primarily located in the intracellular compartment, under conditions of cellular damage or death they can be released into the extracellular milieu and thus become available for binding with P2Y receptors. For this reason, it has been proposed that P2Y receptors and their native ligands mediate danger signaling.[8] However, other mechanisms of release, including vesicular release and activation of adenosine-5′-triphosphate binding cassette transporters, have also been demonstrated.[13] The physiologic roles of P2Y receptor signaling are diverse, including, most notably, platelet aggregation, but it has also been suggested to be involved in immunity, lipid metabolism, the gastrointestinal system, and bone activity, among other things.[14]

Several studies have demonstrated the presence of P2X and P2Y receptors in ocular tissues, including the retina,[15] ciliary body,[16] and lens.[17] These studies indicate that $P2Y_2$ receptors appear to be the main subtype of purinergic receptor located at the ocular surface.[18] Some indirectly imply the presence of $P2Y_2$ receptors by demonstrating an in vitro selective response to exogenously applied $P2Y_2$ receptor agonists, involving an increase in intracellular calcium or stimulation of mucin secretion.[9,19,20] Direct evidence for the presence of $P2Y_2$ receptors in ocular tissues includes the localization of $P2Y_2$ receptors in the conjunctival epithelial goblet and serous cells and meibomian gland acinus and ductal epithelial cells of the rhesus macaque by nonisotopic in situ hybridization.[21]

Animal and human studies have demonstrated that native $P2Y_2$ agonists may be released at the ocular surface in response to trauma. In response to mechanical shear stress, cultured rabbit corneal epithelial cells have been shown to release nucleotides.[22] Increasing blink rate and corneal mechanical stimulation in human subjects led to increased concentrations of diadenosine polyphosphates in tear secretions,[23] as did 5 minutes of contact lens wear in a group of contact lens-naive subjects.[24]

Animal studies have suggested that ocular surface application of $P2Y_2$ agonists promotes aqueous tear secretion. Diquafosol increased aqueous tear secretion in rats that had undergone surgical excision of their exorbital lacrimal glands.[25] This was attributed to stimulation of net ion transport across the conjunctiva, as had been demonstrated in isolated rabbit conjunctiva.[26] In vivo studies conducted on rabbits demonstrated that $P2Y_2$ agonists increased Schirmer values.[27,28]

$P2Y_2$ agonists increased mucin secretion in isolated rabbit and human conjunctiva.[19] In vivo, $P2Y_2$ agonists also increased secretion of mucin in rabbits, as measured by an impression cytology technique.[29]

$P2Y_2$ receptor agonists decreased fluorescein penetrance into rat cornea, as measured by a fluorophotometric method. This increase in corneal barrier function was felt to be secondary to increases in tear fluid and mucin, perhaps improving tight junctional resistance.[25] Additionally, in vivo epithelial wound healing was accelerated by an unknown mechanism in the rabbit cornea by the application of the $P2Y_2$ agonists uridine-5′-triphosphate and diadenosine tetraphosphate, and inhibited by the application of $P2Y_2$ antagonists.[30]

Through its intracellular calcium-raising effect, $P2Y_2$ receptor agonism is thought to open chloride channels in the apical membrane, leading to chloride flux and water transport.[25,26] In goblet cells, the mechanism is less well defined, but presumably the increased intracellular calcium is coupled to the release of mucin from secretory granules.[7]

Review of published clinical studies

Much of the clinical trial data relating to topical diquafosol for the treatment of DED has been presented in abstract

form at meetings and press releases and not reported in the literature. In the following review, only published clinical trial data have been included. The key clinical trials are summarized in Table 1.

Methods

A literature search was conducted using PubMed to identify all existing studies of diquafosol in patients with DED. The search was conducted without date limitations. Keywords used for the search included MeSH terms: dry eye syndromes and purinergic P2Y receptor agonists connected by "and" with the following MeSH and natural-language terms: diquafosol, aqueous deficiency, Sjögren's syndrome, dry eye, and keratoconjunctivitis sicca. The abstracts for all studies meeting initial search criteria were reviewed; relevant studies were included. The reference list of all articles gathered was also reviewed for other potentially relevant articles.

Safety

No serious adverse events have been reported in the literature related to diquafosol. The initial Phase I trials investigating human safety and tolerability included two separate

Table 1 Key clinical trials evaluating topical diquafosol therapy for dry eye disease

Study	Year of publication	Study design	Number of patients (n), study arms	Predominant type of dry eye studied	Duration (weeks)	Results
Tauber et al[34]	2004	Randomized controlled trial	n=527 Three arms: 1) 1% diquafosol 2) 2% diquafosol 3) placebo	ADDE	24	Schirmer scores and corneal fluorescein staining significantly improved with both 1% and 2% diquafosol. Minor improvement in "clearing of foreign body sensation" on secondary (but not primary) analysis.
Matsumoto et al[33]	2012	Randomized controlled trial	n=286 Three arms: 1) 1% diquafosol 2) 3% diquafosol 3) placebo	ADDE	6	Corneal and conjunctival staining scores significantly improved with both 1% and 3% topical diquafosol with a dose-dependent effect. Eleven ocular symptoms were assessed. Only one ("dry eye sensation") improved with diquafosol.
Takamura et al[35]	2012	Randomized controlled trial	n=286 Two arms: 1) 3% diquafosol 2) 0.1% sodium hyaluronate	ADDE	4	Mean fluorescein staining scores and TBUT improved for both groups. No inferiority or superiority detected between groups. Rose bengal staining improved for both groups, with diquafosol superior to hyaluronate. Eleven ocular symptoms were assessed; superiority of diquafosol was demonstrated for one ("heaviness").
Kamiya et al[36]	2012	Prospective, consecutive interventional case series	n=32 (64 eyes) For each patient: 1) one eye served as treatment group (sodium hyaluronate and diquafosol) 2) the other eye served as control (sodium hyaluronate)	ADDE insufficiently responsive to sodium hyaluronate monotherapy	4	Diquafosol eyes showed improvement in TBUT and corneal staining but not Schirmer test. Twelve symptoms were measured. Three ("dry eye sensation, pain, and foreign body sensation") improved in diquafosol eyes.

Abbreviations: ADDE, aqueous tear-deficient dry eye; TBUT, tear film break-up time.

dose-escalation studies: one involving 50 healthy human subjects without any ocular disease,[31] the other involving 60 subjects with mild to moderate dry eye.[32] Adverse findings included mild eyelid erythema, mild conjunctival erythema, and transient mild conjunctival discharge. In subsequent Phase II and III clinical studies, no statistically significant difference was found in the adverse event rate between diquafosol and placebo after 6 weeks[33] or 24 weeks[34] of treatment, and between diquafosol and sodium hyaluronate after 4 weeks of treatment.[35] Some of the common ocular adverse events reported were burning and stinging on instillation (7%) with a 2% diquafosol formulation, and irritation (6.3%–12.5%), eye pain (1.4%–4.2%), and conjunctival hyperemia (0.7%–1.0%) with a 3% formulation.[33,35] The 3% diquafosol ophthalmic solutions used in some studies were preserved with benzalkonium chloride;[35,36] many other studies did not explicitly state which preservative was used in the trial solutions.

Efficacy

Aqueous tear-deficient dry eye

There are three randomized controlled trials (RCTs) reported in the literature comparing diquafosol with either placebo or sodium hyaluronate in the treatment of DED predominantly related to ADDE. In addition, one study evaluated the role of diquafosol as an additive therapy to sodium hyaluronate, one assessed the impact of diquafosol on visual function, and another provided longer-term (6 months) data.

Randomized controlled trials

In one placebo-controlled RCT (n=527), Tauber et al[34] demonstrated statistically significant improvement in some objective measures of dry eye, including fluorescein corneal staining score and Schirmer testing, but not in symptoms of DED. Inclusion criteria included 1) at least a 6-month history of DED, 2) specific dry eye symptoms, 3) a minimum corneal fluorescein staining score, and 4) an unanesthetized Schirmer score ≤7 mm. Contact lens wearers were excluded from this study, as were subjects with significant meibomian gland dysfunction or lid margin inflammation. Qualifying subjects were randomized to one of three groups: 1) placebo, 2) 1% diquafosol, or 3) 2% diquafosol.[34] The frequency of drop application was four times daily. At week 6, both 1% and 2% diquafosol groups demonstrated statistically significant superiority over placebo in improvement of corneal staining score (1% and 2% groups) and conjunctival staining score (2% group). Improvement in tear volume in both diquafosol groups, as measured by unanesthetized Schirmer testing, was statistically significant. The major subjective endpoint, improvement in clearing of foreign body sensation, did not reach statistical significance on primary analysis; however, a significant difference in favor of diquafosol was detected on secondary analysis of week 6, 8, and 10 data.

In a more recent placebo-controlled RCT (n=286), Matsumoto et al[33] also demonstrated improvements in objective markers but little improvement in symptoms of DED. In this study, inclusion criteria were: 1) unanesthetized Schirmer score ≤5 mm and 2) any degree of corneal fluorescein staining after a 2-week washout period. Contact lens wearers were excluded. On the basis of the documented inclusion criteria and baseline patient characteristics revealing that 59 of the 286 (20.6%) subjects had Sjögren's syndrome, patients in this cohort were probably predominantly affected by ADDE, although no explicit exclusion criteria relating to meibomian gland dysfunction were documented. Subjects were randomized to receive 1) placebo, 2) 1% diquafosol, or 3) 3% diquafosol. Frequency of drop application was six times daily. The study duration of 6 weeks was preceded by a 2-week washout period. In this study, the primary endpoint was specified as fluorescein staining score at week 4 of treatment. Compared with placebo, both diquafosol groups demonstrated statistically significant dose-dependent improvements in fluorescein corneal staining scores at week 4 (1% P=0.037; 3% P=0.002). At week 6, the 3% diquafosol group but not the 1% group had significantly better fluorescein corneal staining compared with placebo (P=0.005). Compared with placebo, rose bengal corneal and conjunctival staining scores demonstrated significant improvement with both concentrations of diquafosol at the week 4 and week 6 time points. Although 3% diquafosol improved tear film break-up time (TBUT), this was not statistically significant. Among eleven ocular symptoms assessed, only one of these ("dry eye sensation") was significantly improved by diquafosol, with both treatment groups showing significant improvement over placebo at weeks 4 and 6.

Takamura et al[35] compared 3% diquafosol with 0.1% sodium hyaluronate in an RCT (n=286) with two treatment arms in a 1:1 ratio. Patients qualified for inclusion based on: 1) unanesthetized Schirmer test ≤5 mm, 2) fluorescein staining scores ≥3 (out of nine points), and 3) rose bengal staining scores ≥3 (out of 15 points) following a 2-week run-in period in which no topical therapy except one drop of vehicle was applied six times daily. Again, no explicit exclusion criteria relating to meibomian gland dysfunction were documented. Treatment involved drops applied six times daily for 4 weeks. After 4 weeks, both groups showed significant

improvement in fluorescein staining (diquafosol –2.12±0.14, sodium hyaluronate –2.08±0.13) and TBUT compared with baseline. There was, however, no statistically significant intergroup difference. By contrast, the diquafosol group showed significantly improved rose bengal staining when compared with sodium hyaluronate at 4 weeks (P=0.010). Among eleven measured subjective parameters, only one ("heaviness") was significantly improved in the diquafosol group compared with in the sodium hyaluronate group at week 4.

Additive effect of 3% diquafosol and 0.1% sodium hyaluronate

Kamiya et al[36] analyzed the additive effect of 3% diquafosol and 0.1% sodium hyaluronate in 64 eyes of 32 consecutive patients with Sjögren's syndrome or without Sjögren's syndrome with ADDE who were insufficiently responsive to sodium hyaluronate monotherapy alone at a frequency of one drop six times a day. Each eye of the 32 patients was randomized to receive sodium hyaluronate (the "control group"), with the fellow eye receiving diquafosol (Diquas) and sodium hyaluronate (the "diquafosol group"). Statistically significant improvement in TBUT, fluorescein, and rose bengal staining scores was seen in the diquafosol group at 2 weeks and 4 weeks compared with baseline. In contrast, the control group did not achieve statistically significant improvements to these endpoints at either 2 weeks or 4 weeks. Neither group improved tear volume as measured by the Schirmer test. In this study, twelve subjective symptoms were measured using a visual analog scale graded between 0 and 100. Of the twelve subjective symptoms, only three (dry eye sensation, pain, and foreign body sensation) were improved significantly in the diquafosol group compared with baseline. The control group did not have any statistically significant change in subjective symptoms.

Functional evaluation

Another study (n=24) assessed the impact of diquafosol therapy on tear function and visual performance.[37] Patients with short TBUTs (≤5s) were included; those with meibomian gland disease, blepharitis, lid malposition, nasolacrimal duct disease, or contact lens wear were excluded. Patients were divided into two groups based on the presence (n=11) or absence (n=13) of symptoms related to DED. Both groups were treated with diquafosol (Diquas) applied six times daily for 1 month. Higher order aberrations, as measured by wavefront aberrometry, were significantly reduced in the symptom-positive group only. Functional visual acuity, measured using a proprietary device, improved in both groups.

Preliminary long-term efficacy data

Preliminary long-term efficacy data for diquafosol in patients with ADDE have been published by Koh et al.[38] Fifteen eyes of 15 subjects (Sjögren's syndrome n=13, no Sjögren's syndrome ADDE, n=2) were treated with 3% diquafosol (Diquas) for a period of 6 months at a frequency of six times daily. Twelve ocular symptoms were evaluated, with each symptom rated on a four-point scale at 1 month, 3 months, and 6 months compared with baseline. Significant improvement occurred at all time points. Mean corneal staining scores were significantly reduced at 1 month, 3 months, and 6 months compared with baseline. Conjunctival staining was significantly reduced at 3 months and 6 months. Both TBUT and tear meniscus height (measured by anterior-segment optical coherence tomography) were significantly increased at all time points compared with baseline. Despite the latter increase, Schirmer values were not significantly altered at any time point.

Evaporative dry eye

More recently, the role of diquafosol in treating EDE has been explored. Arita et al[39] analyzed the effect of 3% diquafosol on 19 eyes of ten patients with obstructive meibomian gland dysfunction. Treatment was with four times daily 3% diquafosol (Diquas), and follow-up lasted at least 4 months (range 4–16 months). Improvements were seen in ocular symptoms, lid margin abnormalities, superficial punctate keratopathy, meibum grade, break-up time, and meniscus height in all 19 eyes. Statistical analysis was applied to quantitative noncontact meibography, which demonstrated an increase in meibomian gland coverage in all 19 eyelids from 36.9%±10.1% to 41.5%±9.2% after treatment (P<0.0001).

Discussion

Studies evaluating diquafosol therapy for ADDE demonstrated significant improvement in signs of DED but only partial improvement in symptoms.

In these studies, diquafosol consistently improved ocular surface staining. Two moderately large RCTs demonstrated that compared with placebo, diquafosol significantly reduced fluorescein corneal staining scores after a treatment period of between 4 weeks and 6 weeks using 1%,[33] 2%,[34] and 3%[33] concentrations. This improvement in fluorescein corneal staining occurred in a dose-dependent manner.[33] When diquafosol 3% was compared with sodium hyaluronate, after 4 weeks there appeared to be no statistically significant difference in fluorescein corneal staining.[35] However, rose bengal staining was significantly improved with diquafosol when compared with sodium hyaluronate.

In comparison, improvements in TBUT were less consistent. Fluorescein TBUT data were not documented in the Tauber et al[34] study. In the Matsumoto et al[33] study, no significant difference from TBUT was demonstrated between 1% and 3% diquafosol and placebo at any time point. In the Takamura et al[35] study, improvements from baseline in TBUT were documented in both the 3% diquafosol and 0.1% sodium hyaluronate groups, but there was no statistical difference between the two groups. Although a smaller study than the RCTs listed previously, Koh et al[38] demonstrated improvements in TBUT at 1 month, 3 months, and 6 months, indicating, perhaps, a longer-term improvement in tear film stability.

In patients with DED recalcitrant to sodium hyaluronate therapy alone, the addition of diquafosol improved fluorescein and rose bengal corneal staining scores as well as TBUT.[36] This may suggest that certain subgroups of dry eye unresponsive to one therapy alone may respond with either alternative or additional treatment. Although an additional therapy may simply address different disease pathways simultaneously, these pathways may interact in a dynamic manner, and one therapy may potentiate or facilitate the effect of the other.

Although diquafosol appears to consistently improve ocular surface staining, improvements in DED subjective symptom scores have been disappointing. In the Tauber et al[34] study, "clearing of foreign body sensation" at week 6 (defined as a primary endpoint) was not significantly different from placebo, although some improvement in symptoms was detected on secondary analysis. Matsumoto et al[33] found that only one out of eleven subjective symptoms evaluated ("dry eye sensation") improved significantly with diquafosol versus placebo. Similarly, Takamura et al[35] found that only one of the eleven subjective symptoms evaluated ("heaviness") improved significantly with diquafosol against sodium hyaluronate. Kamiya et al[36] found significant improvements in three out of twelve subjective symptoms with the addition of diquafosol to sodium hyaluronate.

The discrepancy between improvement in signs and symptoms on treatment with topical diquasofol is relatively consistent throughout these clinical studies. However, previous studies have demonstrated that simply by improving the signs of DED, symptoms may not be alleviated, implying a poor correlation between the two.[4,40,41] The reason for this is unclear and probably multifactorial. Due to its subjective nature, measuring patient symptoms is inherently difficult. In DED, challenges include a multifactorial etiology. It has been noted that both symptom frequency and intensity may differ among different etiologies of DED.[4] The influence of psychological factors on the perception and expression of symptoms is also intertwined with the immeasurable effect of language and culture.[4] These difficulties present significant ongoing challenges to clinical researchers and would be ameliorated by the introduction of a standardized method of assessing symptoms in all DED studies. Even in relation to the commonly used clinical tests for eliciting what are regarded as "objective" signs, there exists a poor relationship between tests intended to assess similar characteristics of the tears or ocular surface. This has been attributed to measurement error, as well as to the inherent variability of the tear and ocular surface status in an individual over time.[40] Although objective signs of amelioration with treatment are important for providing a solid rationale for introducing new therapies, the alleviation of sometimes distressing symptoms rightly also assumes primacy in DED management. This is reflected in the inclusion of ocular symptoms in all international definitions.[42]

Studies utilizing various methods of corneal esthesiometry coupled with confocal microscopy to assess the change in corneal sensation and nerve morphology in patients with DED have demonstrated inconsistent findings. Some suggest that dry eyes are hyperesthetic,[43,44] whereas others suggest that they are hypoesthetic.[45,46] The reasons for these conflicting results are unresolved. Potentially, they are due to different subtypes, chronicities, and/or severities of DED being studied. Still, there is a consensus that corneal nerve function and morphology are altered in DED, in part as a response to chronic inflammation.[43–46]

All of the larger RCTs specified their primary endpoints at between 4 weeks and 6 weeks. Given the chronicity of DED, we postulate that even if short-term treatment with diquafosol restored corneal epithelial integrity (as measured by improved staining scores) and improved the thickness and stability of the overlying tear film, normalization of the inflammatory changes to the cornea and recovery of corneal nerve responsiveness may lag behind the surface improvements to some extent. In this regard, it is possible that improvements in patient symptoms may be borne out in longer-term studies. Koh et al[38] demonstrated significant improvement to dry eye symptom scores over a 6-month treatment period. However, this was a small study (n=15), and additional larger long-term studies would be useful. It would be of great interest to study the effect of diquafosol treatment on the corneal nerves using confocal microscopy and esthesiometry, and relate this to changes in symptomatology over time. To the authors' knowledge, no animal or

human study has examined the influence of P2Y$_2$ agonists on corneal nerve structure or function.

Diquafosol may have a role in treating EDE related to meibomian gland dysfunction.[39] More studies with larger numbers are required to further evaluate its efficacy in improving signs and/or symptoms.

Conclusion

The published clinical trial data surrounding the use of diquafosol in the management of DED suggest that diquafosol consistently improves ocular surface staining and may improve tear film volume and stability. However, this is generally not accompanied with a major improvement in symptoms related to DED, although a few specific subjective symptoms improved across different studies.

Further studies may be useful to clarify its role in specific subgroups of DED, such as meibomian gland dysfunction, Sjögren's syndrome, or DED recalcitrant to more traditional therapies. Longer-duration studies, as well as studies examining the effect of diquafosol on corneal sensation and nerve structure, and potential synergistic interplay between diquafosol and other therapeutic agents such as ocular lubricants, traditional topical immunomodulatory agents such as prednisolone and cyclosporine, and newer targeted molecular agents would also be of great interest.

Progress in the understanding of dry eye pathophysiology and advances in diagnostic methods will help us better understand the place of diquafosol in the overall picture of DED management, overcome the discrepancy between signs and symptoms, and find new markers to gauge efficacy in future clinical trials.

Disclosure

The authors have no conflicting relationship or financial interest in any aspect of this review.

References

1. The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye WorkShop (2007). *Ocul Surf*. 2007;5(2):75–92.
2. McCarty CA, Bansal AK, Livingston PM, Stanislavsky YL, Taylor HR. The epidemiology of dry eye in Melbourne, Australia. *Ophthalmology*. 1998;105(6):1114–1119.
3. Lin P-Y, Tsai S-Y, Cheng C-Y, Liu J-H, Chou P, Hsu W-M. Prevalence of dry eye among an elderly Chinese population in Taiwan: the Shihpai Eye Study. *Ophthalmology*. 2003;110(6):1096–1101.
4. Nichols KK. Patient-reported symptoms in dry dye disease. *Ocul Surf*. 2006;4(3):137–145.
5. Schiffman RM, Walt JG, Jacobsen G, Doyle JJ, Lebovics G, Sumner W. Utility assessment among patients with dry eye disease. *Ophthalmology*. 2003;110(7):1412–1419.
6. Buchholz P, Steeds CS, Stern LS, et al. Utility assessment to measure the impact of dry eye disease. *Ocul Surf*. 2006;4(3):155–161.
7. Nakamura M, Imanaka T, Sakamoto A. Diquafosol ophthalmic solution for dry eye treatment. *Adv Ther*. 2012;29(7):579–589.
8. Burnstock G. Introductory overview of purinergic signalling. *Front Biosci (Elite Ed)*. 2011;3:896–900.
9. Jacobson KA, Balasubramanian R, Deflorian F, Gao Z-G. G protein-coupled adenosine (P1) and P2Y receptors: ligand design and receptor interactions. *Purinergic Signal*. 2012;8(3):419–436.
10. Guzman-Aranguez A, Santano C, Martin-Gil A, Fonseca B, Pintor J. Nucleotides in the eye: focus on functional aspects and therapeutic perspectives. *J Pharmacol Exp Ther*. 2013;345(3):331–341.
11. Crooke A, Guzman-Aranguez A, Peral A, Abdurrahman MKA, Pintor J. Nucleotides in ocular secretions: their role in ocular physiology. *Pharmacol Ther*. 2008;119(1):55–73.
12. Pintor J, Carracedo G, Alonso MC, Bautista A, Peral A. Presence of diadenosine polyphosphates in human tears. *Pflugers Arch*. 2002;443(3):432–436.
13. Bodin P, Burnstock G. Purinergic signalling: ATP release. *Neurochem Res*. 2001;26(8–9):959–969.
14. Abbracchio MP, Burnstock G, Boeynaems JM, et al. International Union of Pharmacology LVIII: update on the P2Y G protein-coupled nucleotide receptors: from molecular mechanisms and pathophysiology to therapy. *Pharmacol Rev*. 2006;58(3):281–341.
15. Neal M, Cunningham J. Modulation by endogenous ATP of the light-evoked release of ACh from retinal cholinergic neurones. *Br J Pharmacol*. 1994;113(4):1085–1087.
16. Farahbakhsh NA, Cilluffo MC. P2 purinergic receptor-coupled signaling in the rabbit ciliary body epithelium. *Invest Ophthalmol Vis Sci*. 2002;43(7):2317–2325.
17. Riach RA, Duncan G, Williams MR, Webb SF. Histamine and ATP mobilize calcium by activation of H1 and P2u receptors in human lens epithelial cells. *J Physiol (Lond)*. 1995;486(Pt 2):273–282.
18. Burnstock G, Knight GE. Cellular distribution and functions of P2 receptor subtypes in different systems. *Int Rev Cytol*. 2004;240:31–304.
19. Jumblatt JE, Jumblatt MM. Regulation of ocular mucin secretion by P2Y2 nucleotide receptors in rabbit and human conjunctiva. *Exp Eye Res*. 1998;67(3):341–346.
20. Kimura K, Nishimura T, Satoh Y. Effects of ATP and its analogues on [Ca2+]i dynamics in the rabbit corneal epithelium. *Arch Histol Cytol*. 1999;62(2):129–138.
21. Cowlen MS, Zhang VZ, Warnock L, Moyer CF, Peterson WM, Yerxa BR. Localization of ocular P2Y2 receptor gene expression by in situ hybridization. *Exp Eye Res*. 2003;77(1):77–84.
22. Srinivas SP, Mutharasan R, Fleiszig S. Shear-induced ATP release by cultured rabbit corneal epithelial cells. *Adv Exp Med Biol*. 2002;506(Pt A):677–685.
23. Peral A, Carracedo G, Acosta MC, Gallar J, Pintor J. Increased levels of diadenosine polyphosphates in dry eye. *Invest Ophthalmol Vis Sci*. 2006;47(9):4053–4058.
24. Guzman-Aranguez A, Crooke A, Peral A, Hoyle CHV, Pintor J. Dinucleoside polyphosphates in the eye: from physiology to therapeutics. *Prog Retin Eye Res*. 2007;26(6):674–687.
25. Fujihara T, Murakami T, Fujita H, Nakamura M, Nakata K. Improvement of corneal barrier function by the P2Y(2) agonist INS365 in a rat dry eye model. *Invest Ophthalmol Vis Sci*. 2001;42(1):96–100.
26. Li Y, Kuang K, Yerxa B, Wen Q, Rosskothen H, Fischbarg J. Rabbit conjunctival epithelium transports fluid, and P2Y2(2) receptor agonists stimulate Cl(−) and fluid secretion. *Am J Physiol Cell Physiol*. 2001;281(2):C595–C602.
27. Pintor J, Peral A, Hoyle CHV, et al. Effects of diadenosine polyphosphates on tear secretion in New Zealand white rabbits. *J Pharmacol Exp Ther*. 2002;300(1):291–297.
28. Murakami T, Fujihara T, Nakamura M, Nakata K. P2Y2 receptor stimulation increases tear fluid secretion in rabbits. *Curr Eye Res*. 2000;21(4):782–787.
29. Murakami T, Fujihara T, Nakamura M, Nakata K. P2Y(2) receptor elicits PAS-positive glycoprotein secretion from rabbit conjunctival goblet cells in vivo. *J Ocul Pharmacol Ther*. 2003;19(4):345–352.

30. Pintor J, Bautista A, Carracedo G, Peral A. UTP and diadenosine tetraphosphate accelerate wound healing in the rabbit cornea. *Ophthalmic Physiol Opt*. 2004;24(3):186–193.
31. Mundasad MV, Novack GD, Allgood VE, Evans RM, Gorden JC, Yerxa BR. Ocular safety of INS365 ophthalmic solution: a P2Y(2) agonist in healthy subjects. *J Ocul Pharmacol Ther*. 2001;17(2):173–179.
32. Yerxa BR, Mundasad M, Sylvester RN, Garden JC, Cooper M, Kellerman DJ. Ocular safety of INS365 ophthalmic solution, a P2Y2 agonist, in patients with mild to moderate dry eye disease. *Adv Exp Med Biol*. 2002;506(Pt B):1251–1257.
33. Matsumoto Y, Ohashi Y, Watanabe H, Tsubota K, Diquafosol Ophthalmic Solution Phase 2 Study G. Efficacy and safety of diquafosol ophthalmic solution in patients with dry eye syndrome: a Japanese phase 2 clinical trial. *Ophthalmology*. 2012;119(10):1954–1960.
34. Tauber J, Davitt WF, Bokosky JE, et al. Double-masked, placebo-controlled safety and efficacy trial of diquafosol tetrasodium (INS365) ophthalmic solution for the treatment of dry eye. *Cornea*. 2004;23(8):784–792.
35. Takamura E, Tsubota K, Watanabe H, Ohashi Y, Diquafosol Ophthalmic Solution Phase 3 Study G. A randomised, double-masked comparison study of diquafosol versus sodium hyaluronate ophthalmic solutions in dry eye patients. *Br J Ophthalmol*. 2012;96(10):1310–1315.
36. Kamiya K, Nakanishi M, Ishii R, et al. Clinical evaluation of the additive effect of diquafosol tetrasodium on sodium hyaluronate monotherapy in patients with dry eye syndrome: a prospective, randomized, multicenter study. *Eye*. 2012;26(10):1363–1368.
37. Kaido M, Uchino M, Kojima T, Dogru M, Tsubota K. Effects of diquafosol tetrasodium administration on visual function in short break-up time dry eye. *J Ocul Pharmacol Ther*. 2013;29(6):595–603.
38. Koh S, Ikeda C, Takai Y, Watanabe H, Maeda N, Nishida K. Long-term results of treatment with diquafosol ophthalmic solution for aqueous-deficient dry eye. *Japanese Journal of Ophthalmology*. 2013;57(5):440–446.
39. Arita R, Suehiro J, Haraguchi T, et al. Topical diquafosol for patients with obstructive meibomian gland dysfunction. *Br J Ophthalmol*. 2013;97(6):725–729.
40. Johnson ME. The association between symptoms of discomfort and signs in dry eye. *Ocul Surf*. 2009;7(4):199–211.
41. Nichols KK, Nichols JJ, Mitchell GL. The lack of association between signs and symptoms in patients with dry eye disease. *Cornea*. 2004;23(8):762–770.
42. Methodologies to diagnose and monitor dry eye disease: report of the Diagnostic Methodology Subcommittee of the International Dry Eye WorkShop (2007). *Ocul Surf*. 2007;5(2):108–152.
43. Tuisku IS, Konttinen YT, Konttinen LM, Tervo TM. Alterations in corneal sensitivity and nerve morphology in patients with primary Sjögren's syndrome. *Exp Eye Res*. 2008;86(6):879–885.
44. De Paiva CS, Pflugfelder SC. Corneal epitheliopathy of dry eye induces hyperesthesia to mechanical air jet stimulation. *Am J Ophthalmol*. 2004;137(1):109–115.
45. Labbe A, Liang Q, Wang Z, et al. Corneal nerve structure and function in patients with non-sjogren dry eye: clinical correlations. *Invest Ophthalmol Vis Sci*. 2013;54(8):5144–5150.
46. Benitez-Del-Castillo JM, Acosta MC, Wassfi MA, et al. Relation between corneal innervation with confocal microscopy and corneal sensitivity with noncontact esthesiometry in patients with dry eye. *Invest Ophthalmol Vis Sci*. 2007;48(1):173–181.