

Review

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Review

Applications of Diquafosol Sodium in Ophthalmology: A Comprehensive Review on Therapeutic Utility

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Abstract: Diquafosol is a purinergic P2Y₂ receptor agonist that is garnering much interest for its potential therapeutic benefits in dry eye disease (DED) management and is used commonly in Asia. Diquafosol's mechanism of action involves enhancing fluid secretion from conjunctival epithelial cells and promoting mucin secretion, thereby improving tear film stability and ocular surface lubrication. Clinical trials have demonstrated its efficacy in effectively targeting all three DED subtypes, aqueous-deficient, decreased-wettability, and evaporative dry eye, by increasing tear production leading to improvements in objective markers and reducing subjective symptoms of DED. Adverse effects are generally mild and transient, including ocular irritation and discomfort. This review explores future research directions including its efficacy in certain subtypes of DED yet to be thoroughly explored, inter-product superiority, creation of various formulations, recommended treatment duration and its potential combination therapies. Overall, this review seeks to provide an overview of the pharmacological properties, clinical efficacy, safety profile, clinical utility, long-term usage, and prospects of diquafosol in managing DED. Diquafosol represents a promising novel option in the armamentarium against DED to address the underlying pathophysiology of this common ocular condition.

Keywords: “dry eye”, “diquafosol”, “ocular surface disorders”, “tear film-oriented therapy”, “tear film oriented diagnosis”, “topical non-steroidal immunosuppressants”, “Ocular Graft versus Host Disease”, “soft contact lens users”, “LASIK”, “post-cataract surgery”

1. Introduction

Dry eye disease (DED) is a chronic, multifactorial ocular surface disease [1] and is particularly common in Asia, with a pooled prevalence of 21.7% among males and 16.4% among females [2]. In China alone, a meta-analysis estimated that symptomatic DED affects approximately 31.4% of the population, corresponding to 394 million individuals [3]. DED carries a significant symptomatic burden and is associated with reduced quality of life and vision [4]. Patients with severe DED have reported that loss in health utilities were comparable to patients on dialysis, experiencing severe angina or a disabling hip fracture [5]. At a population level, these issues translate into substantial

economic costs, with estimated annual healthcare expenditures for DED reaching USD\$104-167 billion in China and USD\$55 billion in the United States respectively [6,7].

Several factors contribute to the development of DED, including systemic and ocular diseases, medication intake, genetic, environmental and lifestyle choices. The Tear Film and Ocular Surface Society Dry Eye Workshop II (TFOS DEWS II) defines DED as a multifactorial disease of the tear and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface [8]. It highlights tear film hyperosmolarity and inflammation as key drivers of epithelial damage. The Asia Dry Eye Society (ADES) defines DED as a multifactorial disease characterised by an unstable tear film causing a variety of symptoms or visual impairment, potentially accompanied by ocular surface damage [9]. In contrast to the TFOS DEWS II, ADES focuses on tear film instability as the primary mechanism of DED and suggests that inflammation occurs secondary to epithelial damage caused by an unstable tear film.

While perspectives on mechanisms leading to DED may differ, both highlight the critical role of tear film stability – the ability to maintain a smooth, continuous, and uniform layer over the corneal surface before breaking up [10]. Deficiencies in any of the key components of the tear film result in distinct DED subtypes, including aqueous-deficient, evaporative and decreased wettability, each characterised by variations in tear film dynamics and break-up patterns [10].

Diquafosol sodium, a first-in-class mucin secretagogue, has introduced a paradigm shift in the treatment of DED in Asia. Although primarily indicated to improve surface wettability, its effectiveness has been demonstrated across all DED sub-types. It is one of the few therapies that enhances lipid layer thickness (LLT) and goblet cell density while reducing conjunctival epithelial damage [11]. Diquafosol has reported superior efficacy over many tear-replacement solutions in improving tear production, ocular surface staining scores and tear film stability [12,13]. Emerging research highlights diquafosol's effectiveness in managing an array of ocular surface disorders. This review aims to summarise its utility and provide recommendations for clinical use based on existing evidence, and offer insights into the future directions and innovations to optimise the management of ocular surface diseases.

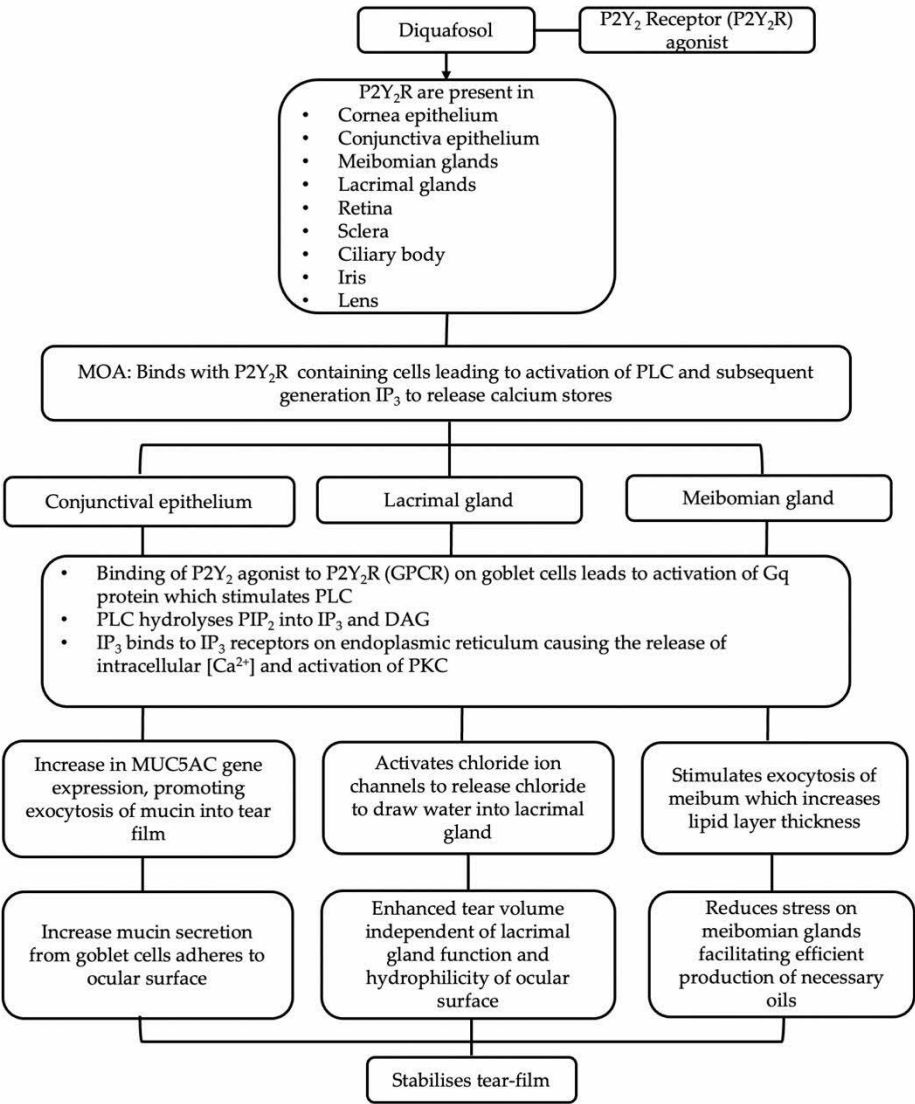
2. Methods

A detailed search of the available literature was conducted in PubMed, EMBASE and COCHRANE from inception to September 2024. Key search terms included but were not limited to “dry eye”, “diquas”, “diquafosol”, “ocular surface disorders”, “tear film oriented therapy”, “tear film oriented diagnosis”, “tear-replacement solutions”, “topical corticosteroids”, “intense pulsed light”, “ocular graft versus host disease”, “soft contact lens users”, “LASIK”, “cataract surgery”. Both animal and human clinical studies were included in this review. Additionally, references were hand-searched for further relevant articles.

3. Pharmacologic Properties

3.1. Mechanism of Action

Diquafosol is a stable derivative of uridine 5'-triphosphate (UTP) and a potent P2Y₂ receptor (P2Y₂R) agonist that plays a significant role in regulating tear and mucin secretion. In the eye and ocular adnexae, P2Y₂R are expressed by the corneal and conjunctival epithelium, goblet cells and meibomian glands [14]. Activation of P2Y₂R elevates intracellular calcium ion concentrations and facilitates fluid transport from the serosal to mucosal side via chloride channel activation to enhance tear fluid secretion from conjunctival epithelial cells and mucin secretion from conjunctival goblet cells [15]. Diquafosol also augments gene expression of membrane-associated mucin 1 (MUC1), 4 (MUC4) and 16 (MUC16) in corneal epithelial cells and MUC1, MUC16 and membrane-associated mucin 5AC (MUC5AC) in conjunctival epithelial cells to stimulate mucin secretion [16]. Figure 1 summarises the action of diquafosol on various receptors in the ocular region to stabilise the tear film.



Abbreviations
MOA = mechanism of action
GPCR = G-protein coupled receptors
PLC = phospholipase C
PIP2 = phosphatidylinositol 4,5-bisphosphate
IP3 = Inositol triphosphate
DAG = diacylglycerol
PKC = protein kinase C

Figure 1. Mechanism of action of diquafosol.

3.2. Commercially Available Formulations

Diquafosol 3% is presently available in two commercial formulations, a formulation containing chlorhexidine gluconate solution as a preservative (Diquas®) and a non-preservative, single vial formulation (Diquas®-S). Table 1 lists the active ingredients in Diquas® and Diquas®-S. When compared against Diquas®, administration of Diquas®-S has been reported to lead to greater improvements in dry eye symptoms, tear break-up time (TBUT) and meibomian gland function across parameters such as LLT, lid margin abnormalities, meibomian gland dysfunction stage, meibum expressibility and quality and meibomian gland dropout among post-cataract surgery patients after three months of usage [17]. However, Diquas®-S is presently available in a limited number of countries, with cost and logistical considerations likely limiting its commercial availability.

Table 1. Composition of Diquas® and Diquas®-S.

Diquas®	Diquas®-S
Chlorhexidine gluconate (preservative)	Dibasic sodium phosphate hydrate
Dibasic sodium phosphate hydrate	Disodium edetate hydrate
Disodium edetate hydrate	Sodium chloride
Sodium chloride	Potassium chloride
Potassium chloride	Hydrochloric acid
Sodium hydroxide	Sodium hydroxide
Dilute hydrochloric acid	

3.3. Adverse Effects

Diquafosol is a relatively safe medication with no serious adverse events associated with its usage reported across several major clinical trials [18–24]. A meta-analysis estimated the odds of developing adverse events from using topical diquafosol to be 1.7 times that of hyaluronic acid (odds ratio: 1.71, 95% confidence interval (CI): 1.08 to 2.71, $p=0.02$; $I^2=18\%$) [13]. Reported side effects, such as ocular irritation (6.3%), discharge (2.8%), foreign body sensation (2.8%), conjunctival hyperaemia (1.4%), pain (1.4%), and pruritus (1.4%) are generally mild and transient [19]. An observational study by Ohashi and coworkers reported an adverse drug reaction rate of 10.7%, primarily involving non-serious symptoms of eye discharge, irritation, pain, pruritus, foreign body sensation, with the highest incidence occurring during the first month of treatment [25]. A study by Nakamura and coworkers reported that symptoms of diquafosol-induced eye irritation and pain disappeared within seven days in about 50% of cases and within 28 days in 80% of cases following continued diquafosol instillation [11]. No studies till date have evaluated the mechanisms leading to a transient worsening of DED symptoms during diquafosol initiation. It is postulated that activation of P2Y₂R in the acute phase induces a local pro-inflammatory state over the ocular surface [26]. Additionally, on compromised ocular surfaces with corneal epithelial erosions, diquafosol may bind to P2Y₂R in nerve terminals and the purinergic pain receptor P2X₃ to induce pain and discomfort [26]. Therefore, the authors recommend active management of ocular surface inflammation prior to commencement of topical diquafosol for DED. Additionally, patients ought to be primed regarding possible ocular discomfort and worsening dry eye symptoms upon commencement of diquafosol and encouraged to persist with treatment if minor side effects manifest.

Systemically, research has reported P2Y₂R to worsen inflammation [26]. The activation of P2Y₂R is reported to induce proinflammatory states in gastrointestinal, respiratory and neurological cells, and has also been implicated in cancer pathogenesis [27–29]. However, P2Y₂R agonists have also been demonstrated to promote wound healing through recruitment of leukocytes to sites of tissue damage, facilitating differentiation and proliferation of structural cells [30]. Nevertheless, topical administration of diquafosol is unlikely to yield any systemic adverse reactions as it is rapidly metabolised at the ocular surface. Random sampling of 25 patients receiving topical diquafosol for six months showed no elevation of systemic concentration of derived metabolites relating to diquafosol [31]. Thus far, no studies have evaluated its safety during pregnancy.

3.4. Effects on Tear Stimulation

Diquafosol administration has been reported to increase tear fluid secretion in murine dry eye models. A study conducted on Goto-Kakizaki rats; a spontaneous murine model of type 2 diabetes with corneal neuropathy, which were exposed to constant airflow towards the face reported that diquafosol administration significantly increased Schirmer’s test after 15 minutes ($p<0.01$), reduced corneal fluorescein staining score at four ($p<0.05$) and six weeks ($p<0.01$) [32]. Exact values were not reported by the authors. This suggests the possible role of diquafosol in stimulating tear production, despite the presence of impaired corneal neuronal function. Another in-vivo murine study investigated the effect of diquafosol administration on corneal epithelial defects, with percentage of

wound closure monitored via image analysis [33]. Rats were anaesthetised followed by the creation of a 3 mm central corneal epithelial defect with a burr. Instillation of diquafosol facilitated earlier epithelial healing compared to control eyes at 12 and 24 hours respectively (percentage wound closure of diquafosol-treated eyes at 12 and 24 hours: $63.4 \pm 2.0\%$ and $98.1 \pm 1.1\%$; percentage wound closure of control eyes at 12 and 24 hours: $42.7 \pm 2.5\%$ and $82.3 \pm 3.2\%$). However, no mention of use of topical agents or vehicle in the control group was reported. This is further supported by in-vitro experiments demonstrating that diquafosol induces extracellular signal-regulated kinase cell proliferation, epithelial growth factor receptor phosphorylation and increased intracellular calcium, suggesting that the accelerated corneal epithelial healing process may be attributed to intracellular calcium-mediated epithelial growth factor receptor signalling pathways through the activation of P2Y₂R [33].

Studies in human subjects have reported similar improvements in tear secretion. Administration of diquafosol in both healthy and DED patients demonstrated increased radius of curvature of the central lower tear meniscus for up to 30 minutes after instillation [34]. The radius of curvature of the lower tear meniscus shares a linear relationship with the cumulative tear volume over the ocular surface, serving as a surrogate measure of aqueous tear volume [35,36]. This was also observed in patients with aqueous deficient DED secondary to Sjögren's syndrome, where instillation of diquafosol significantly increased the central lower tear meniscus radius curvature at 15 minutes [37]. These results suggest the role of diquafosol in promoting tear fluid secretion of conjunctival epithelial cells, independent of lacrimal gland function [33,37].

3.5. Effects on Lipid Secretion

Diquafosol has also been demonstrated to stimulate holocrine-like lipid secretion from meibocytes via activation of P2Y₂R. Tissue sections of meibomian gland specimens from superoxide dismutase-1 (Sod1) mice and wild-type (WT) mice were stained with Oil Red O stain and analysed digitally using an image processing software to quantify the number of pixels corresponding to lipid droplets [38]. Administration of diquafosol six times daily for two weeks increased the total number of lipid droplets (20 ± 15 droplets pre-treatment to 100 ± 80 droplets two weeks post-treatment, $p < 0.01$ in Sod1 mice and 25 ± 10 droplets pre-treatment to 60 ± 100 droplets two weeks post-treatment, $p < 0.05$ in WT mice), which was attributed to increased meibum secretion. There were also documented improvements in corneal fluorescein staining score, attributed by the authors to improved tear film stability and the impact of diquafosol on corneal epithelial healing. This was supported by an in-vitro study of cultivated rabbit meibomian gland cells, suggesting that diquafosol induced intracellular calcium signalling in a dose-dependent manner, increasing total cholesterol cellular release [39]. Clinically, administration of diquafosol in human eyes increased LLT and tear film stability. Interferometry measurements of tear film LLT in normal human eyes showed a statistically significant increase in mean thickness from 62.3 ± 31.1 nm to 77.0 ± 39.5 nm ($p < 0.001$), 79.3 ± 40.5 nm ($p < 0.001$), and 77.7 ± 43.6 nm ($P = 0.009$) at the 15, 30 and 60 minute mark respectively [40]. A study among dry eye patients revealed similar improvements in LLT, from an initial 49.4 ± 16.2 nm to 70.6 ± 28.2 nm ($p < 0.001$) and 63.9 ± 30.0 nm ($p = 0.042$) at 30 and 60 minutes after diquafosol instillation [41]. In comparison, instillation of artificial tears did not result in significant increase in LLT after treatment, with measurements reported at 52.9 ± 22.8 nm, 52.3 ± 20.3 nm and 50.3 ± 19.8 nm at 30, 60 and 90 minutes, respectively ($p > 0.05$ for all) [41].

3.6. Effects on Mucin Secretion

Diquafosol is primarily a mucin secretagogue that improves corneal wettability by stimulating expression of human epithelial mucins via intracellular extracellular signal-regulated kinase [16]. Mucins are categorised into two groups: membrane-associated mucins and goblet-cell secreted mucin. MUC1, MUC4, MUC16 are produced by both corneal and conjunctiva epithelia while MUC5AC is a secretory mucin produced by goblet cells [42]. Membrane-associated mucins form a dense protective glycocalyx barrier over the ocular surface epithelia, facilitating debris clearance and

improves the lubricity effect of the tear film, thereby reducing friction generated between the eyelid and cornea interfaces [43,44]. Secretory mucins assist in clearing ocular surface pathogens and, improves surface lubricity by forming a highly hydrated mucus gel that reduces friction [44,45]. In a rabbit study comparing the effects of diquafosol, rebamipide and artificial tears, only diquafosol was found to increase MUC5AC levels in rabbits' tears after 15 minutes ($p<0.01$) [46]. Similar observations in canine models have been documented with statistically significant increase in tear film MUC5AC concentrations at 300min ($p=0.033$) following administration of diquafosol, although neither Schirmer's nor phenol red thread testing demonstrated any statistically significant differences [47]. Another study by Lee and coworkers demonstrated increased expression of MUC1 and MUC16 by human conjunctival epithelial cells following instillation of diquafosol [16]. These in-vivo and in-vitro studies support the efficacy of diquafosol in inducing secretion of membrane-associated and secretory mucins on the ocular surface.

4. Therapeutic Efficacy

4.1. Dry Eye Disease

Multiple studies have reported the efficacy of diquafosol in alleviating symptoms and signs of DED. Ohashi and coworkers report significant symptomatic improvements and reduction in Dry Eye-related Quality of Life Score (DEQS) values in patients receiving topical diquafosol for a duration of 12 months [25]. Randomised double-masked parallel-group trials also reported findings of greater improvement in foreign body sensation and eyelid heaviness among patients receiving diquafosol compared to placebo artificial tears [19,20]. A randomised controlled trial involving patients with DED reported comparable improvements in corneal fluorescein staining score (-2.1 ± 1.5 in diquafosol, -2.0 ± 1.3 in sodium hyaluronate at week four, 95% CI: -0.303 to 0.181) between patients who utilised diquafosol compared to sodium hyaluronate eyedrops ($P>0.05$) [21]. However, improvements in conjunctival rose bengal staining score was more significant among diquafosol users (-2.5 ± 2.0 in diquafosol, -2.0 ± 1.9 in sodium hyaluronate at week four, $p=0.019$). Both treatment groups experienced improvements in TBUT by 1.046 ± 1.797 s and 0.832 ± 1.775 s respectively with no statistically significant differences between findings [21]. Meta-analyses of randomised controlled trials exploring outcomes of diquafosol administration for the treatment of DED have reported its efficacy in alleviating subjective ocular symptoms and improving dry eye indices compared to artificial tears [13,48]. Sun and co-workers analysed nine randomised controlled trials that recruited patients with DED and reported significant improvements in Ocular Surface Disease Index (OSDI) (mean difference (MD): -3.59 , 95% CI: -4.68 to -2.50 , $p<0.001$; $I^2=6\%$), Schirmer's test (MD: 1.08 mm, 95% CI: 0.41 to 1.76 , $p=0.002$; $I^2=0\%$), TBUT (MD: 0.60 s, 95% CI: 0.20 to 0.99 , $p=0.003$; $I^2=63\%$), corneal fluorescein staining score (MD: -0.20 , 95% CI: -0.37 to -0.03 , $p=0.02$; $I^2=58\%$), and rose bengal staining score (MD: -0.62 , 95% CI: -0.88 to -0.35 , $p<0.001$; $I^2=15\%$) [13].

Diquafosol has also been shown to inhibit nuclear factor-kappa B (NF- κ B) signalling and other inflammatory factors induced by hyperosmotic stress in in-vitro studies using human corneal epithelial cells, suggesting its role in the management of inflammation in DED [49]. A study conducted on DED patients receiving cyclosporine 0.1%, cyclosporine 0.05% or diquafosol measured downregulation of tear proteomes (AFM, ALCAM, CFB, H1-4, PON1, RAP1B, and RBP4) across all three groups after 12 weeks of treatment, suggesting a reduction in inflammation [50]. Clinical parameters, including conjunctival fluorescein staining score and TBUT improved following administration of all medications compared to baseline. However, corneal fluorescein staining score improved more significantly in patients receiving cyclosporine compared to diquafosol from baseline to 12 weeks (cyclosporine 0.1%: 4.89 ± 1.26 to 1.39 ± 1.37 , $p<0.001$; cyclosporine 0.05%: 4.69 ± 1.12 to 1.31 ± 1.52 , $p<0.001$ vs diquafosol: 4.31 ± 0.93 to 2.15 ± 1.42 , $p<0.001$). A prospective non-randomised observational study of patients with DED comparing administration of only cyclosporine 0.1% once daily versus cyclosporine 0.1% once daily in combination with diquafosol six times daily reported that combination therapy led to a greater degree of improvement in TBUT compared to patients

receiving only cyclosporine 0.1% (combination: 2.13 ± 2.41 s vs 1.07 ± 1.71 s; $p=0.001$) [51]. Therefore, administration of diquafosol and cyclosporine in combination appears to produce synergistic effects over the ocular surface. Thus far, evidence remains inconclusive on whether patients with poor response to artificial tears should be preferentially started on topical diquafosol or cyclosporine. A study exploring patients' considerations and preferences around medications in DED management has reported that costs and side effect profiles are major attributing factors to choice of therapy [52]. While diquafosol offers a more favourable side effect profile, physicians should be mindful that initiation of diquafosol in the early phases, particularly in patients with significant inflammation, may exacerbate ocular surface symptoms. This benefit must be weighed carefully against diquafosol's cost and the need for more frequent administration compared to cyclosporine.

4.2. Meibomian Gland Dysfunction

Meibomian gland dysfunction is a chronic process characterised by structural abnormalities of the meibomian gland, terminal duct obstruction and changes in meibum quality [53]. A reduction in meibum secretion and changes to both its viscosity and lipid composition disrupts the tear film lipid layer, contributing to increased evaporative loss.

Several studies have identified the potential of utilising diquafosol in the management of patients with meibomian gland dysfunction. A longitudinal study of patients with obstructive meibomian gland dysfunction receiving diquafosol four times daily for four months or more reported an improvement in measured mean meibomian gland area from $36.9 \pm 10.1\%$ pre-treatment to $41.5 \pm 9.2\%$ post-treatment ($p < 0.001$) [54]. Ocular surface symptoms, lid margin abnormalities; such as plugging of meibomian orifices, and TBUT also improved after instillation of diquafosol. Moreover, diquafosol has been reported to significantly increase LLT [40]. A study analysing DED patients receiving either topical diquafosol, normal saline, sodium hyaluronate 0.1% or gatifloxacin 0.3% found that only diquafosol led to a significant increase in LLT (Mean change in LLT from baseline to 20 minutes after instillation of eyedrops: 12.6 ± 2.0 nm for diquafosol ($p < 0.001$), 1.2 ± 2.2 nm for normal saline ($p = 0.301$), 1.5 ± 2.0 nm for sodium hyaluronate ($p = 0.495$), and 0.5 ± 3.2 nm for gatifloxacin ($p = 0.884$)) [55]. These results suggest a possible therapeutic role for diquafosol in the treatment of evaporative DED.

4.3. Aqueous-Deficient Dry Eye Disease

Aqueous-deficient DED is a subtype of DED characterised by decreased tear production by the lacrimal and accessory glands, affecting up to one-third of patients diagnosed with DED [56]. Aqueous-deficient DED can arise due to a plethora of pathological mechanisms that can ultimately result in lacrimal gland damage. Donthineni and coworkers have suggested a classification comprising of four major groups: immune-mediated lacrimal gland inflammation (such as Sjogren's syndrome), conjunctival cicatrization (mucous membrane pemphigoid and Stevens-Johnson syndrome), neurogenic causes (for example diabetes mellitus) and lacrimal gland loss (arising from trauma, age, or congenital alacrima) [57].

Diquafosol has been shown to be effective in alleviating both subjective and objective clinical parameters of aqueous-deficient DED. A study recruiting patients with mild-to-moderate aqueous-deficiency DED reported that patients receiving diquafosol experienced significant improvements in subjective dry eye symptoms evaluated by the authors based on a composite severity scoring system (exact scores were not reported, $p < 0.01$), corneal fluorescein staining score (4.1 ± 1.8 to 1.1 ± 1.1 , $p < 0.01$) and tear meniscus height (126 ± 24 μ m to 171 ± 48 μ m, $p < 0.01$) with no major adverse reactions reported [58]. Usage of diquafosol has also been reported to improve the optical quality of the tear film, with higher-order aberrations used as a surrogate measurement in patients receiving diquafosol for four weeks. These patients experienced improvements in higher-order aberrations measured from a wavefront sensor at four weeks compared to baseline (0.180 ± 0.06 μ m to 0.148 ± 0.039 μ m, $p = 0.035$), with improvements in subjective dry eye symptom scores (24.3 ± 6.7 to 15.3 ± 6.0 , $p < 0.001$), corneal fluorescein staining score (4.6 ± 1.4 to 1.9 ± 1.1 , $p < 0.001$) and TBUT (1.6 ± 0.8 s to 3.1 ± 0.8 s, $p < 0.001$),

although no significant improvements were demonstrated with conjunctival fluorescein staining score (5.3 ± 1.3 to 4.8 ± 1.1 , $p=0.078$) and Schirmer's test (1.7 ± 2.0 mm to 1.2 ± 1.8 mm, $p=0.228$) [59].

Patients with Sjogren's syndrome also benefit from diquafosol, with its use associated with significant increase in central lower tear meniscus radius curvature 15 minutes after diquafosol instillation (0.16 ± 0.07 mm to 0.21 ± 0.08 mm, $p<0.001$), while no improvements were reported in patients receiving solely artificial tears [37]. Another observational study of female patients with Sjogren's syndrome who were symptomatic despite treatment with artificial tears reported that addition of diquafosol to the existing treatment regimen resulted in improvements in both subjective symptoms, as well as objective parameters including tear meniscus radius (baseline vs 12 months: $p<0.001$), TBUT (baseline vs 12 months: $p<0.05$), corneal fluorescein staining score (baseline vs 12 months: $p<0.001$) and conjunctival fluorescein staining score (baseline vs 12 months: $p<0.05$) at regular intervals up till the 12th month of diquafosol treatment [60]. Importantly, all patients in the study who experienced symptomatic flares were also permitted to instil topical corticosteroids up to twice daily, which may have confounded these findings. However, the proportion of patients receiving topical corticosteroids was not reported.

4.4. Ocular Graft-Versus-Host Disease (oGVHD)

Graft-versus-host disease (GVHD) is a complex immunological condition that arises from donor T-cell mediated responses towards recipient antigens, inciting tissue damage and cytokine-mediated activation of antigen-presenting cells [61]. Chronic GVHD involves additional processes such as thymic injury, B cell autoantibody synthesis, and the formation of profibrotic lesions [62]. oGVHD represents a significant complication of GVHD, with cicatrising changes and desiccation increasing the risk of corneal ulceration and perforation. oGVHD may affect all tear film components. Alloreactive T-cells promote myofibroblast proliferation which leads to fibrosis and destruction of lacrimal glands, with a consequent reduction in aqueous tear production [63]. T-cell infiltration, endothelial injury, neovascularisation and fibroblast activation have been observed in the meibomian glands of murine chronic GVHD models [64]. Another study reported that allogenic GVHD murine models had a significantly higher meibomian gland plugging score (1.5 vs 0 , $p<0.001$) and more noticeable meibomian gland atrophy scored on the meiboscale (93% vs 47% atrophy) compared to the control group with no purified splenic T cells. These results suggest that meibomian gland changes occur with oGVHD which can reduce both the quantity and quality of meibum secretion in stabilising the lipid tear film layer [65]. Furthermore, mucin secretion is also impaired in oGVHD patients. Ogawa and coworkers suggest that epithelial mesenchymal transition, characterised by replacement of epithelial cell with mesenchymal markers incites basal epithelia secretion of abnormal collagen bundles contributing to conjunctival fibrosis that damages the mucin secretory function of goblet cells [66]. This is supported by findings in murine GVHD models in which a reduction in both the area and thickness of corneal glycocalyx has been reported [67]. Cicatrising changes that lead to eyelid abnormalities such as ectropion, entropion, lagophthalmos and trichiasis can further compromise the ocular surface [63].

Diquafosol has been studied as a treatment for oGVHD primarily for its mucin-secreting properties, but is also postulated to augment other components of the tear film. In a retrospective study of patients with mild to moderate chronic GVHD-induced DED using diquafosol for up to 17 months, marked improvements in corneal fluorescein staining score (5.9 ± 0.6 to 1.3 ± 1.1 , $p<0.001$), corneal and conjunctival rose bengal staining score; an aggregate score of staining of the cornea, temporal and nasal conjunctiva (4.7 ± 1.6 to 2.0 ± 1.5 , $p=0.008$), and TBUT (2.6 ± 0.9 s to 4.6 ± 1.6 s, $p=0.009$) were reported [68]. An anecdotal report of a 61-year-old lady with severe DED secondary to chronic GVHD showed that combination therapy of topical diquafosol six times daily with topical rebamipide four times daily resulted in a substantial reduction in an aggregated visual analogue score which measures 12 symptoms: asthenopia, pain, discharge, foreign body sensation, epiphora, burning, ocular itching, dull sensation, conjunctival injection, dullness, dryness, and photophobia from 33 points at baseline to 8.5 points. Improvements in objective clinical findings include corneal

fluorescein staining score (4 to 1.5), cornea and conjunctiva rose bengal score, an aggregate of nasal, temporal conjunctiva and cornea (4 to 1), and TBUT (2.3 s to 10 s) [69]. However, no improvement on Schirmer's test (3.0 mm to 3.0 mm) was identified compared to baseline. Further large-scale studies are required to ascertain its effectiveness in treating oGVHD.

4.5. Glaucoma Medication and Preservatives-Related Ocular Surface Disease

It has been reported that up to 59% of glaucoma patients experience ocular surface symptoms with 78% of patients in an examined cohort exhibiting signs of ocular surface disease [70]. The pathogenesis is multifactorial but has been significantly attributed to medicamentosa arising from the active ingredient, its excipients and associated preservatives [71]. Beta blocker formulations, particularly timolol maleate, have been reported to disrupt tear film stability, reduce tear secretions and incite cicatrising changes over the conjunctival epithelium [72–76]. Prostaglandin analogues induce obstructive meibomian gland dysfunction which exacerbates ocular surface disease and may contribute to poor compliance to glaucoma therapy [74,77]. Moreover, prostaglandin-associated periorbitopathy can induce periorbital and eyelid changes such as trichiasis, and periorbital fat loss that further alter and compromise the ocular surface [78]. Benzalkonium chloride (BAK) is a commonly used preservative that has been demonstrated to disrupt tear film homeostasis and alter corneal sensitivity in a dose-dependent manner [79,80]. A study examining corneal sensitivity in glaucoma patients using BAK-containing eyedrops reported a reduction in corneal sensitivity; measured with a Cochet-Bonnet esthesiometer, in a dose-dependent fashion. Findings of corneal sensitivity were 56.2 ± 5.2 mm, 50.3 ± 12.5 mm and 44.3 ± 13.6 mm among those treated with none, one and two or more instillations of preserved eyedrops, respectively [81]. In-vivo confocal microscopy has also been applied, which demonstrates a significant reduction in sub-basal corneal nerve density among patients treated with BAK-containing medications as compared to BAK-free formulations [82]. BAK has also been shown to incite cornea, conjunctival, meibomian gland and trabecular meshwork cells toxicity [83–85]. A reduction in goblet cell density following brief exposure of the ocular surface to BAK containing formulations has been demonstrated using impression cytology [86]. In-vitro studies with human corneal-limbal epithelial cell cultures showed that prolonged exposure to 0.0025% and 0.01% BAK for an hour decreases the amount of functional mucin, eventually causing complete destruction of the mucous layer and diffuse damage to superficial corneal epithelial cells [87]. Instillation of BAK-preserved latanoprost upregulates tear inflammatory cytokines levels including IL-2, IL-5, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, basic fibroblast growth factor, and platelet-derived growth factor compared to preservative-free formulations of latanoprost [88]. Several clinical studies have reported an association between BAK-containing medications and both worsening OSDI scores and poorer glaucoma filtering surgery outcomes [89,90]. It has been postulated that BAK induces conjunctival subepithelial inflammation and fibrosis [91], which aggravates the wound healing process in glaucoma filtering surgeries [92].

Diquafosol has been suggested to be useful in the management of glaucoma medication and preservative-related ocular surface symptoms due to its ability to promote tear and mucin secretion and stimulate meibomian gland function. Diquafosol contains chlorhexidine at a concentration of 0.0001-0.1% as a preservative, as detailed in the product insert. Chlorhexidine gluconate has been found to be safe and effective as an ocular surface antiseptic at concentrations between 0.05-0.1% [93]. While higher concentrations of chlorhexidine gluconate at 2-4% have been shown in rabbit studies to exhibit dose-dependent toxic effects such as corneal epithelial oedema, corneal de-epithelisation, conjunctival chemosis, bulbar conjunctival hyperaemia and anterior stromal oedema [94,95], application of concentrations of 1% or less did not result in delays in corneal re-epithelisation among experimental rabbit corneal abrasion models, with mild conjunctivitis as the only complication reported [96]. This suggests that diquafosol, even in its chlorhexidine gluconate preservative-containing formulation, is safe and produces minimal preservative-induced ocular surface complications. A clinical study recruited glaucoma patients suffering from DED and reported improvements in mean OSDI score (52.17 ± 13.02 to 48.77 ± 13.27 , $p=0.041$), TBUT (3.79 ± 1.94 s to

4.70±2.81 s, $p=0.009$) and Schirmer's test (4.52±2.11 mm to 5.64±2.79 mm, $p=0.001$) up to 52 weeks following initiation of diquafosol [97]. Impression cytology demonstrated a sustained increase in goblet cell density which begun four weeks after commencing diquafosol, and lasting up to the 52 week mark (Baseline: 445.1±92.2 cells/mm², 4 weeks: 511.0±110.8 cells/mm², 12 weeks: 520.5±121.8 cells/mm², 36 weeks: 504.8±160.3 cells/mm², 52 weeks: 512.4±177.3 cells/mm², $p<0.05$ for all). There was however no information provided regarding the type of anti-glaucoma medication used. Another study consisting of normal tension glaucoma patients who were either on a preservative-containing prostaglandin analogue, preservative-free prostaglandin analogue, or a combination of BAK-containing prostaglandin analogue (BAK-PGA) and diquafosol (BAK-PGA + DQS) suggested that diquafosol was protective against meibomian gland dropout that was associated with BAK-PGA [98]. In the BAK-PGA group, meibomian gland dropout increased significantly at nine and 12 months after initiation of topical therapy compared to baseline, while no statistically significant changes were observed among patients using solely BAK-free formulations or a BAK-PGA + DQS combination. Additionally, another study by Liu and coworkers on patients who underwent glaucoma surgery demonstrated that diquafosol administration in the early postoperative-period following trabeculectomy improved measured tear meniscus height (0.24±0.16 mm to 0.28±0.15 mm, $p=0.025$), TBUT (5.08±3.01 s to 9.02±3.20 s, $p<0.05$) and LLT (4.13±1.36 nm to 4.81±1.45 nm, $p=0.007$) at eight weeks postoperatively compared to baseline [99].

4.6. Cataract Surgery

Cataract surgery has been associated with a worsening of tear film parameters and increased dry eye symptoms postoperatively. A study examined patients three months after undergoing cataract surgery and demonstrated statistically significant deterioration of tear film parameters and increased dry eye symptoms [100]. Miyake and coworkers examined patients at one month after cataract surgery and reported that 31% of patients met the criteria for a diagnosis of DED [101]. This has been attributed to a range of factors, including medicamentosa, the surgical environment such as phototoxic effects from the operating microscope and surface desiccation and manipulation [102–107]. In patients with pre-existing ocular surface compromise, cataract surgery can alter meibum quality and exacerbate tear film instability and worsen symptoms [108,109].

In a study comparing patients receiving preservative-free diquafosol, preservative-containing diquafosol and preservative-free sodium hyaluronate commencing postoperatively from day one for three months, both groups of patients receiving diquafosol experienced significant improvements in measured TBUT at one month postoperatively compared to preoperative values (preservative-free: 4.6±2.2 s to 6.3±3.6 s, $p<0.001$, preservative-containing: 5.0±2.5 s to 7.0±2.8 s, $p<0.001$) [17]. These findings were compared to patients receiving sodium hyaluronate, where a decrease in TBUT from 4.6±1.8 s to 3.7±1.4 s ($p=0.038$) at one-month post-surgery was identified. At three months postoperatively, only patients receiving preservative-free diquafosol maintained significant improvements in TBUT compared to preoperative values (6.5±3.5 s vs 4.6±2.2 s, $p=0.038$) with significantly increased TBUT than patients receiving sodium hyaluronate (4.7±2.3 s vs 4.6±1.8 s, $p=0.038$). Similarly, OSDI and corneal fluorescein staining scores significantly improved in all patients receiving diquafosol compared to sodium hyaluronate. Schirmer's test results remained unchanged across all groups throughout the study period. A study demonstrated that diquafosol administered six times daily postoperatively after cataract surgery resulted in significantly improved TBUT (4.88±2.52 s to 6.69±2.23 s at three months, $p<0.001$), corneal fluorescein staining score (values at three months were not reported, $p=0.045$), and conjunctival lissamine green staining score (1.55±1.19 to 0.36±0.76 at three months, $p=0.001$) compared to baseline [110].

Preoperative management of DED is important in ensuring accurate keratometry measurements, which can in-turn influence IOL calculations [111]. Trattler and coworkers reported that among patients scheduled for cataract surgery, 77% of eyes had evidence of corneal fluorescein staining while 63% of patients had a measured TBUT of five seconds or less, although the proportion of symptomatic patients was not reported [112]. Tear film instability can affect keratometric

measurements. A study of dry eye patients scheduled for cataract surgery reported that IOL calculations may vary by as much as 0.5D between measurements across different visits [113]. Epitropoulos and coworkers have further reported that 8% of eyes with hyperosmolar tears had a difference of more than 0.50 D in measured mean keratometry values across two visits ($p=0.049$) and 17% of eyes had a vector astigmatism difference of more than 1.0 D ($p=0.01$). A multicentre prospective study of preoperative cataract surgery patients with DED found that administration of DQS-LX significantly improved the ocular surface and reliability of keratometry measurements [114]. In this study, patients were diagnosed using the Japanese version of the OSDI (J-OSDI) and underwent baseline biometry measurements and subsequently received DQS-LX thrice daily for four weeks. Post-treatment measurements demonstrated statistically significant improvements in TBUT (2.18 ± 0.80 s pre-treatment to 4.29 ± 1.14 s post-treatment, $p<0.001$) and corneal higher order of aberrations (0.30 ± 0.03 μm pre-treatment to 0.25 ± 0.03 μm post-treatment, $p<0.001$) after starting diquafosol. No statistically significant differences in measurements were seen in non-treated eyes across the measured time-points. Importantly, the duration between last dose of DQS-LX and keratometry measurements were not reported in this paper. Kobashi and coworkers have demonstrated improvements in mean intraocular scattering measured via the objective scattering index, a marker of optical quality, from 2.1 ± 0.7 before treatment to 1.5 ± 0.7 at two weeks ($p<0.001$), and 1.6 ± 0.5 at four weeks ($p<0.001$), after treatment, measured at least two hours after the last instillation of diquafosol [115]. Instillation of diquafosol has also been associated with an increase in LLT and TBUT measured after at least 90 minutes following administration, in support of these findings [41]. Further research on the duration of onset of tear film changes following diquafosol administration will be useful in optimisation of keratometry measurements.

4.7. Contact Lens Wear

Contact lens wear compartmentalises the tear film into a pre- and post-lens tear film. This results in thinning of the tear film, which contributes to its instability and increased friction between the contact lens and the ocular surface [116]. Contact lens wear has also been associated with meibomian gland drop out, and is postulated to be related to either a reduction in volume of the pre-lens tear film or as a result of the direct mechanical effects of the contact lens [117]. These interactions can precipitate inflammation which contributes to further destabilisation of the tear film [116].

Diquafosol has been reported to create a statistically significant increase in pre- and post- ($p<0.01$ at 15 minutes and 30 minutes post-diquafosol instillation) lens tear film volumes in rabbit models of contact lens wear [118]. Topical application of diquafosol in soft contact lens users over a four week treatment period resulted in significant increase in fluorescein intensities of wheat germ agglutinin conjugate of fluorescein (F-WGA), which is used to quantify the presence of membrane-associated mucins (571.8 ± 227.8 $\mu\text{g/ml}$ to 794.6 ± 219.4 $\mu\text{g/ml}$, $p<0.001$), while stable sialic acid and tear protein concentrations suggest that diquafosol increased mucin secretion. This study also reported significant improvement in subjective symptoms measured by DEQS (19.2 ± 12.7 to 10.6 ± 12.5 , $p=0.003$), TBUT (3.6 ± 2.2 s to 5.0 ± 2.1 s, $p=0.003$), conjunctival fluorescein staining score (2.8 ± 1.8 to 2.0 ± 1.4 , $p=0.045$), and corneal fluorescein staining score (0.7 ± 1.0 to 0.3 ± 0.6 , $p=0.021$) [119]. Another study involving soft contact lens wearers on either a daily or fortnightly replacement schedule, reported that diquafosol significantly improved contrast sensitivity ($p<0.05$) following eight weeks of usage six times daily. In this study, both corneal fluorescein staining and conjunctival lissamine green staining scores decreased markedly in the diquafosol group ($p=0.03$ and $p<0.001$, respectively), with significantly lower conjunctival lissamine green staining score in the diquafosol group compared to soft contact lens wearers receiving artificial tears ($p=0.02$). Among contact lens users, Ogami and coworkers reported more significant improvement with diquafosol instillation for symptoms of dryness and blurred vision ($p<0.01$) compared to administration of artificial tears. This was postulated to be due to both good compliance to diquafosol and its ability to promote secretion of water and mucin, thereby stabilising the tear film [120].

Overnight orthokeratology lenses are an increasingly popular management modality for myopia control. However, 30-40% of patients experience ocular discomfort following lens insertion, which has been postulated to be due to tear film instability [121,122]. Similar to soft contact lenses, rigid gas permeable lenses can disrupt the tear film and induce meibomian gland atrophy [123,124]. A study by Xie and coworkers reported a significant increase in OSDI score following three months of orthokeratology lens usage compared to baseline (baseline: 4.13 ± 4.21 vs third month: 7.26 ± 4.52 , $p < 0.05$) [123]. A prospective study of paediatric orthokeratology lens wearers who were prescribed diquafosol four times daily for one month reported improved DEQS (5.54 ± 3.25 to 3.85 ± 2.98 , $p = 0.00$), tear meniscus height (0.20 ± 0.05 mm to 0.21 ± 0.05 mm, $p = 0.01$) and TBUT (6.67 ± 4.71 s to 10.32 ± 6.19 s, $p < 0.001$) compared to baseline [125]. Thus, diquafosol may help alleviate dry eye symptoms associated with paediatric orthokeratology lens wear and improve compliance, which is crucial for effective myopia management in children.

4.8. Keratorefractive Surgery

DED is the most common complication of keratorefractive surgery, with a reported prevalence of up to 75% of patients [126,127]. Pathogenesis of post-keratorefractive surgery dry eye is multifactorial, of which surgical transection of sub-basal corneal nerves; which reduces the cornea-blink reflex and disrupts the ocular surface-lacrimal gland neural loop, is a significant contributor [127]. This reduces the volume of tears secreted and induces tear film instability. Neurogenic inflammation, as evidenced by raised proinflammatory tear mediator levels (IL-6, MMP-9), neuropeptides (Substance P and calcitonin gene-related peptide) and neuromediators (nerve growth factor), also contribute to post-keratorefractive surgery dry eye [128]. Structures of the ocular surface, such as conjunctival goblet cells can inadvertently be damaged by surgical manipulation, such as during the application of suction devices in laser-assisted in situ keratomileusis (LASIK) and small incision lenticule extraction (SMILE) [129]. Other factors include administration of topical therapeutics which may induce a toxic effect on the conjunctiva and cornea [130] as well as central corneal flattening, which affects lid-globe apposition and may result in an abnormal distribution of the tear film and adversely affect meibomian gland function [131].

A prospective study of DED patients who underwent SMILE reported that cornea and conjunctival fluorescein staining score was lower in patients receiving diquafosol compared to sodium hyaluronate (1.20 ± 1.06 vs 1.83 ± 1.41 respectively, $p = 0.026$) [132]. Additionally, the diquafosol group had better OSDI scores (12.98 ± 7.29 vs 16.82 ± 8.25 , $p = 0.029$), improved TBUT (5.83 ± 2.02 s vs 4.24 ± 0.94 s, $p < 0.001$), and Schirmer's test (7.75 ± 3.92 mm vs 5.24 ± 3.42 mm, $p = 0.003$) three months post-surgery. A prospective study investigated the efficacy of diquafosol in treating patients experiencing persistent dry eye symptoms for over 12 months following LASIK [133]. This study compared the effects of diquafosol with those of artificial tears over a 12 week period. Results demonstrated that diquafosol significantly improved both subjective and objective parameters. Although Schirmer's test was not significantly different after addition of diquafosol (7.4 ± 5.1 mm pre-treatment vs 6.8 ± 3.65 mm at 12 weeks post-treatment, $p = 0.48$), TBUT improved from a baseline to one-week ($p = 0.007$), four weeks ($p = 0.001$), and 12 weeks ($p < 0.001$), however, exact improvement in TBUT was not reported. Corneal fluorescein and conjunctival lissamine green staining scores improved within one week of initiating diquafosol treatment ($p < 0.001$). Reported subjective symptoms; measured using a modified OSDI tool, demonstrated a reduction in fatigue, discomfort, dryness, grittiness, and difficulty reading ($p < 0.05$).

In patients undergoing femtosecond LASIK (FS-LASIK), regardless whether dry eye was present, combining diquafosol six times a day and sodium hyaluronate four times a day postoperatively for a month significantly improved postoperative subjective symptoms, ocular surface status, and LLT compared to monotherapy with sodium hyaluronate therapy [134]. Additionally, all patients also received routine post-procedural eyedrops such as antibiotics and anti-inflammatory agents. The use of combination therapy significantly lowered OSDI scores (diquafosol and sodium hyaluronate: 17.55 ± 15.70 to 16.97 ± 9.96 vs sodium hyaluronate: 18.39 ± 17.31 to 28.72 ± 19.65 ,

p=0.024) following FS-LASIK one month postoperatively. While there was no significant difference in corneal fluorescein staining score for the combination group between preoperative and postoperative visits, score increments were significantly lower for the combination group compared to the sodium hyaluronate group at one week (p=0.018) and was not statistically significant at one month after FS-LASIK. Furthermore, in patients without preoperative dry eye symptoms, addition of diquafosol resulted in better corneal sensitivity measured with a Cochet-Bonnet esthesiometer one month after FS-LASIK compared to patients who only used sodium hyaluronate (26.43 ± 20.80 mm vs 12.237 ± 14.86 mm, p=0.041). Confocal microscopic analysis of DED patients has suggested a possible role of diquafosol (administered six times daily for three months) in increasing sub-basal corneal nerve density (Baseline: 829.6 ± 348.0 $\mu\text{m}/\text{mm}^2$ vs three months: 1238.6 ± 410.1 $\mu\text{m}/\text{mm}^2$, p=0.02) [135].

4.9. Long Acting Diquafosol (DQS-LX) Formulation

Diquafosol is usually prescribed up to six times daily for maximal efficacy. Compliance has however been identified as a challenge, with only 10.2% of participants reported to be compliant with the recommended administration frequency in a study [136]. A new long-acting diquafosol formulation which is administered thrice daily has recently been developed via the addition of polyvinylpyrrolidone (PVP). It confers several benefits including increased viscosity and higher levels of lipid-secretion more effectively compared to standard diquafosol formulation [137]. It is postulated that the increased viscosity reduces ocular surface irritation, improves tear fluid stability and reduces corneal and conjunctival epithelial damage. The lipid-increasing effect on the tear film may further minimise friction generated during lid-globe interactions and reduce evaporative loss. DQS-LX was dosed at three times daily as studies have shown that effects on corneal fluorescein staining score and TBUT were similar to when conventional diquafosol was used six times daily [138].

A randomised controlled trial of patients with aqueous deficient DED receiving DQS-LX were reported to experience improvements in corneal fluorescein staining (MD: -0.51, p<0.0001) and conjunctival lissamine green staining scores (MD: -0.33, p=0.0093) after four weeks of administration compared the vehicle control group [138]. Common adverse symptoms reported were eye irritation (3.6%) and discharge (1.8%) of mild severity.

Another study by Ishikawa and coworkers investigating compliance rates of patients using diquafosol reported that switching to a long-acting formulation improved adherence rates from 5.6% among those prescribed a conventional diquafosol formulation six times daily, to 88.9% in patients prescribed DQS-LX to be administered three times daily [139]. In this study, both formulations were found to be equally effective in improving TBUT.

Patients with reduced tear film volume or moderate-severe dry eyes with meibomian gland dysfunction have been reported to prefer the long-acting formulation, while those with allergic findings such as conjunctival papillae expressed their preference for conventional diquafosol [137]. The study cited eye stickiness, discharge, itchiness, and irritation as reasons for reverting to conventional diquafosol. Notably, patients with papillary and follicular changes of the conjunctiva are more symptomatic regardless of the severity of dry eye symptoms [140]. The preference for conventional diquafosol may be attributable to PVP, which increases tear viscosity and prolongs allergen residence time on the ocular surface, exacerbating itch. Additionally, patients with allergic conjunctivitis have been described to experience impaired blink mechanisms which may affect the lacrimal pump mechanism and contribute to tear retention [141]. Likely explanations for this include changes in the palpebral conjunctival anatomy due to papillae formation, as well as persistent itching, which can disrupt normal blinking patterns.

5. Conclusions

Existing evidence suggests that diquafosol is a useful addition to our treatment armamentarium in managing a range of ocular surface conditions, though exact mechanisms underpinning its reported range of benefits have yet to be fully elucidated. Clinicians considering prescribing

diquafosol for their patients should be aware of its potential side effects, particularly when initiating therapy. Future efforts ought to focus on conducting rigorous high-quality trials to evaluate the effectiveness of diquafosol across the spectrum of ocular surface diseases against and alongside other pharmacological and office-based interventions, and effects of long term cessation of diquafosol to optimise treatment outcomes for patients with ocular surface disorders.

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