

Effect of topical 3% diquafosol sodium on eyes with dry eye disease and meibomian gland dysfunction

Shiro Amano
Kenji Inoue

Inouye Eye Hospital, Chiyoda-ku,
Tokyo, Japan

Purpose: To prospectively evaluate the effect of topical diquafosol sodium on eyes with dry eye disease (DED) and meibomian gland dysfunction (MGD).

Patients and methods: The subjects were consecutive patients diagnosed with both DED and MGD at Inouye Eye Hospital between March and September of 2016. The subjects were administered topical 3% diquafosol sodium ophthalmic solution six times a day for 3 months. At each study visit, subjects underwent slit-lamp examination and completed MGD symptoms and dry eye-related quality of life score (DEQS) questionnaires. Meibum quality (meibum score) and meibomian gland loss (meiboscore) were evaluated. Tear lipid layer thickness was measured in both eyes with an ocular surface interferometer immediately after clinic arrival and 20 minutes after instillation of one drop of topical diquafosol.

Results: Thirteen patients (3 men, 10 women) with a mean age of 69.5 ± 8.3 years completed the 3-month study. The number of telangiectasia and plugged meibomian gland orifices significantly decreased from baseline after 1 month of diquafosol use. The meibum score and the meiboscore significantly decreased from baseline at 3 months. Also, the lipid layer thickness was greater after diquafosol administration than before administration at baseline and 1, 2, and 3 months by 12.2, 11.5, 9.5, and 17.0 nm, respectively, but this difference was only significant at 3 months ($p=0.039$). The DEQS ocular symptom ($p=0.065$) and MGD questionnaire ($p=0.081$) scores tended to be lower than baseline at 3 months.

Conclusion: Diquafosol sodium ophthalmic solution improves DED- and MGD-related signs in eyes with MGD.

Keywords: diquafosol, meibomian gland dysfunction, dry eye

Introduction

Diquafosol is a $P2Y_2$ receptor agonist that activates $P2Y_2$ receptors. When used on the ocular surface, rehydration occurs through increased fluid secretion from conjunctival epithelial cells (via accessory lacrimal fluid pump activation) and increased mucin secretion from conjunctival goblet cells.¹⁻⁵ As a result, topical diquafosol solutions have been used to treat dry eye disease.^{6,7} Expression of the $P2Y_2$ receptor occurs in ocular surface tissue, including the corneal epithelium, conjunctival epithelium, lacrimal gland ductal epithelium, meibomian gland sebaceous cells, and meibomian gland ductal cells.^{8,9} Therefore, diquafosol is expected to have at least some effect on the meibomian glands. We previously demonstrated the beneficial effects of topical diquafosol sodium on the ocular surface in subjects with meibomian gland dysfunction (MGD), including decreases in ocular symptoms, lid margin abnormalities, and meibum grade and increases in tear film break-up time (BUT) and meibomian gland area.¹⁰ However, the duration of

Correspondence: Shiro Amano
Inouye Eye Hospital, 4-3 Kandasurugadai,
Chiyoda-ku, Tokyo 101-0062, Japan
Tel +81 3 295 0925
Fax +81 3 295 0917
Email amanoshiro1126@gmail.com

diquafofol use widely varied (4–16 months), likely reducing the reliability of our findings. This study was performed to resolve this issue and to prospectively evaluate the effect of topical diquafofol sodium use on the signs and symptoms of dry eye disease in eyes with MGD.

Patients and methods

This study was reviewed and approved by the institutional review board of Inouye Eye Hospital (Tokyo, Japan). It is registered with the University Hospital Medical Information Network in Japan (number UMIN000018510). The entire study conduct adhered to the tenets of the Declaration of Helsinki, and written informed consent was obtained from all study participants before any study examinations were performed.

Study subjects

Consecutive adult patients diagnosed with both dry eye disease and MGD at Inouye Eye Hospital between March and September 2016 were considered for enrollment. Subjects were excluded from participation if any of the following was found: active ocular infection, ocular inflammation, or history of ocular surgery within 3 months of enrollment.

Diagnosis of MGD

An MGD diagnosis was made using the diagnostic criteria proposed by the MGD Working Group in Japan.¹¹ Briefly, an MGD diagnosis was given when lid margin abnormalities and meibomian gland occlusion signs were both present. Lid margin abnormalities were considered to be present when at least two of the following were present in at least one eye: ≥ 2 telangiectasias, lid margin irregularity (notching), and mucocutaneous junction shifts. Meibomian gland occlusion was considered to be present when at least one eye had meibum secretions that were reduced when moderate pressure was applied with the thumb to the middle-third region of the upper eyelid and ≥ 2 gland orifices appeared to be plugged.

Diagnosis of dry eye disease

Dry eye disease was diagnosed using standard diagnostic criteria used in Japan.¹² Briefly, a patient was given a definitive dry eye disease diagnosis when dry eye symptoms, a tear film abnormality (BUT ≤ 5 seconds or a Schirmer I test value of ≤ 5 mm), and keratoconjunctival epithelial disorders (fluorescein staining score ≥ 3 [maximum score = 9]) were present. When two of the three criteria were met, the patient was given a diagnosis of probable dry eye disease.

Treatment with diquafofol sodium ophthalmic solution

All patients underwent topical administration of 3% diquafofol sodium ophthalmic solution (Diquas[®] ophthalmic solution 3%; Santen Pharmaceutical Co., Ltd., Osaka, Japan). Subjects were instructed to use one drop of topical diquafofol six times day for 3 months.

Study examinations

Examinations were performed before and 1, 2, and 3 months after initiating diquafofol treatment in the order listed. Subjects were asked not to use eye drops for at least 2 hours before study visits to minimize the influence of eye drops on the tear lipid layer thickness (LLT). Immediately after the subjects arrived at our clinic, LLT was measured using the LipiView ocular surface interferometer (TearScience, Morrisville, NC, USA).^{13,14} One drop of diquafofol was then instilled in both eyes and LLT was measured again 20 minutes later.

Subjects completed a symptoms questionnaire (MGD questionnaire), which included rating the presence of 11 ocular symptoms using the following scoring system: 0= never, 1= rarely, 2= sometimes, 3= often, and 4= all of the time. The rated symptoms included discomfort, pressure sensation, fatigue, discharge, stickiness, itching, heavy sensation, dryness, foreign body sensation, burning sensation, and redness. All 11 symptom scores were totaled to calculate the MGD symptom score. Subjects also completed a questionnaire to determine the dry eye-related quality of life score (DEQS).¹⁵ Medication use compliance was evaluated as 100%, 75%–99%, 25%–74%, or $<25\%$.

A slit-lamp examination was performed to thoroughly examine the upper and lower eyelids for lid margin abnormalities (eg, irregular lid margin, telangiectasia, plugged meibomian gland orifices, and mucocutaneous junction shift). Telangiectasias needed to occupy at least one half of the lid margin width to be considered present. Mucocutaneous junction shifts were evaluated based on the location of the Marx line after fluorescein instillation.^{16,17} When all or part of the Marx line touched the meibomian gland orifices in the upper or lower eyelid, a mucocutaneous junction shift was considered to be present.

The ocular surface was evaluated by measuring the tear BUT and corneal and conjunctival fluorescein staining. Tear BUT was measured using a slit-lamp after of fluorescein instillation into the conjunctival sac. The time from normal blinking to the first appearance of a break in the tear film was measured three times using a stopwatch. The mean of

the three measurements was calculated and recorded as the tear BUT. To obtain the fluorescein staining score, the ocular surface was divided into the nasal conjunctival, corneal, and temporal conjunctival sections. Each section was graded on a severity scale of 0–3 (0= no staining, 3= severe staining). Section gradings were summed to obtain the overall epithelial damage score (maximum of 9).^{18,19}

Meibomian gland secretions and orifice plugging were then examined. Moderate pressure was applied to the middle-third region of the upper eyelid using the thumb. Meibum appearance and the ease at which it was expressed were considered when grading meibum (meibum score) using the following semi-quantitative grading system: grade 0= clear meibum that is easily expressed, grade 1= cloudy meibum that could be expressed with mild pressure, grade 2= cloudy meibum that could only be expressed after more than moderate pressure was applied, and grade 3= no meibum expression, even with hard pressure.²⁰ Meibomian gland loss was evaluated using the following meiboscore definitions: 0= no loss, 1= lost gland area accounts for less than one-third of the total meibomian gland area, 2= lost area was between one-third and two-thirds of the total meibomian gland area, and 3= lost area over two-thirds or more of the total meibomian gland area.²¹ Meibomian gland area was measured using a mobile pen-shaped infrared meibography device (Meibom Pen; JFC Sales Plan Co. Ltd., Tokyo, Japan).²² A standard Schirmer I test was performed 5 minutes after administering topical anaesthesia (0.4% oxybuprocaine hydrochloride; Santen Pharmaceutical Co. Ltd.).

Statistical analyses

Data from the right eye of all subjects were used in analyses because the right eye qualified for the study in all cases. Baseline and posttreatment values of continuous variables were compared using a mixed-effects model with eyes (right) or subjects as a random effect. A generalized estimating equation model or McNemar test was used for binominal variables. Changes from baseline in LLT values in each pre- and post-diquafosol application and differences between pre- and post-diquafosol dosing at each visit were evaluated using a mixed-effects model with eyes (right) as a random effect.

Results

Study subjects

Thirteen subjects were ultimately included in the study analyses. Fourteen subjects were enrolled in this study. However, one subject reported pain immediately after the first instillation of 3% diquafosol sodium ophthalmic solution.

Therefore, this subject stopped using the medication and withdrew from the study. Fortunately, the pain quickly resolved and this subject experienced no further adverse events.

The 13 subjects (3 men, 10 women) included in the analyses had a mean age of 69.5 ± 8.3 years (range: 54–83 years). One subject missed the 1-month follow-up visit, and 1 subject refused to undergo further Schirmer tests after the 1-month visit. Hence, the number of subjects was 13 at baseline, 2 and 3 months, and 12 at 1 month. For Schirmer tests, the number of subjects used in the analyses was 13 at pretreatment, 12 at 2 and 3 months, and 11 at 1 month. All subjects had satisfactory medication compliance at each study visit, reporting a topical diquafosol eye drop use of at least 75%. None of the 13 subjects used any ophthalmic solutions other than 3% diquafosol sodium ophthalmic solution during the study. None of the subjects performed self-care of MGD, such as lid hygiene and warm compression, during the study.

Effect of diquafosol on the ocular surface

Table 1 shows the changes over time in ocular surface parameters. The number of telangiectasia and plugged meibomian gland orifices in each eye was significantly lower at 1, 2, and 3 months than at baseline. Also, the meibum score and the total meiboscore were significantly lower at 3 months than at baseline. The Schirmer test results were significantly higher at 3 months than at baseline. Tear BUT also significantly increased and fluorescein staining score significantly decreased 1 month after initiating diquafosol use.

Table 2 summarizes the LLT measurements. Briefly, LLT measured immediately after clinic arrival was not significantly different from baseline at 1, 2, or 3 months. This was also true of LLT measured 20 minutes after diquafosol administration. At each time point, the second LLT measurement was higher than the first LLT measurement, but this difference (17 nm) was only significant at 3 months ($p=0.039$).

Effect of diquafosol on subject symptoms

Subject symptoms associated with dry eye disease and MGD were evaluated at each study visit (Table 3). The DEQS ocular symptom ($p=0.065$) and MGD ($p=0.081$) scores were both marginally, but not significantly, lower at 3 months than at baseline.

Discussion

This study examined the effect of 3% diquafosol sodium ophthalmic solution on the signs and symptoms of dry eye disease in eyes with MGD. The number of telangiectasia and plugged

Table 1 Changes in ocular surface parameters over time

Parameters	Pretreatment	1 month		2 months		3 months	
			p-value		p-value		p-value
Telangiectasia							
%	92.3 (60.9–98.9)	87.7 (59.5–97.2)	0.604	69.2 (40.9–88.0)	0.078	61.5 (34.4–83.0)	0.041
Number/eye	7.3 (5.2–9.4)	5.2 (3.1–7.3)	0.015	3.4 (1.3–5.5)	<0.001	2.8 (0.8–4.9)	<0.001
Anterior shift of MCJ, %	53.8 (28.2–77.6)	40.8 (19.0–66.9)	0.143	38.5 (17.0–65.6)	0.130	38.5 (17.0–65.6)	0.130
Lid margin irregularity, %	38.5 (17.0–65.6)	39.2 (17.4–66.3)	0.387	23.1 (7.6–52.2)	0.133	30.8 (12.0–59.1)	0.300
Gland orifice plugging							
%	100.0	75.0		69.2		69.2	
Number/eye	6.3 (4.5–8.1)	3.4 (1.5–5.2)	<0.001	2.2 (0.4–4.0)	<0.001	1.8 (0.0–3.6)	<0.001
Meibum score	2.3 (1.9–2.7)	2.2 (1.8–2.6)	0.617	2.2 (1.8–2.6)	0.658	1.8 (1.5–2.2)	0.011
Meiboscore							
Upper eyelid	1.4 (0.8–2.0)	1.2 (0.6–1.8)	0.269	1.3 (0.7–1.9)	0.589	1.2 (0.6–1.8)	0.283
Lower eyelid	1.2 (0.8–1.7)	1.2 (0.8–1.7)	0.921	1.0 (0.5–1.5)	0.082	1.0 (0.5–1.5)	0.082
Total score	2.6 (1.7–3.6)	2.5 (1.5–3.4)	0.418	2.3 (1.3–3.3)	0.091	2.2 (1.3–3.2)	0.036
Schirmer I test result, mm	7.5 (6.4–8.7)	8.6 (7.4–9.9)	0.177	7.9 (6.7–9.2)	0.614	10.1 (8.9–11.3)	0.002
Tear breakup time, seconds	3.3 (1.6–5.0)	6.2 (4.5–7.9)	0.0007	6.4 (4.7–8.0)	0.0003	7.5 (5.9–9.2)	<0.0001
Fluorescein staining score	1.5 (0.9–2.2)	0.5 (0–1.2)	0.0004	0.4 (0–1.0)	<0.0001	0.4 (0–1.0)	<0.0001

Notes: Percentages for telangiectasia, shift of MCJ, lid margin irregularity, and plugging are percentages of eyes in which each sign is present. Data are presented as mean (95% CI). As the plugged ratio was 100% before treatment, CIs could not be estimated. p-values show comparisons with pretreatment values.

Abbreviation: MCJ, mucocutaneous junction.

meibomian gland orifices in each eye significantly decreased after 1 month of diquafosol use. Also, the meibum score and the meiboscore were significantly lower than baseline at 3 months. The MGD questionnaire score was also lower than baseline at 3 months, but this change was only marginally significant. These results suggest that topical diquafosol sodium improves pathologic ocular surface changes and symptoms associated with MGD. As gene expression of the P2Y₂ receptor has been observed in meibomian gland sebaceous and ductal cells,^{8,9} it is likely that these improvements were directly related to the effect of diquafosol on meibomian glands. However, it has not been confirmed that topically applied diquafosol penetrates into the meibomian glands, and this should be examined in future studies.

Schirmer I test scores significantly increased after 3 months of topical diquafosol use. Also, at 1 month, tear BUT had significantly increased over baseline and the fluorescein staining score had significantly decreased from baseline. Furthermore, the DEQS ocular symptoms score consistently decreased in each subject, but, when averaged, this change was only marginally significant. These improvements in dry eye-related signs and symptoms agree with the findings of previous studies that reported a high efficacy of topical diquafosol sodium when used to treat dry eye disease.^{6,7} It is thought that diquafosol stimulates water secretion from the conjunctival epithelial cells and mucin secretion from the conjunctival goblet cells via P2Y₂ receptor activation. As some symptoms included in the MGD questionnaire are related to dry eye

Table 2 Tear lipid layer thickness immediately before and 20 minutes after administration of topical diquafosol sodium in eyes with dry eye disease and meibomian gland dysfunction

Parameters		LLT value (nm)	Compared with pretreatment	Compared with first measurement
			p-value	p-value
First measurement (before diquafosol administration)	Pretreatment	81.8 (64.5–99.2)		
	1 month	77.0 (59.3–94.7)	0.563	
	2 months	81.1 (63.7–98.4)	0.925	
	3 months	79.7 (62.3–97.1)	0.791	
Second measurement (20 minutes after diquafosol administration)	Pretreatment	94 (76.6–111.4)		0.138
	1 month	88.5 (70.8–106.2)	0.511	0.177
	2 months	90.5 (73.2–107.9)	0.671	0.247
	3 months	96.7 (79.3–114.1)	0.741	0.039

Note: 95% CIs are shown in parentheses.

Abbreviation: LLT, lipid layer thickness.

Table 3 Severity of dry eye disease symptoms in subjects with meibomian gland dysfunction before and after treatment with diquafosol sodium ophthalmic solution

Parameters			Estimated value	p-value
DEQS	Ocular symptoms	Pretreatment	20.5 (13.0–28.0)	
		1 month	19.2 (11.4–26.9)	0.788
		2 months	14.4 (7.0–21.9)	0.220
		3 months	11.2 (3.7–18.7)	0.065
	Influence on daily life	Pretreatment	10.9 (4.3–17.5)	
		1 month	8.0 (1.2–14.8)	0.493
		2 months	7.9 (1.4–14.5)	0.469
		3 months	9.6 (3.1–16.2)	0.756
	Total score	Pretreatment	14.8 (8.6–20.9)	
		1 month	12.5 (6.1–18.9)	0.572
		2 months	10.5 (4.4–16.7)	0.284
		3 months	10.3 (4.1–16.4)	0.256
MGD questionnaire		Pretreatment	8.5 (5.2–11.7)	
		1 month	9.4 (6.0–12.8)	0.659
		2 months	7.8 (4.5–11.0)	0.739
		3 months	4.8 (1.5–8.0)	0.081

Notes: Data are presented as mean (95% CIs). A higher score indicates a more severe symptomatic disease. p-values shown are for comparison with pretreatment values.

Abbreviations: DEQS, dry eye-related quality of life score; MGD, meibomian gland dysfunction.

disease, improvements in MGD questionnaire score may be attributed to improvements in meibomian gland function and enhancement of aqueous tear and mucin secretion from conjunctival cells.

We measured LLT when subjects arrived at our clinic and 20 minutes after instillation of topical diquafosol. A previous study²³ showed that LLT was significantly increased 15 minutes after the instillation of 3% diquafosol in healthy human eyes and the increase was maintained for 60 minutes. Hence, we thought 20 minutes is enough to observe the effect of diquafosol on LLT. The second LLT measurement was ~10 nm higher than the first at baseline and 1 and 2 months and 17 nm higher at 3 months ($p=0.039$). This suggests that a single instillation of diquafosol increases lipid secretion from the meibomian glands, and that using topical diquafosol for 3 months enhances lipid secretion from the meibomian glands in subjects with MGD. These results are consistent with the simultaneous decrease in the number of plugged meibomian gland orifices, which was significantly lower at 1 month than at baseline. These findings, together with the fact that LLT is significantly associated with MGD severity and meibomian gland dropout grade,^{13,14} suggest that topical diquafosol use over 3 months somewhat restores meibomian gland lipid secretion in patients with MGD.

A recent study by Kam et al reported that uridine-5'-triphosphate (UTP), a P2Y₂ receptor agonist, had no effect on immortalized human meibomian gland epithelial cells.²⁴

The authors also concluded that if diquafosol does influence human meibomian glands, it may be that ductal, not acinar, epithelial cells are affected. In contrast, Wen et al found that P2Y₂ receptor agonists enhance lipid production in primary cultured rabbit meibocytes.²⁵ These discrepant results may have resulted from differences in species and/or culture conditions. Also, UTP easily degrades in aqueous solutions, making it necessary to use UTP immediately after it is dissolved in water.^{5,26} In contrast, diquafosol is stable when dissolved in water.^{5,26} As Kam et al did not mention whether or not UTP solutions were immediately used,²⁴ we do not know for certain whether or not their results are applicable to the actions of diquafosol on meibomian gland acinar cells. Either way, our results suggest that topical diquafosol is beneficial to meibomian glands. Further studies are needed to elucidate which meibomian gland cell types (acinar and/or ductal) are affected by diquafosol.

One of our subjects withdrew from the study because of ocular pain that began immediately after the first instillation of diquafosol sodium. Ocular pain and discomfort were previously observed in a Japanese Phase II clinical trial,⁷ where one subject (1.0%) reported eye pain and seven subjects (7.3%) reported ocular irritation after using 1% diquafosol. Furthermore, 4 subjects (4.2%) reported eye pain and 12 subjects (12.5%) reported ocular irritation after using 3% diquafosol. However, no serious treatment-related adverse events occurred in any subject. In agreement with this, no adverse events occurred in any of our study subjects during the 3-month follow-up period, including the subject who experienced ocular pain. Therefore, our study further supports the safety of using diquafosol sodium ophthalmic solution to treat MGD.

Our study was limited in that it was an uncontrolled study (no placebo group), had a relatively small number of subjects, and had a short follow-up period. Future randomized, placebo-controlled studies with a larger number of participants and a longer follow-up period are needed to confirm the efficacy of topical diquafosol sodium for treating the signs and symptoms in patients with MGD. Another limitation was that the slit-lamp diagnostic criteria, including meibum evaluations, were subjective. These parameters require standardized grading methods or objective measures to allow more accurate comparisons within and between studies.¹⁶

In conclusion, diquafosol sodium ophthalmic solution improves MGD and dry eye-related signs and symptoms in subjects with MGD. Also, the use of topical diquafosol sodium for at least 3 months likely increases lipid secretions from the meibomian glands in patients with MGD.

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Disclosure

Dr Amano reports a grant and personal fees from Santen Pharmaceutical. Dr Inoue reports personal fees from Santen Pharmaceutical. The authors report no other conflicts of interest in this work.

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