Eye (2012) 26, 1363–1368

www.nature.com/eye

CLINICAL STUDY

Clinical evaluation of the additive effect of diquafosol tetrasodium on sodium hyaluronate monotherapy in patients with dry eye syndrome: a prospective, randomized, multicenter study

K Kamiya[1], M Nakanishi[2], R Ishii[3], H Kobashi[4], A Igarashi[1], N Sato[1] and K Shimizu[1]

Abstract

Purpose **To assess the additive effect of diquafosol tetrasodium on sodium hyaluronate monotherapy in patients with dry eye syndrome.**

Methods **This study evaluated 64 eyes of 32 patients (age: 62.6 ± 12.8 years (mean ± SD)) in whom treatment with 0.1% sodium hyaluronate was insufficiently responsive. The eyes were randomly assigned to one of the two regimens in each patient: topical administration of sodium hyaluronate and diquafosol tetrasodium in one eye, and that of sodium hyaluronate in the other. Before treatment, and 2 and 4 weeks after treatment, we determined tear volume, tear film break-up time (BUT), fluorescein and rose bengal vital staining scores, subjective symptoms, and adverse events.**

Results **We found a significant improvement in BUT ($P = 0.049$, Dunnett test), fluorescein and rose bengal staining scores ($P = 0.02$), and in subjective symptoms ($P = 0.004$ for dry eye sensation, $P = 0.02$ for pain, and $P = 0.02$ for foreign body sensation) 4 weeks after treatment in the diquafosol eyes. On the other hand, we found no significant change in these parameters after treatment in the control eyes.**

Conclusions **In dry eyes, where sodium hyaluronate monotherapy was insufficient, diquafosol tetrasodium was effective in improving objective and subjective symptoms, suggesting its viability as an option for the additive treatment of such eyes.**

Eye (2012) **26,** 1363–1368; doi:10.1038/eye.2012.166; published online 10 August 2012

Keywords: diquafosol tetrasodium; $P2Y_2$ receptor antagonist; sodium hyaluronate; dry eye syndrome

Introduction

Dry eye is a common disorder of the ocular surface characterized by dryness and damage to the ocular surface, with an estimated prevalence of 5–30%, depending on the patient's age.[1,2] It is associated with decreased tear production and abnormality of the lipid, protein, and mucin profiles. These changes may result in tear film instability with potential damage to the ocular surface, increased tear film osmolality, and subsequent inflammation of the ocular surface, and are a major cause of ocular discomfort.[1–4] Current medical therapies for the management of dry eye include tear supplementation, retention, stimulation, anti-inflammatory agents, and environmental strategies.[5] A topical application of sodium hyaluronate has been shown to confer both subjective and objective improvement in patients with dry eye syndrome arising from Sjögren's syndrome or

[1]Department of Ophthalmology, University of Kitasato School of Medicine, Kanagawa, Japan

[2]Department of Ophthalmology, Yamato Municipal Hospital, Kanagawa, Japan

[3]Department of Ophthalmology, Kitasato Institute Medical Center Hospital, Saitama, Japan

[4]Department of Ophthalmology, Ebina General Hospital, Kanagawa, Japan

Correspondence:
K Kamiya, Department of Ophthalmology, University of Kitasato School of Medicine, 1-15-1 Kitasato, Minami-ku, Sagamihara, Kanagawa 252-0374, Japan
Tel: +81 42 778 9012;
Fax: +81 42 778 9920.
E-mail: kamiyak-tky@umin.ac.jp

Received: 9 February 2012
Accepted in revised form: 31 May 2012
Published online: 10 August 2012

keratoconjunctivitis sicca,[6–9] and thus, in practice, it is widely used in the treatment of these patients. Sodium hyaluronate has also been reported to provide better protection of the corneal epithelium against dryness than hydroxyethylcellulose or phosphate-buffered saline.[10] However, treatment with sodium hyaluronate alone was inadequate for improving subjective or objective symptoms in some dry eye patients in a clinical setting.

Diquafosol tetrasodium is a $P2Y_2$ purinergic receptor agonist that activates $P2Y_2$ receptors on the ocular surface, leading to rehydration through activation of the fluid pump mechanism of the accessory lacrimal glands on the conjunctival surface.[11–15] $P2Y_2$ agonists are also potent mucin secretagogues and stimulate goblet-cell secretion of ocular mucins. Recently, this new ophthalmic solution has become commercially available in Japan for clinical use, and holds promise for the effective treatment of dry eye syndrome. However, to our knowledge, there have so far been no clinical studies on the additive effects of diquafosol tetrasodium on sodium hyaluronate monotherapy in patients with dry eye syndrome. The purpose of the current study is to prospectively investigate the additive effects of diquafosol tetrasodium in dry eyes in which solo treatment with sodium hyaluronate did not improve these symptoms.

Materials and methods

This prospective study focused on 64 eyes of 32 consecutive patients (5 men and 27 women) in whom dry eye syndrome, due to keratoconjunctivitis sicca or Sjögren syndrome, was diagnosed by specialists at four major medical hospitals (Kitasato University Hospital, Yamato Municipal Hospital, Ebina General Hospital, and Kitasato Institute Medical Center Hospital), and who did not find solo treatment effective with 0.1% sodium hyaluronate eye drops (0.1% Hyalein Ophthalmic Solution, Santen Pharmaceuticals, Osaka, Japan) six times a day. A minimum sample size of 28 eyes in each group was required to have 80% statistical power at the 5% level to detect a 1-s difference in break-up time (BUT) between the two groups, when the SD of the mean difference was 1.8 s. Allowing for a withdrawal rate of 10%, it was estimated that the recruitment of at least 31 patients was required. The mean patient age ($\pm$SD) was 62.6 ± 12.8 years (age range, 31–80 years). Inclusion criteria were as follows: age $\geq$20 years, both eyes diagnosed with dry eye syndrome, and objective and subjective symptoms (Schirmer's I test $\leq$5 mm, tear film BUT $\leq$3 s, fluorescein score $\geq$3, or rose bengal score $\geq$3, and some degree of subjective patient discomfort) remained for the solo treatment with sodium hyaluronate. Any history of previous ocular surgery, ocular trauma, contact lens use, punctual occlusion or diathermy, or eye disease including active inflammation of the eye, was excluded from the study. The study was approved by the Institutional Review Board at Kitasato University School of Medicine, and followed the tenets of the Declaration of Helsinki. Informed consent was obtained from all patients.

The eyes of each patient were randomly assigned by a designated study controller to one of two regimens: topical administration of sodium hyaluronate and diquafosol tetrasodium (3% Diquas ophthalmic solution, Santen Pharmaceuticals) six times a day in one eye, as the study group (diquafosol group), and topical administration of sodium hyaluronate six times a day in the fellow eye, as the control group. The pH and the osmolality of this diquafosol ophthalmic solution was between 7.2 and 7.8, and between 286 and 315 mOsm, respectively. Both sodium hyaluronate and diquafosol tetrasodium contained the same benzalkonium chloride as preservative. Before and 2 and 4 weeks after treatment, we determined the following in both groups: tear volume determined with Schirmer's I test, tear film BUT, fluorescein score, rose bengal score, adverse events, and subjective symptoms (except for 2 weeks after treatment). Natural tear volume was measured using Schirmer's I test, in which the extent of tear flow down a piece of filter paper inserted into the lateral part of the inferior fornix of the eye was measured over a 5-min period without anaesthetic drops. The standard tear film BUT measurement was performed. After 1% fluorescein dye was instilled into the conjunctival sac, the interval between the last complete blink and the appearance of the first corneal black spot in the stained tear film was measured three times and the mean value of the measurements was calculated. The fluorescein score was assessed with a 1% fluorescein solution using the 0–9 scoring system as described by Shimmura *et al*.[16] The staining of the superior cornea, mid-cornea, and inferior cornea was graded on a scale of 0 (no staining) to 3 (intense staining) using a slit-lamp microscope. The rose bengal score was assessed using the 0–15 scoring system. The staining of the nasal and temporal bulbar conjunctiva, the superior cornea, mid-cornea, and inferior cornea was graded on a scale of 0 (no staining) to 3 (intense staining). Subjective symptoms (asthenopia, discharge, epiphora, an itchy sensation, redness, dry eye sensation, pain, foreign body sensation, blurred vision, heavy lids, general discomfort, and photophobia) were also assessed using visual analogue scale (VAS) symptom intensity scores on a scale of 0 (no symptom) to 100 (maximum intensity). Adherence was confirmed by counting the number of empty containers collected upon each visit.

All statistical analyses were performed using StatView version 5.0 (SAS, Cary, NC, USA). One-way analysis of variance (ANOVA) was used for the analysis of the time

course of changes, with the Dunnett test for multiple comparisons. After normal distribution of the data was confirmed with the Kolmogorov–Smirnov test ($P \geq 0.05$), the paired t-test was used for statistical analysis to compare the pre-treated data between two groups, and to compare the pre- and post-treated VAS scores. Unless otherwise indicated, the results are expressed as mean ± SD, and a value of $P < 0.05$ was considered statistically significant.

Results

Patient population

The preoperative demographics of the study population are summarized in Table 1. All patients successfully completed the trial and were eligible for statistical analysis. Before treatment, there were no significant differences in tear volume ($P = 0.27$, paired t-test), BUT ($P = 0.68$), fluorescein score ($P = 0.25$), rose bengal score ($P = 0.15$), or subjective symptom score ($P = 0.06$ for asthenopia, $P = 0.84$ for discharge, $P = 0.06$ for epiphora, $P = 0.07$ for itchy sensation, $P = 0.09$ for redness, $P = 0.06$ for dry eye sensation, $P = 0.06$ for pain, $P = 0.52$ for foreign body sensation, $P = 0.31$ for blurred vision, $P = 0.46$ for heavy lids, $P = 0.10$ for general discomfort, and $P = 0.60$ for photophobia), between the two groups.

Tear volume (Schirmer's I test)

Tear volume (Schirmer's I test) was 7.4 ± 5.2, 8.7 ± 7.0, 7.8 ± 4.2 mm, before and 2 and 4 weeks after treatment, respectively, in the diquafosol group. The variance of the data was not statistically significant ($P = 0.67$, ANOVA). Multiple comparisons demonstrated no significant differences between measurements made before treatment and (a) at 2 weeks after ($P = 0.30$, Dunnett test) and (b) at 4 weeks after ($P = 0.56$). Similarly, it was 8.9 ± 8.0, 9.0 ± 7.7, 8.0 ± 5.4 mm before and 2 and 4 weeks after treatment, respectively, in the control group. The variance of the data was not statistically significant ($P = 0.83$, ANOVA). Multiple comparisons demonstrated no significant differences between measurements made before treatment and those made (a) at 2 weeks after ($P = 0.65$) and (b) at 4 weeks after ($P = 0.84$), treatment.

Break-up time

The tear film BUT was 3.5 ± 1.5, 4.5 ± 1.9, 4.4 ± 2.1 s, before and 2 and 4 weeks after treatment, respectively, in the diquafosol group (Figure 1). The variance of the data was statistically significant ($P = 0.045$, ANOVA). Multiple comparisons demonstrated significant differences between measurements made before treatment and (a) at 2 weeks after ($P = 0.03$), (b) at 4 weeks after ($P = 0.049$). By contrast, it was 3.6 ± 1.7, 3.7 ± 1.6, 4.1 ± 1.7 s, before and 2 and 4 weeks after treatment, respectively, in the control group (Figure 1). The variance of the data was not statistically significant ($P = 0.38$, ANOVA). Multiple comparisons demonstrated no significant differences between measurements made before treatment and (a) at 2 weeks after ($P = 0.51$) or (b) at 4 weeks after ($P = 0.15$), treatment.

Table 1 Preoperative patient demographics

Characteristics	*Mean ± SD*		P-*value*
	Diquafosol group	*Control group*	
Age (years)	64.5 ± 12.4		
Gender (male : female)	M : F = 6 : 26		
Schirmer's I test (mm)	7.4 ± 5.2	8.9 ± 8.0	0.27
Break-up time (s)	3.6 ± 1.7	3.5 ± 1.5	0.68
Fluorescein score (0–9)	3.4 ± 2.6	2.9 ± 2.7	0.25
Rose bengal score (0–15)	2.8 ± 2.2	2.3 ± 2.2	0.15

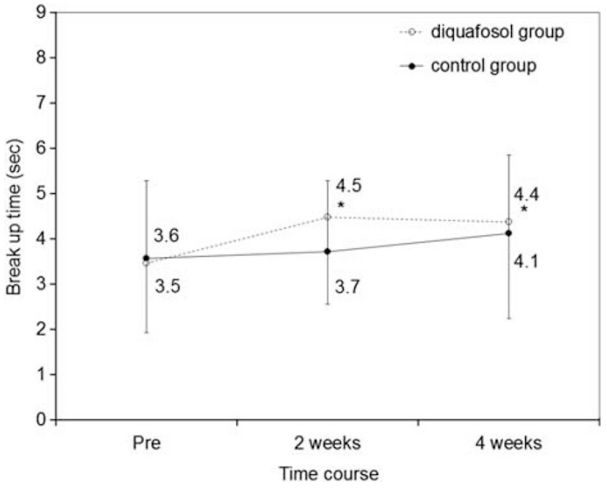

Figure 1 Time course of BUT. There was a significant improvement in the BUT after treatment in the diquafosol group, but no significant change in the control group. The bar indicates the SD. *Significant difference of $P < 0.05$.

Fluorescein staining score

The fluorescein staining score was 3.4 ± 2.6, 2.0 ± 1.8, 2.2 ± 2.0, before and 2 and 4 weeks after treatment, respectively, in the diquafosol group (Figure 2). The variance of the data was statistically significant ($P = 0.01$, ANOVA). Multiple comparisons demonstrated significant differences between measurements made before treatment and (a) at 2 weeks after ($P = 0.007$) and (b) at 4 weeks after ($P = 0.02$). By contrast, it was 2.9 ± 2.7, 2.3 ± 2.4, 2.7 ± 2.5, before and 2 and 4 weeks after treatment, respectively, in the control group (Figure 2).

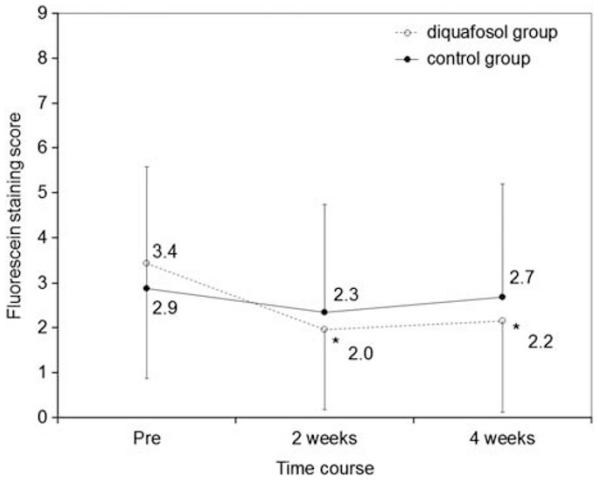

Figure 2 Time course of fluorescein score. There was a significant improvement in the fluorescein score after treatment in the diquafosol group, but no significant change in the control group. The bar indicates the SD. *Significant difference of $P<0.05$.

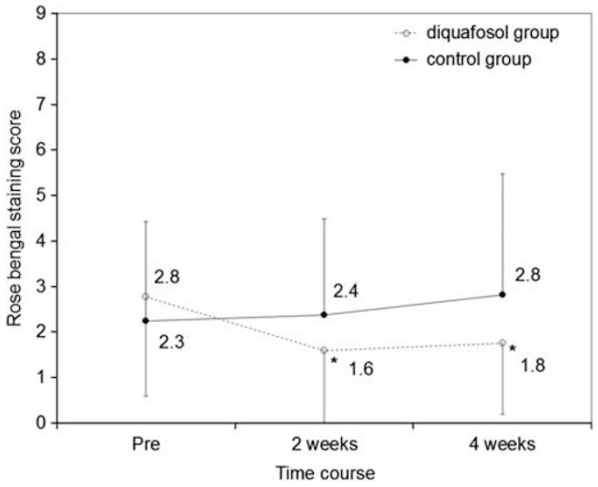

Figure 3 Time course of rose bengal score. There was a significant improvement in the rose bengal score after treatment in the diquafosol group, but no significant change in the control group. The bar indicates the SD. *Significant difference of $P<0.05$.

The variance of the data was not statistically significant ($P=0.70$, ANOVA). Multiple comparisons demonstrated no significant differences between measurements made before treatment and (a) at 2 weeks after ($P=0.32$), or (b) at 4 weeks after0 ($P=0.54$), treatment.

Rose bengal staining score

The rose bengal staining score was 2.8 ± 2.2, 1.6 ± 1.6, 1.8 ± 1.6, before and 2 and 4 weeks after treatment, respectively, in the diquafosol group (Figure 3). The variance of the data was statistically significant ($P=0.02$, ANOVA). Multiple comparisons demonstrated significant differences between measurements made before treatment and (a) at 2 weeks after ($P=0.009$) and (b) at 4 weeks after ($P=0.02$), treatment. By contrast, it was 2.3 ± 2.2, 2.4 ± 2.1, 2.8 ± 2.7, before and 2 and 4 weeks after treatment, respectively, in the control group (Figure 3). The variance of the data was not statistically significant ($P=0.60$, ANOVA). Multiple comparisons demonstrated no significant differences between measurements made before treatment and (a) at 2 weeks after ($P=0.75$) or (b) at 4 weeks after ($P=0.93$) treatment.

Subjective symptoms

In the diquafosol group, we found significant improvements in subjective symptoms, such as dry eye sensation (paired t-test, $P=0.004$), pain ($P=0.02$), and foreign body sensation ($P=0.02$), but no significant improvement in other subjective symptoms (Figure 4a). By contrast, we found no significant changes in any subjective symptoms in the control group (Figure 4b).

Adverse events

Burning and stinging on the instillation of eye drops occurred in one eye (3.1%) in the diquafosol group. Otherwise, no serious adverse events were seen at any time during the observation period in either the diquafosol or the control group.

Discussion

In the present study, our results show that diquafosol tetrasodium was effective not only for the improvement of objective symptoms, such as BUT, fluorescein score, and rose bengal score, but also for that of subjective symptoms, such as dry eye sensation, pain, and foreign body sensation, in eyes in which sodium hyaluronate has a limited role in the improvement of these symptoms. Diquafosol tetrasodium activates $P2Y_2$ receptors on the surface of the eye and on the inner surfaces of the eyelids, enhancing the natural process of tear secretion. Stimulation of tear secretion with diquafosol causes the release of salt, water, mucin and other components of the tear film, resulting in hydration of the surface of the eye.[11–15] Our results also show that adverse events of the additive diquafosol treatment was similar to those of the solo diquafosol treatment, such as burning and stinging on instillation, eye pain, and injection,[12,14] suggesting that the combination treatment of diquafosol and hyaluronate does not induce new adverse events, and thus that it may be clinically well tolerated and beneficial for dry eye patients in whom solo treatment with sodium hyaluronate is insufficient. We found statistically significant improvements of objective and subjective symptoms in the current study, but the differences before

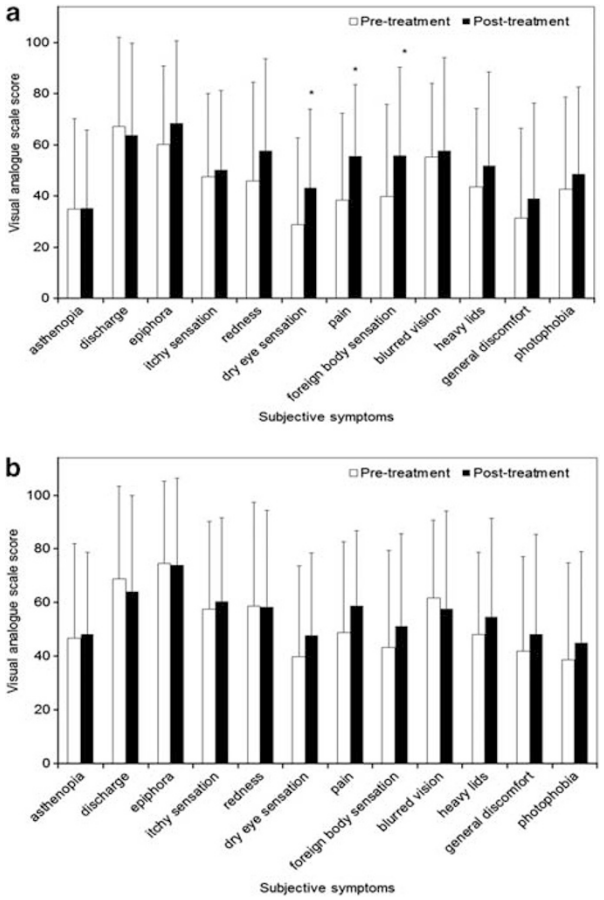

Figure 4 Visual analogue scale scores of subjective symptoms in the diquafosol and control groups. (a) There was a significant improvement in the subjective symptoms, such as dry eye sensation, pain, and foreign body sensation in the diquafosol group. The bar indicates the SD. (b) There was no significant change in any subjective symptoms in the control group. The bar indicates the SD. *Significant difference of $P<0.05$.

and after treatment are not very great. Hence, their clinical significance should be interpreted with some caution, and a large cohort of patients is necessary for confirming these preliminary findings.

The primary goal of this study is to determine whether additive treatment with diquafosol tetrasodium in patients already on sodium hyaluronate monotherapy was clinically useful when the latter therapy was inadequate, and therefore we did not use the vehicle treatment in the control group. Although the exact additive effect of diquafosol tetrasodium remained unanswered, our findings support the view that the combination treatment of diquafosol and hyaluronate improves the subjective and objective symptoms for dry eye syndrome in a clinical setting. Moreover, the use of a punctal plug is another treatment option for patients who respond poorly to monotherapy with sodium hyaluronate. However, several adverse events, such as spontaneous extrusion,[17] migration,[18] pyogenic granuloma,[19,20] biofilm formation,[21] and peripheral corneal ulceration,[22] have been reported after insertion of these plugs. There are also some patients who do not wish to undergo surgical intervention. Hence, we believe that the additive use of diquafosol tetrasodium may become an alternative treatment for dry eye syndrome, especially for patients who cannot continue to use the punctal plug because of adverse events, or for those who do not wish to undergo surgical intervention.

There are two limitations to this study. First, we assessed subjective symptoms in each eye. It has been stated that symptomatic relief or deterioration in one eye can affect the patient's judgment of symptoms in the other eye.[16] Second, although there was no significant improvement of subjective or objective symptoms, we observed a tendency for these symptoms to improve in some eyes in the control group, suggesting that the adherence of eye drops may induce a beneficial effect on these symptoms in some patients. Accordingly, we cannot deny the possibility that the significant improvements of these symptoms in the diquafosol group were attributable to the additive effects of diquafosol tetrasodium as well as to the adherence effect of eye drops. A further study is required to clarify this point.

In summary, our prospective, randomized, multicenter study indicates that diquafosol tetrasodium is effective for the improvement of objective and subjective symptoms in eyes on which sodium hyaluronate was inadequate in patients with dry eye syndrome. Moreover, no serious adverse events of diquafosol tetrasodium occurred throughout the follow-up period. These findings suggest that this new, additive therapy, which requires no surgical intervention, may be a good treatment option for dry eye patients in whom sodium hyaluronate had a limited role in the improvement of subjective and objective symptoms.

Summary

What was known before

- Recently, diquafosol tetrasodium ophthalmic solution has become commercially available in Japan for clinical use, and holds promise for the effective treatment of dry eye syndrome.
- However, to our knowledge, there have so far been no clinical studies on the additive effects of diquafosol tetrasodium on sodium hyaluronate monotherapy in patients with dry eye syndrome.

What this study adds

- This new, additive diquafosol tetrasodium therapy, may be a good treatment option for dry eye patients in whom sodium hyaluronate had a limited role in the improvement of subjective and objective symptoms.

Conflict of interest

The authors declare no conflict of interest.

References

1 Lemp MA. Report of the national eye institute/industry workshop on clinical trials in dry eyes. *CLAO J* 1995; **21**(4): 221–232.
2 Report of the Epidemiology Subcommittee of the International Dry Eye Work Shop: the epidemiology of dry eye disease. *Ocul Surf* 2007; **5**: 93–107.
3 Toda I, Fujishima H, Tsubota K. Ocular fatigue is the major symptom of dry eye. *Acta Ophthalmol* 1993; **71**: 18–23.
4 Tsubota K, Toda I, Yagi Y, Ogawa Y, Ono M, Yoshino K. Three different types of dry eye syndrome. *Cornea* 1994; **13**: 202–209.
5 Report of the Management and Therapy Subcommittee of the International Dry Eye Work Shop: management and therapy of dry eye disease. *Ocul Surf* 2007; **5**: 163–178.
6 DeLuise VP, Peterson WS. The use of topical Healon tears in the management of refractory dry-eye syndrome. *Ann Ophthalmol* 1984; **16**(9): 823–824.
7 Polack FM, McNiece MT. The treatment of dry eyes with Na hyaluronate (Healon). *Cornea* 1982; **1**: 133–136.
8 Sand BB, Marner K, Norn MS. Sodium hyaluronate in the treatment of keratoconjunctivitis sicca. *Acta Ophthalmol* 1989; **67**: 181–183.
9 Stuart JC, Linn JG. Dilute sodium hyaluronate (Healon) in the treatment of ocular surface disorders. *Ann Ophthalmol* 1985; **17**: 190–192.
10 Wysenbeek WS, Loya N, Sira BI, Ophir I, Ben Shaul Y. The effect of sodium hyaluronate on the corneal epithelium. An ultrastructural study. *Invest Ophthalmol Vis Sci* 1988; **29**: 194–199.
11 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**: 96–100.
12 Nichols KK, Yerxa B, Kellerman DJ. Diquafosol tetrasodium: a novel dry eye therapy. *Expert Opin Investig Drugs* 2004; **13**: 47–54.
13 Murakami T, Fujihara T, Horibe Y, Nakamura M. Diquafosol elicits increases in net Cl- transport through P2Y2 receptor stimulation in rabbit conjunctiva. *Ophthalmic Res* 2004; **36**: 89–93.
14 Tauber J, Davitt WF, Bokosky JE, Nichols KK, Yerxa BR, Schaberg AE *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**: 784–792.
15 Dartt DA. Regulation of mucin and fluid secretion by conjunctival epithelial cells. *Prog Retin Eye Res* 2002; **21**: 555–576.
16 Shimmura S, Ono M, Shinozaki K, Toda I, Takamura E, Mashima Y *et al.* Sodium hyaluronate eyedrops in the treatment of dry eyes. *Br J Ophthalmol* 1995; **79**: 1007–1011.
17 Horwath-Winter J, Thaci A, Gruber A, Boldin I. Long-term retention rates and complications of silicone punctal plugs in dry eye. *Am J Ophthalmol* 2007; **144**: 441–444.
18 Rumelt S, Remulla H, Rubin PAD. Silicone punctal plug migration resulting in dacryocystitis and canaliculitis. *Cornea* 1997; **16**: 377–379.
19 Rapoza PA, Ruddat MS. Pyogenic granuloma as a complication of silicone punctal plugs. *Am J Ophthalmol* 1992; **113**: 454–455.
20 Akova YA, Demirhan B, Cakmakci S, Aydin P. Pyogenic granuloma: a rare complication of silicone punctal plugs. *Ophthalmic Surg Lasers* 1999; **30**: 584–585.
21 Yokoi N, Okada K, Sugita J, Kinoshita S. Acute conjunctivitis associated with biofilm formation on a punctal plug. *Jpn J Ophthalmol* 2000; **44**: 559–560.
22 Toufeeq A, Mohammad-Ali FH. Peripheral corneal ulceration as a complication of silicon punctal plug: a case report. *Eye* 2007; **21**: 1437–1438.