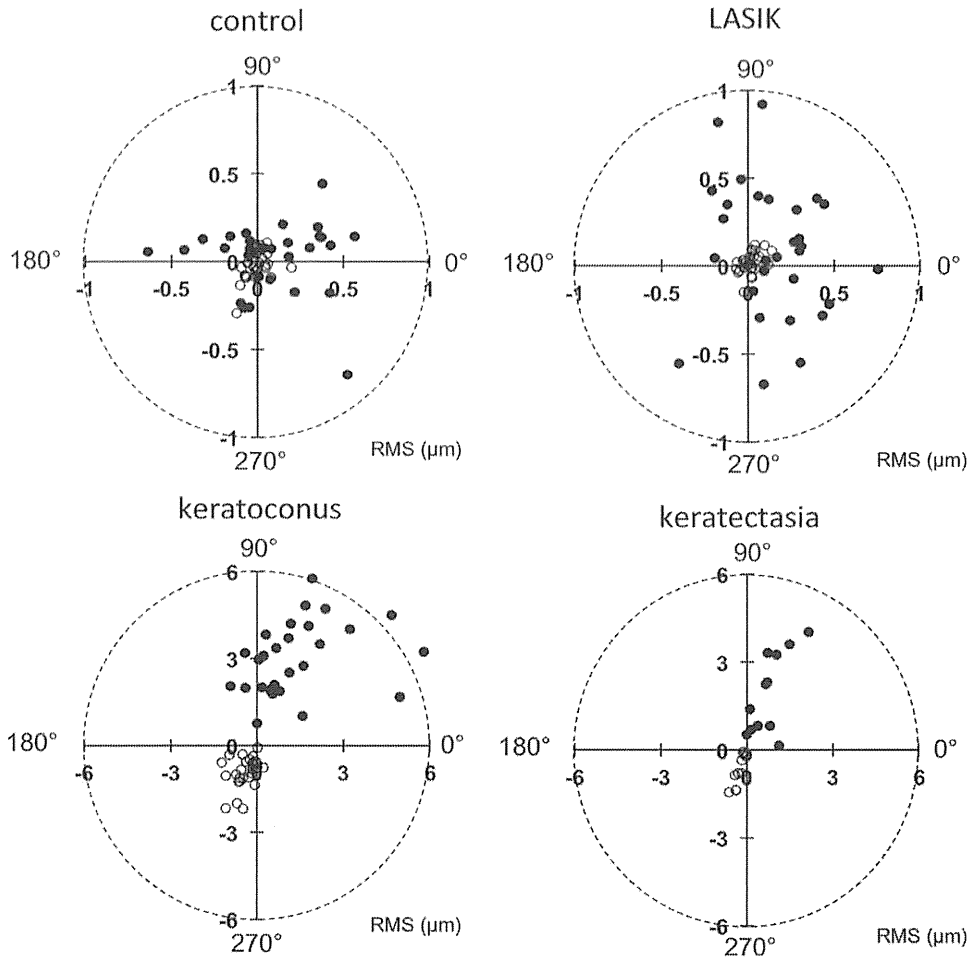

FIGURE 5. Scatterplots for the coma of the anterior and posterior corneal surfaces in control, LASIK, keratectasia, and keratoconic eyes. The axes of the coma caused by the anterior and posterior surfaces were generally in opposite directions in keratectasia and keratoconus.

accumulation of data of post-LASIK eyes with and without keratectasia will be necessary for a more detailed analysis.

A prospective study for clarifying the change of optical characteristics in keratectasia after LASIK will be necessary to establish the interventions needed for the disease, including the inhibition of keratectasia and maintaining or improving the quality of vision for people with the disease.

The preoperative risk factors also were not clear in keratectasia eyes that were examined in this study, because all cases had LASIK in other clinics and were referred to us. It will be interesting to evaluate the differences in the pattern of HOAs in the anterior and posterior corneal surfaces between eyes with preoperative abnormal topography and eyes with thin stromal beds.

In conclusion, keratectasia after LASIK revealed coma-dominant HOAs at both corneal surfaces, suggesting that the cornea in keratectasia had optical properties similar to those in keratoconus. Therefore, the treatment strategy for keratoconus might be applicable to keratectasia to improve the quality of vision and inhibit the progression of keratectasia after LASIK.

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Rigid gas-permeable contact lens–assisted cataract surgery in patients with severe keratoconus

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We describe rigid gas-permeable (RGP) contact lens–assisted cataract surgery in patients with severe keratoconus. During cataract surgery in cases with severe keratoconus, the intraocular images are distorted and visual perspective is lost because of irregular corneal astigmatism. Poor visibility can lead to complications, including posterior capsule rupture and corneal endothelial cell damage. To overcome these problems, an RGP contact lens was placed on the cornea in 2 cases. The image distortion decreased markedly, and the visual perspective improved. Intraocular manipulations such as irrigation/aspiration were performed safely. Improvement in transillumination led to good visualization of the anterior and posterior capsules. No intraoperative or postoperative complications developed in either case. This technique provided excellent visualization during cataract surgery in patients with severe keratoconus.

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Online Video

Keratoconus is a noninflammatory disease characterized by thinning of the central stroma and anterior corneal protrusion.[1] This corneal architectural distortion results in myopia and irregular astigmatism that impair the quality of vision. Keratoconus management is a spectrum of therapy that progresses from no treatment to correction with glasses to contact lenses and finally to surgery. The appropriate treatment depends on the disease and visual requirements. Cases of mild keratoconus are treated with spectacles or contact lenses. Contact lens wear becomes necessary when conic progression increases the degree of irregular astigmatism. Keratoplasty is performed when patients cannot tolerate contact lenses or achieve satisfactory vision with them.

Intraocular images become distorted because of corneal irregular astigmatism in patients with keratoconus (Figure 1). However, the image distortion can be reduced with use of a rigid gas-permeable (RGP) contact lens. When cataract surgery is performed in keratoconus patients, poor visibility due to corneal irregular astigmatism can lead to complications, including posterior capsule rupture and corneal endothelial cell damage. We describe the use of RGP contact lens–assisted cataract surgery to overcome the poor visibility.

TECHNIQUE

After topical anesthesia and sub-Tenon anesthesia are induced with lidocaine 2.0%, an ophthalmic viscosurgical device (OVD) (Healon) is applied to the cornea. An RGP contact lens (Hoya Hard Ex, Hoya Corp.) (7.8 mm base curve, 8.8 mm diameter, 0 diopter power) is sterilized using low temperature (55°C for 210 minutes) ethylene oxide gas sterilization. The RGP contact lens is placed stably in the center of the cornea on the OVD despite corneal irregularity.

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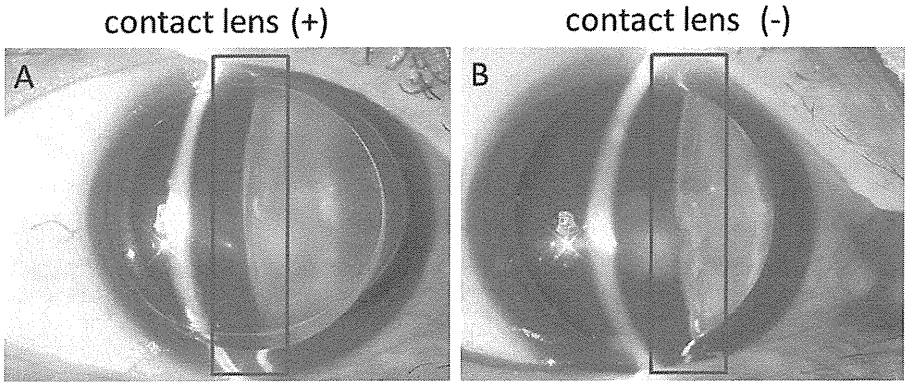

Figure 1. Slitlamp photographs of a patient with keratoconus with and without an RGP contact lens. *A*: The anterior capsule line is straight with the contact lens in place. *B*: The anterior capsule line twists and turns without the contact lens.

A capsulorhexis is created successfully using a 27-gauge bent-tip needle with the RGP contact lens in place. During phacoemulsification without the RGP contact lens, the images are distorted (Figure 2) (Video 1, available at http://jcrsjournal.org). However, the image distortion significantly decreases with use of the RGP contact lens. The intraocular image can be seen clearly and the opacified lenses chopped successfully using a bimanual phacoemulsification procedure as during standard cataract surgery. Phacoemulsification with a torsional oscillation system is performed safely and effectively, and the lens particles are aspirated smoothly in the phaco tip along with the fluidics.

Image distortion is worse without the RGP contact lens while irrigation/aspiration (I/A) is performed in the posterior chamber (Figure 2). The tip of the instrument appears enlarged and crooked without the contact lens; with the contact lens, the view improves markedly. Thus, the residual cortex is clearly visible and removed safely. Visualization of the anterior and posterior capsules is confirmed easily with the contact lens after I/A; however, transillumination is disrupted when the contact lens is removed (Figure 2).

A foldable acrylic intraocular lens (IOL) can be safely inserted through a 2.4 mm sclerocorneal incision using an injector and cartridge, and the IOL is dialed into the capsular bag when the circular capsulorhexis edge is visualized using the RGP contact lens. The RGP contact lens is removed from the cornea using forceps at the end of the procedure.

Results

Rigid gas-permeable contact lens–assisted cataract surgery was performed in 2 patients with severe

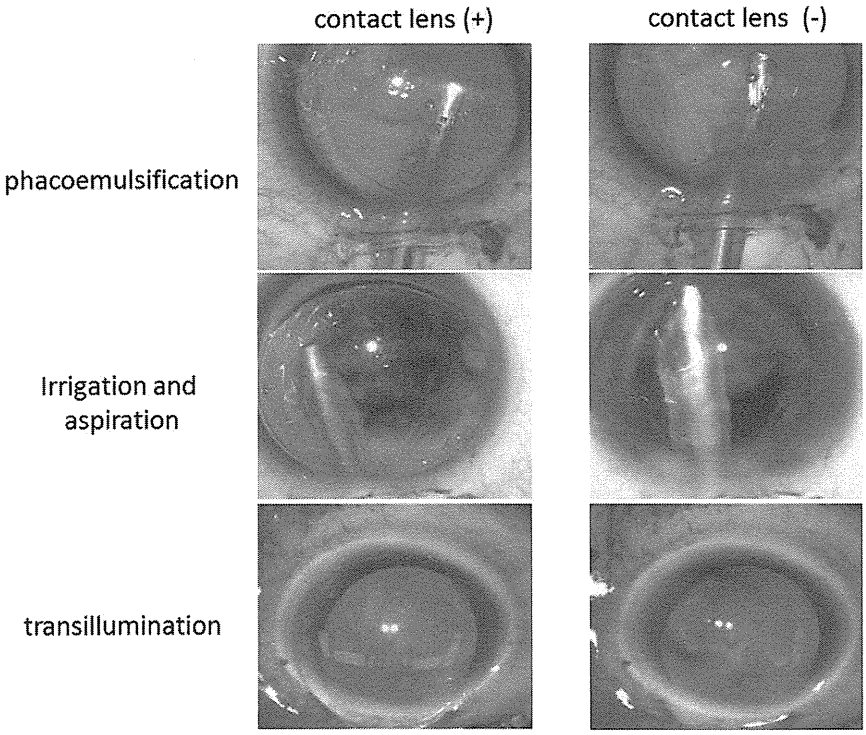

Figure 2. Intraoperative photographs of RGP contact lens–assisted cataract surgery. Image distortion is improved with use of the contact lens during phacoemulsification and I/A. Transillumination also improves with the contact lens in place.

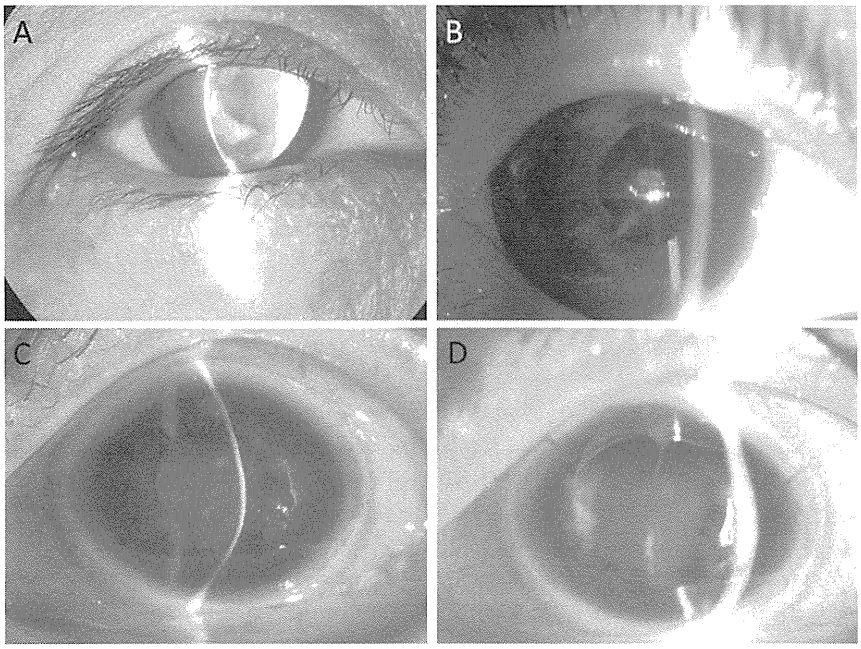

Figure 3. Slitlamp photographs of 2 patients with keratoconus before and after RGP contact lens–assisted cataract surgery. *A*: The right eye has a mature cataract complicated by severe keratoconus. The visual acuity is light perception. *B*: Five months postoperatively, the IOL is well fixated in the capsular bag. The visual acuity is 20/1000. No severe adverse event has occurred. *C*: The left eye had a grade 3 cataract complicated by keratoconus. The visual acuity was 20/667. *D*: Seven months postoperatively, the IOL is fixated in the capsular bag. The visual acuity has improved to 20/100. No severe adverse event has occurred.

keratoconus who requested cataract surgery without keratoplasty (Figure 3). In the first patient, the visual acuity improved from light perception to 20/1000 (Figure 3, *A* and *B*). In the second patient, the visual acuity improved from 20/667 to 20/100 (Figure 3, *C* and *D*). No intraoperative or postoperative adverse events occurred.

DISCUSSION

In our cases, both patients requested only cataract surgery without keratoplasty. Although corneal cross-linking and intrastromal corneal ring segments are possible treatments for keratoconus patients,[3,4] we did not think they were applicable in these cases because the central corneal thicknesses were less than 400 μm and the keratoconus was classified as stage 4 (Amsler-Krumeich classification[2]).

Cataract surgery in patients with severe keratoconus is challenging because of poor intraocular visibility. We overcame the challenge using an RGP contact lens. Although an OVD can be applied to make the corneal surface smooth during cataract surgery, the RGP contact lens offers an advantage. It can provide an ideal optical surface independent of the corneal surface, whereas the corneal surface coated with only OVD can be irregular because of astigmatism. Therefore, the RGP can offer the best visualization in cases with severe keratoconus. The corneal safety of the RGP contact lens has been established during the many years it has been used for optical correction of keratoconus.[5] The RGPs are readily available for use.

Intraocular image distortion and the subsequent lack of visual perspective are the main problems during capsulorhexis, phacoemulsification, and I/A. However, visualization improves with use of an RGP contact lens, which makes it easy to obtain *z*-axis information. The anterior capsule can be controlled with a 27-gauge bent-tip needle during capsulorhexis with an RGP contact lens in place. For a phaco-chop procedure, the emulsification tip should be used to impale the nucleus and knowledge of the depth of the instrument is very important. For divide-and-conquer techniques, the depth of the emulsification tip is important to divide the nucleus effectively. Use of an RGP contact lens facilitates acquiring information regarding the depth. The degree of image distortion in the same patient depends on the distance between the surgical instruments and the cornea. Distortion was the worst during I/A. Thus, this technique has the greatest effect on image improvement during manipulation in the posterior chamber, including I/A. Improvements in image distortion and visual perspective result in efficient and safe manipulation of the residual cortex. The RGP contact lens also improves transillumination. It is very important to confirm the posterior capsule for safe IOL insertion. We recommend that this technique also be used for this purpose.

Kamei et al.[6] reported the effectiveness of an RGP contact lens to protect the cornea from drying during vitrectomy with a wide-angle viewing system. The RGP contact lenses provided visibility similar to or clearer than that obtained with balanced salt solution, an OVD, a vitrectomy contact lens, or a soft contact lens. Because of the ease of use and low cost, an RGP

contact lens is ideal for use during vitrectomy with a wide-angle viewing system. This suggests that RGP contact lenses can also be used to protect the cornea from drying during cataract surgery.

The diameter of the RGP contact lens used in the current study was 8.8 mm, which was smaller than the corneal diameter. Because the visual field is limited to the lens diameter in this technique, a larger lens would offer a larger visual field. A lens with an 11.0 mm diameter or larger can be used for this purpose in a future study.

To our knowledge, this is the first description of RGP contact lens–assisted cataract surgery in patients with severe keratoconus. The technique is simple and can be performed by many surgeons. Rigid gas-permeable contact lens–assisted cataract surgery provides excellent visualization during cataract surgery in patients with severe keratoconus. In our 2 cases, the method was safe and effective.

WHAT WAS KNOWN

- Cataract surgery in patients with severe keratoconus is challenging because of poor intraocular visibility.

WHAT THIS PAPER ADDS

- Rigid gas-permeable contact lens–assisted cataract surgery offers marked improvements in intraocular image distortion, visual perspective, and transillumination during surgery.

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CLINICAL INVESTIGATION

Assessment of vision-related quality of life among patients with cataracts and the outcomes of cataract surgery using a newly developed visual function questionnaire: the VFQ-J11

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Abstract
Objective To investigate vision-related quality of life (VRQoL) and associated factors in patients with cataracts and the outcomes of cataract surgery using the newly developed VRQoL instrument: the visual function questionnaire, 11-item Japanese version (the VFQ-J11).
Methods A total of 457 patients scheduled for cataract surgery at 12 clinical sites from November 2008 through February 2010 were included in the study. The patients completed the VFQ-J11 before and 3 months after surgery. The VFQ-J11 was used to investigate factors associated with VRQoL of the cataract patients, the outcome of cataract surgery, and the predictors of improved VRQoL due to cataract surgery.
Results In a multiple regression model, the VFQ-J11 score was significantly associated with corrected distance visual acuity in the better-seeing eye (better eye VA), and improvement in the VFQ-J11 score after cataract surgery was associated not only with improvement in the better eye VA, but also with improvement in the worse eye VA. Compared to one-eye cataract surgery, both-eyes surgery had a greater impact on VFQ-J11 score improvement.
Conclusions The VFQ-J11 is a good measure of VRQoL in cataract patients. The present study indicates that by including the domains measured in the VFQ, the VFQ-J11 can provide valid data on VRQoL and be less of a burden for patients.

The authors contributed this article on behalf of the Eye Care Comparative Effectiveness Research Team (ECCERT), the members of which are listed in the Appendix.

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Keywords Cataract surgery · VFQ-25 · VFQ-J11 · Vision-related quality of life · Visual function questionnaire · Visual impairment

Introduction

Cataract is the leading cause of blindness in the world [1], and cataract surgery is the most frequent surgical procedure in people aged 65 years or older in the Western world, and in Japan [2, 3]. The value of cataract surgery is firmly established, and as a result, cataracts account for approximately one-third of medical spending on eye care in Japan [4]. As the Japanese population ages, the prevalence of visual impairment is projected to increase from 1.35 % of the population in 2007 to 2.0 % by 2050 [5], hence, visual impairment resulting from cataracts will be on the rise.

With the increase in appreciation for measurable patient-reported outcomes (PROs) such as social functioning, satisfaction and quality of life (QOL), there came the realization that clinical measures like visual acuity alone are not the only outcomes that reflect the benefits of cataract surgery [6–8]. Previous investigations demonstrate that (compared to visual acuity) tasks associated with visual function in daily living and QOL are strong predictors of postoperative satisfaction with vision and postoperative improvement in visual function [9, 10]. Furthermore, improved QOL has been demonstrated even in those with poor post-surgical visual acuity and with good pre-surgical visual acuity of >1.0 following surgery [11, 12], indicating that while visual acuity is the standard measure of effectiveness of surgery for clinicians, it is not necessarily so for patients. The benefits of surgery may be underestimated when assessing visual acuity alone.

Several PRO instruments have been developed to measure vision-related QOL (VRQoL). The visual function questionnaire (VFQ) was originally developed by the National Eye Institute [13]. It is intended to measure visual function in 11 domains: general vision, ocular pain, near activities, distance activities, vision-specific social functioning, vision-specific mental health, vision-specific role difficulties, vision-specific dependency, driving, color vision and peripheral vision. The 25-item version of the VFQ (VFQ-25) has been used in the evaluation of visual impairment in many studies [6, 8, 14, 15]. Although the VFQ-25 is a shorter version of the 51-item National Eye Institute VFQ, it still contains 25 essential items and 13 optional items that generate sub-scales [13]. For patients in clinical settings, responding to many questions about visual function, sometimes asked by an interviewer, can be burdensome. Recently, the Visual Function Questionnaire 11-item Japanese version (VFQ-J11) was developed in order to decrease that burden while maintaining good psychometric properties [16]. Compared to the VFQ-25, the VFQ-J11 has superior responsiveness and criterion-related validity, and is unidimensional, integrating the values of multiple information domains into a single score (i.e., one score for 11 domains that were originally intended to be measured separately in the VFQ) (Table 1) [16]. The purpose of this study was to investigate the factors associated with VRQoL of cataract patients, the outcome of cataract surgery, and the predictors of the improved VRQoL due to cataract surgery, using the VFQ-J11. This is the first multicenter study that used the VFQ-J11 in routine clinical settings for the evaluation of cataract surgery outcomes in Japan.

Table 1 Difference in characteristics between the VFQ-25 and the VFQ-J11

	The VFQ-25	The VFQ-J11
Number of questions (questions)	25	11
Pages in questionnaire (pages)	8	2
Time required to complete (mean ± SD)	10.8 ± 2.3 min	8.7 ± 3.4 min
Domains (number of items)		
General vision	1	1
Well-being, distress	4	1
Near vision	3	3
Distance vision	3	3
Social functioning	2	1
Role limitation	2	1
Dependency	3	1
Ocular pain	2	
Peripheral vision	1	
Color vision	1	
Driving	2	
General health	1	

Subjects and methods

The current study is part of the prospective multicenter observational study conducted by the Eye Care Comparative Effectiveness Research Team (ECCERT) at 12 clinical sites in Japan (see "Appendix"). We previously described the methods and results of a study that evaluated the cost-utility of routine cataract surgery in Japan [12, 17]. Briefly, a prospective observational protocol was developed by ECCERT to evaluate visual and patient-reported outcomes before and after routine cataract surgery. Twelve ophthalmic facilities (7 university hospitals, 3 public hospitals and 2 private surgical clinics) participated in the study. The study protocol was approved by the institutional review board of each facility, and the conduct of the study

followed the tenets of the Declaration of Helsinki. Patients were eligible for inclusion in the study if they were scheduled for first-eye, second-eye or both-eyes cataract surgery and were 50 years of age or older. Patients were excluded if the planned cataract surgery was a combined procedure involving glaucoma, corneal or vitreoretinal surgery. Exclusions were also made if patients had any visually significant coexisting ocular pathology, such as macular diseases, glaucoma, optic atrophy, amblyopia or proliferative diabetic retinopathy with dementia. Each subject gave written informed consent to participate in this study. Patients were recruited from 1 November 2008 through 28 February 2010. A total of 549 cases were initially registered. A full preoperative medical history and an ophthalmic examination of each patient were obtained. The ophthalmic examination included visual acuity, types of lens opacity (nuclear, cortical, or posterior subcapsular cataract), and the presence of any other ocular disease. The visual acuity was reported as a corrected decimal acuity obtained according to the usual routine of each clinic. Three-month clinical outcomes were also reported by the treating ophthalmologist. All patients were asked whether they had a history of systemic comorbidities, including cardiovascular diseases (hypertension, angina, heart failure, myocardial infarction, use of a pacemaker), cancer, diabetes, renal and hepatic diseases, gastrointestinal diseases (gastric ulcer, enteritis, colitis), respiratory diseases, musculoskeletal diseases (rheumatoid arthritis, spine disorder), neurological diseases (paralysis such as stroke, need for ambulatory assistance), and deafness or hearing impairment.

Assessment of the VFQ-J11 was performed before and after the surgery, and the scoring yielded a total functioning score. The VFQ-J11 score is presented as an index between 0 and 100, with 0 representing the worse possible score and 100 the best, and the score is an unweighted average of all attributes of 11 questions. Questionnaires were administered at two time points: prior to undergoing cataract surgery, and 3 months after the surgery. The questionnaires were collected from the 12 clinical sites; however, because of the inability to collect some of the survey forms and no possibility of accounting for missing values, we were able to obtain answers from only 457 cases for the VFQ-J11 at the baseline. The patients were encouraged to complete the follow-up questionnaires mailed to their homes 3 months after the surgery, and to return them by post. Later, however, because of reasons including withdrawal of consent, insufficient information on clinical findings from the responsible physicians, and inability to collect survey forms from patients, the final number of postoperative replies we were able to obtain for the VFQ-J11was 350 cases.

Statistical methods

Data were analyzed using STATA 12 software (STATA Corp; College Station, TX, USA). The results are given as the mean and standard deviation (SD), as the mean and 95 % confidence interval (CI) or as the median. The significance of the difference in visual acuity before and after cataract surgery was analyzed with Student's paired *t*-test for dependent samples. The significance of the difference in VFQ-J11 score before and after cataract surgery was analyzed with the Wilcoxon rank-sum test. The two-sample Wilcoxon rank-sum test was used to compare VFQ-J11 score in one-eye surgery with both-eyes surgery. Multiple linear regression models were used to assess the associated factors affecting VFQ-J11 score at the baseline for all patients. The regression model with robust (Eicker–Huber–White) standard errors was adjusted for age, sex, corrected distance visual acuity in the better-seeing eye (better eye VA), corrected distance visual acuity in the worse-seeing eye (worse eye VA), refractive error (the spherical equivalent in diopters), types of lens opacity, ocular past history, other ocular diseases and the number of systemic comorbidities. To assess the predictive factors affecting improved VFQ-J11 score before and after cataract surgery, a random-effects panel model was used to assess differences across patients as well as across time-periods. The factors analyzed were age, sex, past ocular history, other ocular diseases, the number of systemic comorbidities, type of operation (both-eyes vs one-eye operation), and the change in better eye VA, worse eye VA, refractive error, and the type of lens opacity. Visual acuity data obtained by the decimal unit (counting fingers was categorized as an acuity of 0.004, hand motion as 0.002, and light perception as 0.001) were converted to logarithm of minimal angle of resolution (log MAR) units for statistical analysis. A p value of less than 0.05 was considered statistically significant.

Results

Out of 549 patients enrolled in the study, complete data of 457 patients on visual acuity and the VFQ-J11 before surgery could be used in the analysis at the baseline. The age of the patients ($n = 457$) enrolled in the study ranged from 51 to 88 years (average 70.6 ± 7.8 years; 182 men and 275 women). Table 2 summarizes the characteristics of the samples at the baseline. The baseline mean better eye VA (log MAR) and worse eye VA (log MAR) were 0.15 ± 0.26, and 0.48 ± 0.50, respectively. The baseline mean VFQ-J11 score ranged from 4.5 to 98.2 (average 67.9 ± 17.5).

The preoperative VFQ-J11 scores obtained from the 457 cases at the baseline were stratified according to the better eye VA (decimal) (Table 3). The VFQ-J11 score was positively correlated with 6 different visual stratifications.

Table 2 Patients' characteristics before surgery

Characteristic	*n*	Mean ± SD	Median
Age (years)	457	70.6 ± 7.8	71
Women	275	71.3 ± 7.3	72
Men	182	69.4 ± 8.3	70
VA (log MAR)	457		
Better eye		0.15 ± 0.26	0.1
Worse eye		0.48 ± 0.50	0.3
VFQ-J11 score	457	67.9 ± 17.5	69.6

VA visual acuity, *log MAR* logarithm of minimal angle of resolution

Table 3 VFQ-J11 score associated with visual acuity in the better-seeing eye

Visual acuity (decimal)	VFQ-J11 score	SD	*n*
<0.1	34.1	25.4	5
<0.2	38.1	15.2	7
<0.4	50.7	21.2	27
<0.8	64.0	15.7	158
<1.0	71.4	14.4	111
≥1.0	75.0	14.5	149
Overall	67.9	17.5	457

As the better eye VA decreased, the corresponding VFQ-J11 score decreased at every visual stratification level (Table 2). Out of 457 cases, 336 cases (74 %) had nuclear cataract, 325 (71 %) cases had cortical cataract, and 185 (40 %) cases had posterior subcapsular cataract. The proportion of the mixture of nuclear and cortical opacity, nuclear and posterior subcapsular opacity, and cortical and posterior subcapsular opacity were 48, 33, and 26 %, respectively. Twenty-one percent of the cases had a mixture of nuclear, cortical, and posterior subcapsular opacity.

Factors associated with preoperative VFQ-J11 score are given in Table 4. In a multiple linear regression model adjusting for age, sex, the worse eye VA (log MAR), refractive error, types of lens opacity and the history of systemic comorbidities, the better eye VA (log MAR) showed a significant correlation with VFQ-J11 score. The score changed by 37 for each change in visual acuity of 1.0 (log MAR) ($p < 0.001$; Table 4). Although the history of systemic comorbidities (having more than 2 systemic comorbidities) also had a significant effect, the standardized regression coefficient was small (−0.12) compared to that of the better eye VA (−0.43), and the overall model was significant ($n = 416$, $F < 0.0001$, $R^2 = 0.23$).

Before the surgery, 12 patients cancelled their operations, and follow-up questionnaires were received from 412 patients. However, we had no method for dealing with missing values, and, therefore, incomplete questionnaires were excluded. Finally, 350 cases with complete VFQ-J11 data were available to be used in the analysis of final outcome.

Table 4 Factors associated with VFQ-J11score

Variable	Univariate analysis			Multivariate analysis*		
	Coefficient	(95 % CI)	*p* value	Coefficient	(95 % CI)	*p* value
Age (per +1 years old)	−0.20	(−0.41 to −0.01)	0.06	0.11	(−0.11 to 0.33)	0.33
Female gender (vs male)	−2.21	(−5.49 to −1.07)	0.19	−0.53	(−3.57 to 2.51)	0.73
VA better (per +1 LogMAR VA)	−30.19	(−35.60 to −24.78)	<0.001	−37.04	(−46.17 to −27.92)	<0.001
VA worse (per +1 LogMAR VA)	−10.20	(−13.27 to −7.13)	<0.001	0.21	(−3.91 to 4.34)	0.92
Refractive error (per +1 diopter)[a]	0.88	(0.49 to 1.28)	<0.001	0.36	(−7.10 to 0.27)	
Nuclear cataract (present vs absent)	−4.33	(−7.96 to −0.71)	0.02	−3.42	(−0.09 to 0.81)	0.12
Cortical cataract (present vs absent)	−0.56	(−4.11 to 2.99)	0.76			
PSC cataract (present vs absent)	−4.47	(−7.72 to −1.22)	0.01	−0.57	(−3.92 to 2.78)	0.74
Ocular past history (present vs absent)	−0.76	(−6.35 to 4.83)	0.79			
Other ocular diseases (present vs absent)	−0.13	(−6.29 to 6.02)	0.97			
Systemic comorbidity[b]	−3.08	(−6.85 to 0.69)	0.11	−2.91	(−6.53 to 0.72)	0.12
Systemic comorbidities[c]	−5.46	(−9.59 to −1.32)	0.01	−4.55	(−8.38 to −0.72)	0.02

VA better LogMAR visual acuity in better-seeing eye, *VA worse* logMAR visual acuity in worse-seeing eye, *PSC* posterior subcapsular

* Only variables with a *P* value of less than 0.25 in the univariate analysis were included in the multivariate model

[a] For bilateral cataracts, mean refractive error (the spherical equivalent in diopters) in both eyes were used

[b] One systemic comorbidity vs no systemic comorbidity

[c] More than two systemic comrbidities vs no systemic comorbidity

Table 5 Before-and-after VFQ-J11 score change by cataract surgery

Characteristic	1st eye surgery	2nd eye surgery	Both eyes surgery
Age (mean ± SD)	67.9 ± 7.8	69.4 ± 8.4	71.3 ± 7.7
n	109	37	204
VFQ-J11 score (mean ± SD)			
Before surgery	73.08 ± 15.94	73.08 ± 15.14	65.12 ± 17.99
After surgery	88.61 ± 12.07	86.06 ± 12.27	87.28 ± 13.58
VFQ-J11 score change	15.53 ± 16.52	12.97 ± 15.37	22.16 ± 19.11
P value*	$p < 0.001$	$p < 0.001$	$P < 0.001$

* The significance of the differences in VFQ-J11 score before and after cataract surgery was analyzed with a Wilcoxon rank-sum test

Table 6 Predictors associated with VFQ-J11 score improvement

Variable	Random-effects panel model analysis		
	Coefficient	(95 % CI)	*p*-value
Age (per +1 years old)	0.03	(−0.13 to 0.18)	0.74
Female (vs male)	0.81	(−1.55 to 3.16)	0.50
VA better (per +1 logMAR VA)	−41.60	(−49.93 to −33.27)	<0.001
VA worse (per +1 logMAR VA)	−5.14	(−9.07 to −1.21)	0.01
Refractive error (per +1 diopter)[a]	0.27	(−0.10 to 0.65)	0.16
Nuclear cataract (present vs absent)	−4.32	(−6.80 to −1.84)	<0.001
Cortical cataract (present vs absent)	−3.84	(−6.20 to −1.48)	0.01
PSC cataract (present vs absent)	−3.56	(−6.54 to −0.57)	0.02
Ocular past history (present vs absent)	−0.84	(−4.72 to 3.05)	0.67
Other ocular diseases (present vs absent)	−2.57	(−7.15 to −2.00)	0.27
Systemic comorbidity[b]	−0.19	(−2.84 to 2.46)	0.89
Systemic comorbidities[c]	−0.80	(−3.82 to 2.23)	0.61
Operation (both-eye vs. one-eye)	2.82	(0.23 to 5.41)	0.03

VA better LogMAR visual acuity in better-seeing eye, *VA worse* logMAR visual acuity in worse-seeing eye, *PSC* posterior subcapsular

[a] For bilateral cataracts, mean refractive error (the spherical equivalent in diopters) in both eyes were used

[b] One systemic comorbidity vs no systemic comorbidity

[c] More than two systemic comorbidities vs no systemic comorbidity

Intraoperative techniques were phacoemulsification and aspiration with intraocular lens implantation in all cases. Both the postoperative mean better eye VA (log MAR) and worse eye VA (log MAR) significantly improved to −0.06 ± 0.10 and 0.01 ± 0.20, respectively ($p < 0.01$, paired *t*-test).

The study population consisted of 3 subgroups: group 1, in which both eyes were operated on ($n = 204$); group 2, in which only the first eye was operated on ($n = 109$); and group 3, in which the second eye was operated on (the first eye had been operated on previously) ($n = 37$). In the entire group of 350 patients, the overall VFQ-J11 score showed a statistically significant improvement from 68.4 ± 17.5 at base line to 87.6 ± 13.0 at 3 months after the surgery ($p < 0.01$, Wilcoxon signed-rank test). In the subgroup analysis, all groups showed a statistically significant improvement in VFQ-J11 score (Table 5). The VFQ-J11 score change from both-eyes operations was significantly higher than that from a one-eye operation (combined first eye and second eye operations) ($p < 0.001$, two-sample Wilcoxon rank-sum test).

Predictors associated with improved VFQ-J11 score are given in Table 6. Random-effects panel analysis after adjusting for age, sex, past ocular history, other ocular diseases, the number of systemic comorbidities and the change in refractive error, change in the better eye VA, change in the worse eye VA, type of lens opacity, and both-eyes surgery (compared to one-eye surgery) showed a significant correlation with improved VFQ-J11 score. The score changed by 42 for each log MAR change of 1.0 in the better eye VA ($p < 0.001$; Table 6), and 5 for each log MAR change of 1.0 in the worse eye VA ($p = 0.01$; Table 6). The type of lens opacity also had a significant effect on the change in VFQ-J11 score, and nuclear cataract had a greater effect (4.3) than cortical cataract (3.8) and posterior subcapsular cataract (3.6).

Discussion

This is the first study using the VFQ-J11 in routine clinical settings for the evaluation of cataract surgery outcomes in Japan. The VFQ-25 has been widely used to measure visual functioning in many contexts for more than 10 years [6, 8, 13–15]. A new instrument of VRQoL assessment, the VFQ-J11, was recently developed. Compared to the VFQ25, it poses less of a burden to respondents, while maintaining good psychometric properties [16]. The VFQ-J11 (8.7 ± 3.4 min., the mean of scores from 20 questionnaires of the pilot study, unpublished data) took a shorter time to complete than the VFQ-25 (10.8 ± 2.3 min., the mean of scores from 10 questionnaires, source Y. Suzukamo, personal communication) (Table 1). Moreover, compared to the VFQ-25 score, the VFQ-J11 score correlated more strongly with visual functions such as visual acuity and visual field [16]. For patients and interviewers in real clinical settings, usually, using a questionnaire can be burdensome, and the response rate of the survey depends on the cooperation and the

understanding of the patients. If the plan is to conduct the survey in the waiting area of medical facilities or by telephone, the number of questions in the questionnaire and the time required to complete should be kept to a minimum. In these circumstances, the VFQ-J11 can be more useful than the VFQ-25. In the current study, the sensitivity of the VFQ-J11 score to changes in visual function after cataract surgery was quite high, and even in patients with a better eye VA of >1.0 (decimal), improvement in the mean VFQ-J11 score was 13.2 after cataract surgery ($p < 0.001$, Wilcoxon signed-rank test). This means that while for clinicians visual acuity is the most precise and standard way of measuring the effectiveness of surgery, it is not so for the patients. Although visual acuity is the most important VRQoL-associated factor, the emphasis for patients is on daily life activities and overall subjective QOL.

In the current study, better eye VA and the presence of multiple systemic comorbidities (having more than 2 systemic comorbidities) in cataract patients were associated with VFQ-J11 score. Utility (patients' preferences for their health outcomes) associated with ophthalmic disease has been most highly correlated with better eye VA [18]. Data from the analysis presented here also indicate the same relationship between VA and VFQ-J11 score. However, VA is only one factor affecting visual function; thus, better eye VA is the main determinant of vision-related social activity. In the present study, the correlation between VFQ-J11 score and better eye VA, although statistically significant, was modest ($r = -0.49$). This finding emphasizes that many factors such as visual field, color perception, contrast sensitivity, etc. contribute to VRQoL. Although the association with the presence of systemic comorbidity was shown to be significant, the standardized regression coefficient was very small (−0.12) compared to that of better eye VA (−0.43). Yamada et al. [14] report that the presence of systemic comorbidities affects the VFQ-25 score, and particular attention should also be paid to the use of the VFQ-J11 in patients with systemic comorbidities.

Several studies demonstrate greater improvement in subjective visual function in patients who undergo cataract surgery in both eyes than in those who undergo surgery in one eye [19, 20]. We similarly found that cataract surgery in both eyes compared to just one eye results in even greater improvement in subjective visual function measured by the VFQ-J11.

In this study, predictors associated with the improved VFQ-J11 score were improvement in better eye VA and worse eye VA, both-eyes surgery (compared to one-eye surgery), and the type of lens opacity after adjusting for other factors such as age, sex and change in refractive error. Interestingly, improvement in worse eye VA was associated with improvement in VFQ-J11 score in our study. Since VRQoL is reported to depend mainly on better eye VA [18, 21], our analysis suggests the importance of worse eye VA in the improvement of VRQoL. This may influence cost-effectiveness analyses which often assume a difference in the usefulness of treatment of the better-seeing vs worse-seeing eye. The type of lens opacity also has a significant effect on the change in VFQ-J11 score, with nuclear cataracts having a greater effect than cortical and posterior subcapsular cataracts. This finding extends the previous finding by Clemons and co-workers [22] of an association of nuclear but not cortical opacity with lower VRQoL.

Some limitations in this study must be considered. Although we used the common inclusion and exclusion criteria established for selection of our patients, we did not randomly select the study population at each clinical site. In addition, owing to questionnaire incompletion and patient withdrawal from the study, complete data for the VFQ-J11 were obtained from 350 patients out of 457 who were approached. Despite this potential source of error in the outcome measures, our samples were representative of the current population of patients who undergo cataract surgery in Japan, since the sample was large and obtained from 12 different clinical sites that included university hospitals, general hospitals, and private practices. Although compared to the sample included in the analysis, the sample excluded from the analysis had a higher mean age (73 vs 70), lower postoperative better eye VA (1.1 vs 1.2), and lower postoperative worse eye VA (0.9 vs 1.0). This indicates that the difference between patients who answered all questions on the follow-up questionnaire and those who did not was clinically insignificant. Another limitation of this study was the relatively small sample size used for analysis in the moderate-to-severely impaired vision groups (VA < 0.4). This could explain the large standard deviation in VFQ-J11 score observed in this group. Additionally, this study included only cataract patients. Further studies should focus on the main causes of visual impairment in Japan, such as glaucoma, diabetic retinopathy, age-related macular degeneration and pathologic myopia.

In summary, VFQ-J11 is a very useful measure of VRQoL in patients with cataract, and is sensitive to the clinical effect of cataract surgery. VFQ-J11 score is significantly associated with better eye VA in cataract patients, and improvement in VFQ-J11 score after cataract surgery is associated not only with improvement in better eye VA, but also with improvement in worse eye VA. Improvement in the VFQ-J11 score is greater after both-eyes surgery than after one-eye surgery. By including the domains measured in the VFQ, the VFQ-J11 can provide valid data on VRQoL while being less of a burden for patients.

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RESEARCH REPORT

Diquafosol Tetrasodium Increases the Concentration of Mucin-like Substances in Tears of Healthy Human Subjects

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ABSTRACT

Purpose: This study was undertaken to determine the effect of topical application of diquafosol tetrasodium on proteins and mucin-like substances from tears of clinically healthy subjects.

Methods: Tears were collected from both the eyes of 10 healthy volunteers. Diquafosol tetrasodium solution (3%) was applied once to the right eye and 0.9% sodium chloride solution (saline) once to the left eye. Tear samples were collected by Schirmer test strips before application and 5, 15, 30 and 60 min after application. Sialic acid, a marker of mucin-like substances, and major tear proteins including secretory IgA, lactoferrin, lipocalin-1, and lysozyme were measured by high performance liquid chromatography.

Results: Levels of total protein, sIgA and lysozyme were transiently decreased in both groups but returned to baseline levels within 15 min after application. The concentration of lactoferrin and lipocalin-1 did not change significantly in both groups. Sialic acid in tears was significantly decreased 5 min after saline application, but significantly increased 5 min after diquafosol application. No significant difference in sialic acid was seen after 15 min in both groups.

Conclusions: Topical application of saline and diquafosol resulted in transient decrease of tear proteins possibly due to wash out or dilution effects. In contrast, diquafosol application significantly increased sialic acid, although the effect was transient. This suggests diquafosol stimulates the secretion of mucins from ocular tissues of healthy human subjects.

Keywords: Diquafosol tetrasodium, dry eye, mucins, sialic acid, tears

BACKGROUND

Diquafosol tetrasodium is a $P2Y_2$ purinergic receptor agonist.[1] $P2Y_2$ receptors are present at various sites in the ocular surface, including epithelial cells and goblet cells of the conjunctiva. In rabbit conjunctiva, binding of diquafosol to $P2Y_2$ receptors increases intracellular calcium and activates chloride ion transport driving fluid transport across the epithelial layer.[2–4] Increases in intracellular calcium induced by diquafosol also stimulate conjunctival goblet cell degranulation and the subsequent release of mucins to the ocular surface.[5–7]

Topical instillation of diquafosol reportedly improves corneal epithelial staining scores in rat and rabbit models of dry eye.[8,9] Diquafosol is also effective at reducing staining of the cornea and conjunctiva, and improving symptoms in dry eye patients.[10–12] Diquafosol was approved for clinical use by the Ministry of Health, Labour and Welfare, Japan, in December 2011. Since then, diquafosol has become a new modality for the treatment of dry eye in Japan.

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Diquafosol is believed to exert its therapeutic effect in dry eye patients by activating the $P2Y_2$ receptor in the conjunctiva, thereby promoting the secretion and content of aqueous and mucin in the tear fluids.[1,13] Although these stimulatory effects of diquafosol on conjunctival tissue have been demonstrated in animal models,[8,9,14] they have not fully been investigated in healthy human subjects to date. The aim of the current study was to determine the effect of topical application of diquafosol on proteins and mucins in tears from a group of clinically healthy subjects.

METHODS

Chemicals

Three percent of diquafosol tetrasodium solution (Diquas ophthalmic solution 3%) was obtained from Santen Pharmaceutical Co. (Osaka, Japan). 0.9% Sodium chloride solution (saline) was obtained from Otsuka Pharmaceutical Co. (Tokyo, Japan). Phosphate buffered saline (PBS), bovine serum albumin (BSA), sodium phosphate dibasic, lactoferrin from human milk and lysozyme from human neutrophils were obtained from Sigma-Aldrich Co. (Tokyo, Japan). Secretory immunoglobulin A (sIgA) from human colostrum was obtained from Acris Antibodies Inc. (San Diego, CA), and recombinant human lipocalin-1 was from R&D Systems Inc. (Minneapolis, MN). Advanced protein assay reagents were purchased from Cytoskeleton Inc. (Denver, CO). Methanol, sodium chloride and acetic acid (HPLC grade) were obtained from Wako Chemical Industries (Osaka, Japan). Acetonitrile (HPLC grade) was obtained from Honeywell Japan Inc. (Tokyo, Japan). 1,2-Diamino-4,5-methylenedioxybenzene (DMB), coupling solution (acetic acid, β-mercaptoethanol and sodium hydrosulfite) was obtained from Takara Bio Inc. (Otsu, Japan).

Subjects

Ten healthy volunteers (six males and four females) aged 26–50 years (34.9 ± 7.3 years, mean ± SD) with no history of eye disease, except for refractive errors, were enrolled in the study. At the screening visit, one of the authors (SH) performed routine ocular examination of all subjects, followed by an examination of the ocular surface, including Schirmer testing and measurement of tear film break-up time (BUT). Two microliters of 1% fluorescein solution was used for vital staining. For all the subjects there was more than 5 mm of Schirmer strip wetting, more than 5 s in tear film BUT, and no apparent fluorescein staining of the cornea and conjunctiva. All the subjects were free from any topical medication. Guidelines according to the World Medical Association Declaration of Helsinki were followed. The subjects received a full explanation of the procedures and provided their informed consent for participation prior to the experiment. The protocol was approved by the institutional review board of National Hospital Organization Tokyo Medical Center (R10-022), and all the subjects provided written informed consent.

Tear Samples

Fifty microliters of 3% diquafosol solution was topically applied in the right eye and 50 µl of saline solution in the left eye. Tears were collected by inserting a Schirmer test strip (Alcon, Inc., Fort Worth, TX) in the lower outer fornix and participants were asked to close their eyes. Topical anesthesia was not used to eliminate possible compositional changes of tears induced by anesthetic eyedrops.[15] After 1 min the strips were removed, immediately placed in a 1.5 ml Eppendorf tube, and stored at −80 °C until used for assay.

Tear samples were collected before the application (base line), and 5, 15 30 and 60 min after application to both the eyes. Each sample was collected on a different consecutive day at the same time (between 4 and 6 PM) in the same place to avoid any possible contribution of cumulative effects and diurnal variation.[16,17]

Total Tear Protein Assay

The Schirmer strip was soaked in 200 µl of PBS for 30 min to elute tear proteins before the assay. Total tear proteins were determined using the Bradford method, with BSA as a standard. A volume of 2 µl of each tear protein extract was mixed with 300 µl of advanced protein assay reagent. The total tear protein concentration of each sample was detected using a plate reader (Benchmark Plus, Bio-Rad Ltd., Hercules, CA) set at 590 nm.

Major Tear Protein Assay

A volume of 3 µl of each tear protein extract was fractionated by HPLC.[18] HPLC conditions used for the determination of sIgA, lactoferrin, lipocalin-1 and lysozyme were described previously.[15,19] An HPLC system consisting of a SCL-10Avp HPLC controller (Shimadzu Co., Kyoto, Japan), a LC-10ADvp solvent delivery system, a SPD-10Avp UV-VIS detector, and a LC solution chromatography software (Shimadzu) was used. Elution was performed with a TSK 3000SWXL column, 7.8 mm i.d. × 300 mm (Tosoh Inc., Tokyo, Japan), with a mobile phase of 0.5 M sodium

chloride and 0.1 M sodium phosphate, pH 5.0. The flow rate was 0.7 ml/min and the peaks were detected at 230 nm. Standards for each protein were used to determine the test concentrations. sIgA from human colostrum, lactoferrin from human milk, lysozyme from human neutrophils and recombinant human lipocalin-1 were used as standards. The results were expressed as mg/ml.

Sialic Acid Assay

Procedures for analyzing sialic acids were based on the report of Yasueda et al.[20] A volume of 10 µl of each eluted tear protein extract was mixed with 10 µl of 4 M acetic acid and kept at 80 °C for 3 h to release sialic acid. A mixture of 200 µl of DMB solution, coupling solution (containing acetic acid, β-mercaptoethanol and sodium hydrosulfite) and water (ratio 1:5.4) were added, and the mixture was kept at 50 °C for 2.5 h in the dark to develop fluorescent labeling. The reaction mixture was cooled in ice water to stop the reaction and 5 µl was submitted for HPLC analysis.

An HPLC system consisting of a SCL-10Avp HPLC controller (Shimadzu Co.), a LC-10ADvp solvent delivery system, a RF-10AxL fluorescence detector, and LC solution chromatography software was used. Elution was performed using a COSMOSIL5C18-AR-2 column, 4.6 mm i.d. × 150 mm (Nakarai Tesque, Kyoto, Japan) with a mobile phase of acetonitrile-methanol-water (2:14:84). The flow rate was 0.9 ml/min at 40 °C, and the peak of Neu5Ac was detected by fluorescence (excitation 375 nm, emission 448 nm). The Neu5Ac standard for quantification was obtained from the Sialic Acid Fluorescence Labeling Kit (Takara Bio Inc.). The final results were expressed as µg/ml, or µg/mg protein.

Statistical Analysis

Data were analyzed using InStat 3 software (GraphPad Software, Inc., La Jolla, CA). The results are presented as mean and standard deviation (SD). The significance of the differences was analyzed with Wilcoxon signed-rank test. A p value < 0.05 was considered statistically significant.

RESULTS

Tear Proteins

The mean 1-min Schirmer strip wetting value before application was 15.3 ± 5.8 mm in the right eye (diquafosol group) and 16.3 ± 8.1 mm in the left eye (saline group). As shown in Table 1, 1-min Schirmer strip wetting values were stable and showed no significant differences between the groups throughout the study (Wilcoxon signed-rank test).

Comparison of total tear protein concentration before and 5 min after topical application showed a significant decrease in both the groups. The concentration of total protein recovered at 15 min was maintained during our observation time of 60 min for both the groups.

HPLC was used to determine the concentration of each of the four major tear proteins: sIgA, lactoferrin, lipocalin-1 and lysozyme. The concentration of lactoferrin and lipocalin-1 did not change significantly in both groups. sIgA significantly decreased at 5 min in both groups. While sIgA recovered within 15 min in the diquafosol group, the decrease persisted at 15 min in the saline group and recovery was observed at 30 min. A significant decrease in lysozyme concentration was seen 5 min after topical application in the saline group but not in the diquafosol group.

TABLE 1 Changes in tear volume and protein concentrations after topical applications of 3% diquafosol solution and saline.

	Group	Pre	5 min	15 min	30 min	60 min
1-min Schirmer strip wetting (mm)	Diquafosol	15.3 ± 5.8	14.6 ± 6.4	13.6 ± 8.8	15.7 ± 8.2	13.8 ± 15.1
	Saline	16.3 ± 8.1	15.8 ± 7.2	15.5 ± 7.2	14.7 ± 9.0	15.1 ± 6.5
Total protein (mg/ml)	Diquafosol	6.1 ± 3.2	5.0 ± 2.3*	6.2 ± 2.3	6.1 ± 2.2	6.3 ± 2.3
	Saline	6.4 ± 3.1	4.9 ± 1.8*	6.0 ± 1.7	6.9 ± 2.0	6.7 ± 2.2
Lactoferrin (mg/ml)	Diquafosol	2.0 ± 0.9	1.8 ± 0.9	2.0 ± 0.6	2.0 ± 0.9	2.2 ± 0.7
	Saline	1.9 ± 0.7	1.7 ± 0.7	2.1 ± 0.5	2.3 ± 0.5	2.2 ± 0.6
Lipocalin-1 (mg/ml)	Diquafosol	2.1 ± 1.7	1.7 ± 1.1	2.1 ± 1.2	2.0 ± 1.4	2.0 ± 1.3
	Saline	1.8 ± 1.0	1.5 ± 0.6	1.7 ± 0.9	2.0 ± 0.9	1.9 ± 1.0
sIgA (mg/ml)	Diquafosol	0.74 ± 0.46	0.57 ± 0.50**	0.59 ± 0.28	0.67 ± 0.39	0.76 ± 0.34
	Saline	0.89 ± 0.54	0.58 ± 0.32*	0.54 ± 0.17*	0.73 ± 0.31	0.81 ± 0.44
Lysozyme (mg/ml)	Diquafosol	1.2 ± 0.6	1.3 ± 0.4	1.2 ± 0.4	1.3 ± 0.5	1.2 ± 0.3
	Saline	1.3 ± 0.4	1.0 ± 0.3*	1.1 ± 0.3	1.3 ± 0.3	1.3 ± 0.4

Data are presented as mean ± S.D. Results were compared with values before application.
*$p < 0.05$.
**$p < 0.01$ (Wilcoxon signed-rank test).

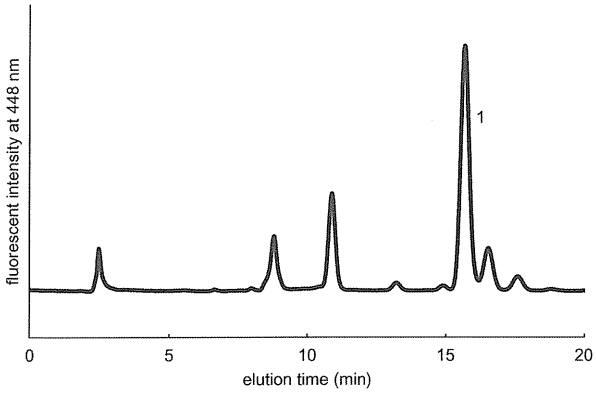

FIGURE 1 A typical high performance liquid chromatogram of fluorescent-labeled sialic acid. Measurement of the Neu5Ac peak (1) representing sialic acid was well separated from the other peaks.

Sialic Acid Concentration

Measurement of the Neu5Ac peak, representing sialic acid, was well separated from other peaks by HPLC (Figure 1). Compared with baseline data (91.3 ± 31.9 μg/ml), the concentration of sialic acid decreased significantly in the saline group at 5 min (74.4 ± 28.2 μg/ml) after saline application (Figure 2A). In contrast, the concentration of sialic acid increased significantly in the diquafosol group at 5 min (113.4 ± 42.8 μg/ml) when compared with the baseline value (90.1 ± 44.7 μg/ml). However, the sialic acid concentration returned to baseline levels in both groups after 15 min.

When sialic acid concentration was adjusted by total protein in tears (μg/mg protein), it remained stable before and after the topical application of saline (Figure 2B). The concentration of sialic acid increased significantly in the diquafosol group at 5 min post-application.

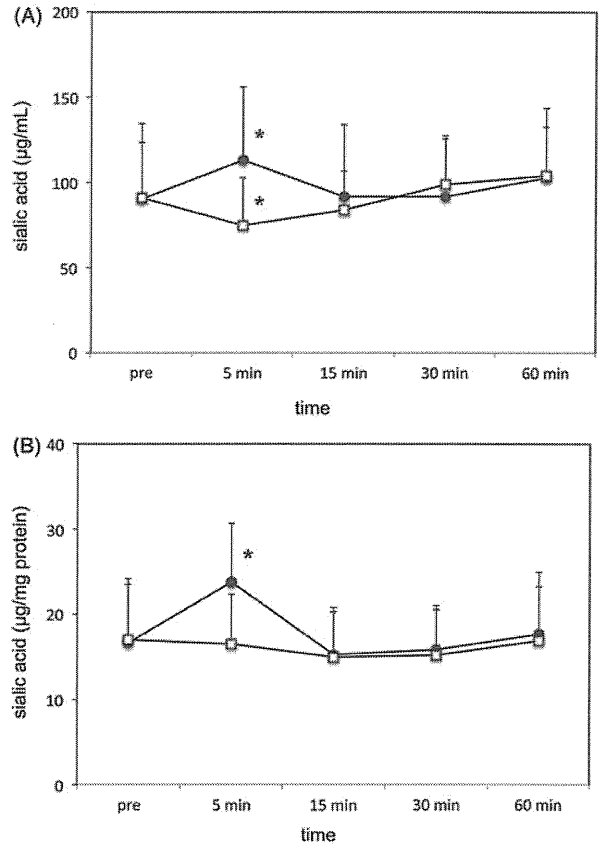

FIGURE 2 (A) Sialic acid concentration (μg/ml) in tears before and after the topical application of diquafosol (closed circle) and saline (open square). Compared with the baseline data, the concentration of sialic acid decreased significantly in the saline group and increased significantly in the diquafosol group at 5 min post-application. Sialic acid concentration returned to the baseline levels in both groups after 15 min. (B) Sialic acid concentration was adjusted by total protein in tears (μg/mg protein). It remained unchanged before and after the topical application of saline (open square). The concentration of sialic acid increased significantly in the diquafosol group (closed circle) at 5 min post-application. $^*p < 0.05$ (Wilcoxon signed-rank test).

DISCUSSION

In the present study, Schirmer strip wetting values at 1 min, which were used for tear sample collections as well as for tear volume estimation, were stable and similar in both the groups after topical application of diquafosol or saline. The result was rather unexpected because diquafosol is believed to promote the secretion of aqueous content in tear fluids. Diquafosol reportedly increased tear volume in normal healthy cats and rabbits.[2–4,14] The stimulatory effect of diquafosol on fluid secretion might be masked by increased tear excretion into the lachrymal ducts to prevent lacrimation in human subjects. Another possibility is that diquafosol does not elicit fluid secretion from the conjunctiva in human eyes with normal tear volume. Topical application of diquafosol to dry eye patients with decreased tear volumes might have a different result.

Previously, we reported that topical application of saline resulted in a transient decrease in protein concentration in the tears of healthy subjects.[15] The decrease was attributed to the washout and dilution effects caused by the application of eye drops. The results of the current study confirmed that a single drop of ophthalmic solution has a significant impact on tear protein concentration.

The protein constitution of tear fluids is known to be different from serum and extracellular fluids. The main components of tears include lactoferrin, lipocalin-1, lysozyme and sIgA, which are relatively tear-specific proteins.[16–18] Of the four major proteins in tears, lactoferrin, lipocalin-1 and lysozyme are products of acinar tissues in the lachrymal glands. sIgA is

produced by a combination of dimeric IgA from plasma cells and a secretory component synthesized in the ductal cells of lachrymal glands. Diquafosol is thought to promote fluid transport across the epithelial layer of the conjunctiva.[2–4] Fluid secreted from the conjunctival epithelium should not contain major tear proteins. Therefore, we expected to observe decreased levels of the four tear major proteins in the diquafosol treated group compared with the saline group. In the current study, however, compositional changes in tear proteins were similar in both groups. Shichijo and associates also reported that diquafosol had no effect on protein concentration in the tear fluid of normal rabbits, although the composition of tear proteins was not examined.[4] Thus, diquafosol might stimulate the secretion of fluids from the accessory lachrymal glands of Klause and Wolfring. Further studies are required to clarify the issue.

The most important finding in the current study is that a transient but significant increase of sialic acid concentration in tears was observed after diquafosol application. Sialic acid is considered to be a good marker for monitoring mucins in biological samples because it is often found in the nonreducing termini of mucous carbohydrate chains.[20,21] Ocular mucins are thought to play a crucial role in maintaining tear film stability. Therefore, they have gained considerable attention in current research on dry eye.[13,21–25] Compared with baseline data, the concentration of sialic acid significantly decreased in the saline group at 5 min. In contrast, the concentration of sialic acid significantly increased in the diquafosol group at 5 min after application. Therefore, the stimulatory effect of diquafosol on mucin secretion appears to be significant and can overcome the washout and dilution effects caused by eye drop instillation.

Diquafosol has been reported to stimulate the secretion of mucins, which mainly consist of MUC5AC, from the conjunctival tissue of rabbits.[5–7] Our results are in accordance with those of Shichijo et al.[6,7] We were not able to specify the type of mucins present, because we adopted sialic acid as a general marker of mucins. However, based on previous reports,[5–7] it is likely that secreted mucins are the major source of sialic acid in tears induced by diquafosol. Secreted mucins from the conjunctival goblet cells are thought to form a thick and loose "mucous blanket" and cover the ocular surface epithelia.[13,25] Although the increase of sialic acid in tears induced by diquafosol is transient, a "mucous blanket" formed by secreted mucins might remain on the ocular surface for a longer period and help to maintain tear film stability. A clinical study that showed significant improvement of BUT in dry eye patients when treated with diquafosol might support this hypothesis.[12] The distribution of secreted mucins in the tear film and their turnover are not fully understood. Further studies are planned to investigate the significance of the transient increase of mucins in tears induced by diquafosol.

CONCLUSIONS

In conclusion, the current study showed that a single application of saline and diquafosol resulted in a transient decrease of tear proteins possibly due to wash out and dilution effects of eye drop administration. In contrast, a significant increase of sialic acid was observed in tears after diquafosol application. Although the effect of diquafosol on sialic acid concentration in tears was transient, these results suggest that diquafosol can stimulate the secretion of mucins from the ocular tissues of healthy human subjects.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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