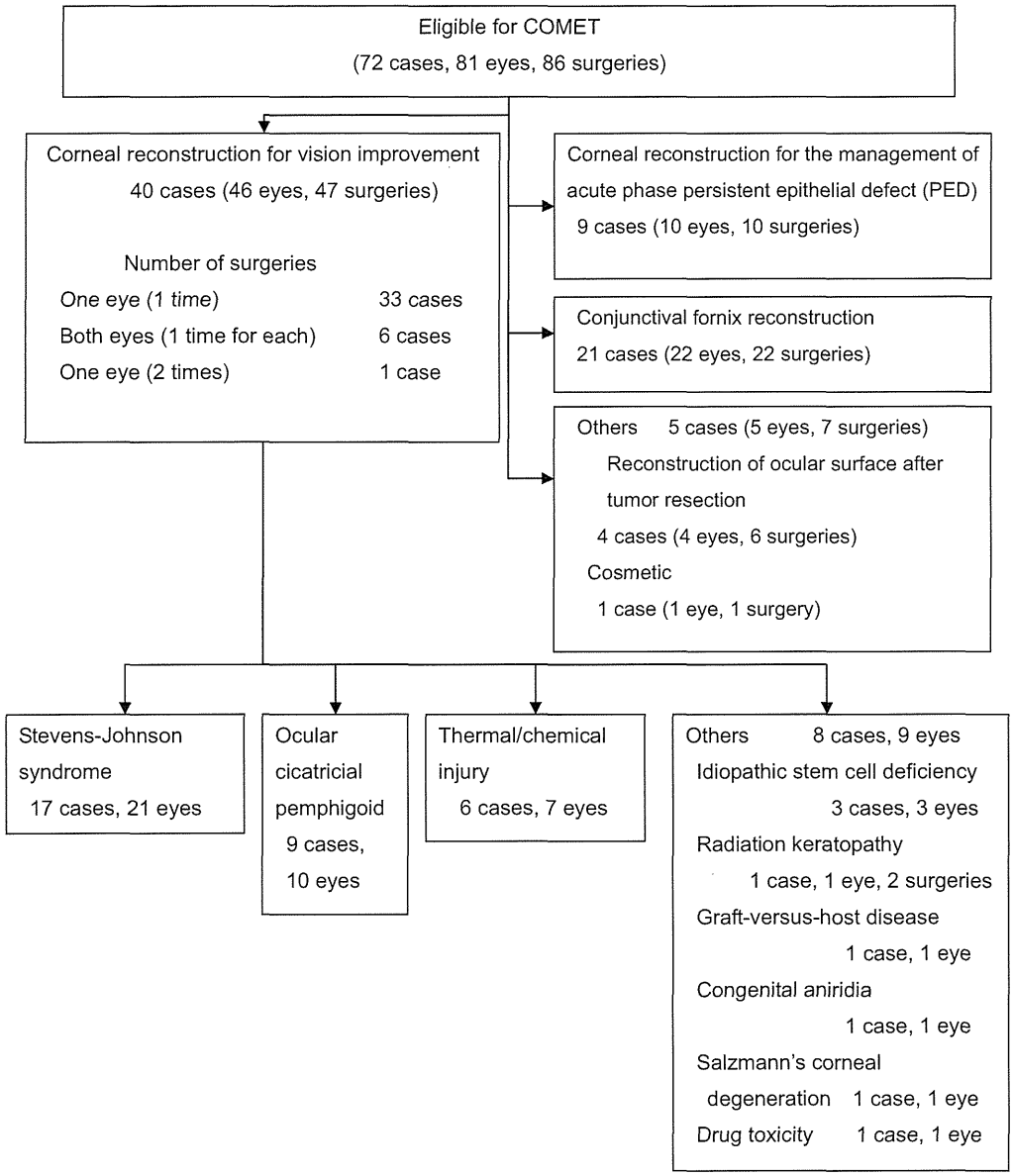

Figure 1. Diagram showing flow of study. Seventy-two patients (81 eyes) underwent cultivated oral mucosal epithelial sheet transplantation (COMET) between June 2002 and December 2008, and 40 patients (46 eyes) were analyzed for visual improvement in this study. Both corneal reconstruction and conjunctival fornix reconstruction were carried out in 3 cases, in the same eye in 1 case, and counted separately.

All statistical analyses were conducted at the Translational Research Informatics Center (Kobe, Japan) with the use of SAS software, version 9.1 (SAS Inc, Cary, NC) or JMP software, version 8.2 (SAS Inc). *P* values of less than 0.05 were considered statistically significant.

Results

Patient Characteristics

Between 2002 and 2008, 47 COMETs (46 eyes in 40 patients) were performed on 21 eyes with SJS, 10 eyes with OCP, 7 eyes with thermal or chemical injury, and 9 eyes with other causes of LSCD (Fig 1). Although 23 eyes (48.9%) previously had been treated with ocular surgery, all of these previous treatments had failed and recurrence of fibrovascular ingrowth on the cornea was observed. Of the 47 surgeries performed, symblepharon and keratinization of the cornea were present in 37 eyes (78.7%) and 10 eyes (21.3%), respectively, thus indicating that most of the eyes were inflicted with end-stage severe OSDs (Table 1).

Outcomes of Cultivated Oral Mucosal Epithelial Sheet Transplantation

Cultivated autologous oral mucosal epithelial sheets were generated successfully from all patients. In all patients, COMET was performed successfully and no epithelial damage was observed during surgery. Cultivated oral mucosal epithelial sheet transplantation was combined with amniotic membrane transplantation in 34 (72%) of the 47 surgeries and with cataract surgery in 11 eyes (23%; Table 2, available at http://aaojournal.org). In 10 patients

Table 1. Baseline Characteristics in Patients Who Underwent Autologous Cultivated Oral Mucosal Epithelial Transplantation

	Total	Stevens-Johnson Syndrome	Ocular Pemphigoid	Thermal/Chemical Injury	Others
No. of COMETs	47	21	10	7	9
Age (yrs)					
Median	57.0	43.0	73.5	50.0	34.0
Range	9–86	14–71	62–86	27–79	9–75
Duration of illness (yrs)					
Median	12.3	17.9	3.5	6.0	5.08
Range	0.3–40.0	3.0–38.0	0.3–15.0	0.5–24.0	0.5–40.0
Prior ocular surgery (%)	23 (48.9)	9 (42.9)	4 (40.0)	3 (42.9)	7 (77.8)
Planned 2-step operations (%)	10 (21.3)	2 (9.5)	0 (0)	6 (85.7)	2 (22.2)
Symblepharon (%)	37 (78.7)	18 (85.7)	10 (100.0)	6 (85.7)	3 (33.3)
Keratinization (%)	10 (21.3)	8 (38.1)	1 (10.0)	0 (0)	1 (11.1)
Preoperative visual acuity*					
Median	2.40	2.4	2.70	2.70	2.40
Range	1.11–3.00	1.40–3.00	1.52–2.70	1.22–2.70	1.10–2.70
Preoperative ocular surface grading score					
Median	14.0	15.0	17.0	13.0	8.0
Range	5.0–21.0	8.0–21.0	10.0–21.0	7.0–17.0	5.0–19.0

COMET = autologous cultivated oral mucosal epithelial transplantation.
*Logarithm of the minimum angle of resolution units.

with severe corneal stromal opacity, a 2-step surgical approach was planned, with COMET followed by penetrating keratoplasty or deep lamellar keratoplasty.[25] Three patients underwent the second surgery before the 24th postoperative week and 5 patients underwent the surgery after the 24th week, but 2 patients did not undergo the second surgery during the study period.

The median preoperative logMAR BCVA was 2.40, and in 31 of the eyes (66%), visual acuity was poorer than 20/2000 (<0.01, logMAR >2). The median preoperative ocular surface grading score was 18.0 (range, 5 to 21). The median patient follow-up period with observation of the primary outcome was 28.7 months after transplantation (range, 6.2 to 85.6 months). Because of heterogeneous etiologic mechanisms, the outcomes in each category are described separately.

Disease-Specific Outcomes

Stevens-Johnson Syndrome. Seventeen patients with SJS underwent COMET (Table 2, available at http://aaojournal.org). The BCVA improved significantly at 4, 12, and 24 weeks after surgery ($P = 0.0005$, $P = 0.0010$, and $P = 0.0117$, respectively; Fig 2A). The ocular surface grading score also improved significantly at 4, 12, and 24 weeks after surgery ($P<0.0001$ for each time point; Fig 2B).

Ocular Cicatricial Pemphigoid. Nine patients (10 eyes) with OCP underwent COMET (Table 1). All 9 patients were older than 60 years, older than many of the patients in this study with other diseases (Table 2, available at http://aaojournal.org). The BCVA was improved significantly at the 4th postoperative week ($P = 0.0156$), but this improvement later disappeared (Fig 2A). In contrast, improvement of the ocular surface grading score was sustained throughout the follow-up period ($P = 0.0020$, $P = 0.0020$, and $P = 0.0078$, respectively; Fig 2B).

Thermal or Chemical Injury. Seven patients (7 eyes) with thermal or chemical injury underwent COMET. Their BCVA did not change until the 24th postoperative week; however, the ocular surface grading score in all 7 patients improved significantly ($P = 0.0156$ for each visit; Fig 2A, B). Although penetrating keratoplasty or deep lamellar keratoplasty surgery was planned for 6 of these 7 patients, only 2 patients underwent this second surgery before the 24th postoperative week visit. Both the BCVA and ocular surface score improved in all 7 patients after the planned surgeries were performed.

Others. Eight other patients underwent COMET: 3 with idiopathic stem cell deficiency, 1 with radiation keratopathy, 1 with graft-versus-host disease, 1 with congenital aniridia, 1 with Salzmanns corneal degeneration, and 1 with drug-toxicity–induced LSCD. In 6 of these 8 patients, BCVA was improved significantly; however, no improvement was seen in 2 of these patients (Table 2, available at http://aaojournal.org; Fig 2A). The 2 patients with no improvement had severe dryness on the ocular surface and had the highest ocular surface grading score in this group. In addition, severe lagophthalmos was present in the 1 patient with radiation keratopathy because of severe lid scarring after irradiation for retinoblastoma. One other patient with graft-versus-host disease had longstanding inflammation on the ocular surface. In both of these 2 cases, keratinization and symblepharon progressed gradually after COMET. Six patients who demonstrated improvement had a low preoperative ocular surface grading score, yet this score was improved considerably in all patients at the 24th postoperative week (Table 2; Fig 2B).

Critical Visual Improvement Rate

The critical visual improvement rate for SJS, OCP, and thermal or chemical injury was 50.0% (7/14), 42.9% (3/7), and 20.0% (1/5), respectively, although the second planned surgery[25] (penetrating or deep lamellar keratoplasty) had yet to be carried out at the 24th postoperative week in 7 of 10 eyes. The clinical observations on both preoperative and postoperative anterior segment slit-lamp photographs are shown in Figure 3 (available at http://aaojournal.org). All patients demonstrated an improvement in their BVCA to 0.01 or more, from a baseline condition of vision loss.

Factors Influencing Visual Improvement

Multivariate stepwise logistic regression analysis was used to estimate the factors influencing postoperative visual acuity after COMET, and the following factors were chosen as variables:

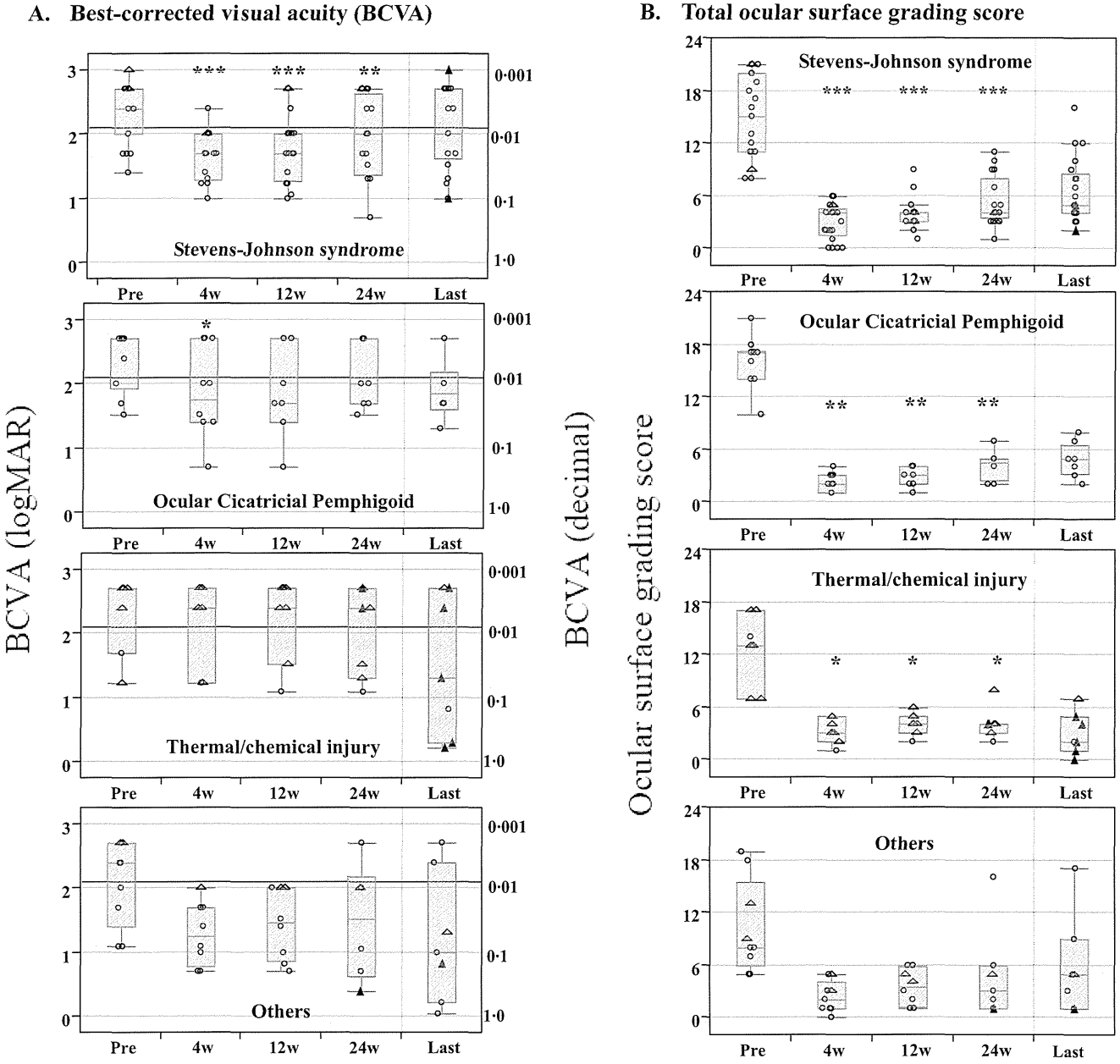

Figure 2. Graphs showing preoperative (Pre) and postoperative clinical outcomes. **A**, Best-corrected visual acuity (BCVA). The BCVA values for each patient are shown grouped according to the cause of corneal dysfunction: Stevens-Johnson syndrome (SJS), ocular cicatricial pemphigoid (OCP), thermal or chemical injury, and others. The change in BCVA from baseline at each visit, except for the last visit, was analyzed using the Wilcoxon signed-rank test in each disease category (SJS, OCP, thermal or chemical injury) except others. Open circles represent cases treated with autologous cultivated oral mucosal epithelial transplantation (COMET) only. Triangles represent cases treated with a planned 2-step surgical combination of COMET followed by penetrating keratoplasty (PK) or deep lamellar keratoplasty (DLKP). Open triangles are before the second operation, and closed triangles are after the second operation. The horizontal line within each box represents the median value, the bottom and top lines of the box represent the 25th and 75th percentiles, respectively, and the horizontal lines below and above the box represent the lowest and highest values, respectively (or are located 1.5 times the interquartile range away from the box). $*P<0.05$, $**P<0.01$, $***P<0.001$ (2-sided). **B**, Total ocular surface grading score. Ocular surface grading scores for each patient were calculated and are shown according to each cause of corneal dysfunction: SJS, OCP, thermal or chemical injury, and others. Scores for 8 components of the ocular surface were calculated by the grading system. The total scores before surgery and at the 4th, 12th, and 24th postoperative weeks and at last follow-up examination were calculated. Open circles represent patients treated with COMET only. Triangles represent patients treated with a planned 2-step surgical combination of COMET followed by PK or DLKP. Open triangles are before the second operation, and closed triangles are after the second operation. The change in ocular surface grading score from baseline at each visit, except for the last visit, was analyzed using the Wilcoxon signed-rank test in each disease category (SJS, OCP, thermal or chemical injury) except others. The horizontal line within each box represents the median value, the bottom and top lines of the box represent the 24th and 75th percentiles, respectively, and the horizontal lines below and above the box represent the lowest and highest values, respectively (or are located 1.5 times the interquartile range away from the box). $*P<0.05$, $**P<0.01$, $***P<0.001$ (2-sided). w = weeks.

Table 3. Summary of Adverse Events in Patients Who Underwent Autologous Cultivated Oral Mucosal Epithelial Transplantation

Event	Total	Disease Category			
		Stevens-Johnson Syndrome	*Ocular Cicatricial Pemphigoid*	*Thermal or Chemical Injury*	*Others*
Hepatic dysfunction	1	1			
Drug-induced allergy	1				1
Persistent epithelial defect	16	10	3	2	1
Corneal stromal melting after the epithelial defect	2		1		1
Ocular infection (keratitis, endophthalmitis)	2	2			
Infiltration	3	2	1		
Elevation of IOP resulting from steroid use	4		1	2	1

IOP = intraocular pressure.
No life-threatening serious adverse events were observed.

disease category, patient age, 2-step surgery, combination with amniotic membrane transplantation, combination with cataract surgery, preoperative logMAR BCVA, and the 8 components of the ocular surface grading system. Corneal neovascularization and symblepharon were found to be correlated significantly with logMAR improvement at the 24th postoperative week ($P = 0.0023$ and $P = 0.0173$, respectively). Visual prognosis was better in the eyes with slight symblepharon than in the eyes with severe symblepharon. In contrast, it was better in the eyes with severe neovascularization than in the eyes with slight neovascularization.

Adverse Events

A summary of the adverse events in the 40 patients who underwent COMET is shown in Table 3. No life-threatening serious adverse events were observed in any of the transplantations. Systemically, moderate liver dysfunction occurred in 1 patient (2.5%; 95% confidential interval [CI], 0.1 to 13.2), but liver function normalized after the discontinuationof systemic drugs.

Postoperative persistent epithelial defects occurred in the eyes of 16 (40.0%) of the 40 patients (95% CI, 24.9 to 56.7), and rather frequently in the SJS eyes (60.0% of SJS patients). Corneal stromal melting after the epithelial defect occurred in 2 patients (5.0%; 95% CI, 0.6 to 16.9), but neither eye became perforated. All of these patients were treated successfully. Slight to moderate corneal infection occurred in 2 patients (5.0%; 95% CI, 0.6 to 16.9); however, both patients healed without scarring. A suspected infection with cell infiltration on the cornea[28] occurred in 3 patients, yet in each patient, it healed within 1 week after receiving a topical instillation of antibiotics. Although a slight elevation of intraocular pressure resulting from steroid use was seen in 4 patients (10.0%; 95% CI, 2.8 to 23.7), this returned to the normal range after reduction of the steroid dose. None of the patients required glaucoma surgery.

Discussion

Severe OSD has proven to be one of the most difficult disorders to treat, and for many patients, vision loss is the end result.[29,31] Keratoprosthesis surgery is one possible way to obtain visual improvement in end-stage severe OSDs; however, serious complications such as endophthalmitis, glaucoma, and tissue melting can arise, especially in SJS or OCP, and can lead to permanent vision loss.[32,33]

At the beginning of 2002, the authors performed ocular surface reconstruction using tissue-engineered autologous oral mucosal epithelial sheets for the first time.[23] In a report of the initial results from the first 12 cases, the successful long-term engraftment of cultivated oral mucosal cells and their transparency was confirmed.[24] Since then, COMET has been used to treat OSD patients, with careful consideration of the surgical indications.[24–26,34] The authors performed 86 COMET operations between 2002 and the end of 2008 for visual improvement, epithelialization of persistent epithelial defects, or conjunctival reconstruction (Fig 1).

In this study, the clinical efficacy and safety of 47 COMETs were evaluated for visual improvement. In 23 eyes (48.9%), previous ocular surgery such as corneal transplantation or amniotic membrane transplantation already had been carried out unsuccessfully at other hospitals. Symblepharon was involved in 37 eyes (78.7%) and keratinization was involved in 10 eyes (21.3%). Symblepharon indicates conjunctival involvement, and pathologic keratinization means that the eye is at the end stage of a severe OSD with chronic inflammation.[3,35] Most of these eyes had severe tear deficiency, which is an important prognostic parameter for surgical outcome.[36] Although such eyes commonly are considered to have contraindications for ocular surface reconstruction, COMET offered substantial visual improvement even for patients with such advanced disease.

In more than half of the eyes, preoperative visual acuity was limited to counting fingers or hand movements. It is striking that such patients were able to come to the hospital without assistance during the 24 weeks after undergoing COMET. For this reason, critical visual improvement rate is proposed as a clear end point for measuring surgical outcome. Considering that most of the eyes in this study were at the end stage of a severe OSD, these results are very favorable and encouraging.

In this study, the preoperative ocular surface grading score was higher (more diseased) in patients with SJS and OCP than in those with thermal or chemical injuries or other

diseases. It should be noted that visual improvement was statistically significant in SJS. In contrast, visual acuity was not improved at the 24th postoperative week in patients with thermal or chemical injury, despite the improvement in total ocular surface grading score. The corneal stroma was damaged severely in most cases of thermal or chemical injury, and such patients obtained visual improvement after undergoing the planned second surgery with penetrating keratoplasty or deep lamellar keratoplasty. In general, the prognosis of penetrating or deep lamellar keratoplasty alone for severe OSDs is very poor.[2] However, the findings of this study show that patients with severe OSDs with corneal stromal opacity can obtain visual improvement after undergoing the surgical combination of COMET and penetrating or deep lamellar keratoplasty.

Best-corrected visual acuity was not improved at the 24th postoperative week in patients with OCP, despite significant improvement of the ocular surface grading score. Because OCP is a progressive autoimmune disease, pathologic keratinization or thickening of the epithelium occurred readily after COMET, thus disrupting visual acuity.

No serious systemic complications occurred in any of the patients. The incidence of postoperative persistent epithelial defects was relatively high, yet still similar to or lower than that reported with other therapies.[6,36–38] Considering that corneal perforation is a common complication after corneal reconstruction in severe OSDs,[38–40] it is noteworthy that no perforation occurred and that none of the eyes demonstrated vision loss after COMET. Ocular surface reconstruction with a combination of COMET and amniotic membrane transplantation was needed to achieve the total replacement of cicatrized tissue. Because cultured epithelial cells on amniotic membrane attach to a basement membrane with hemidesmosomes,[22] these cells can avoid being dropped off and actually survive, regardless of an unstable tear film and the mechanical trauma of blinking. When used as the substrate for oral mucosal cells, amniotic membrane may play a role in protecting the cornea from melting.

Multivariate stepwise logistic regression analysis showed that symblepharon and neovascularization are prognostic factors for visual improvement. Although disease-specific outcomes showed different patterns as described above, disease category was not related to visual prognosis. However, the sample size may be too small to perform such subgroup analyses. Multivariate stepwise logistic regression analysis also was performed for all 86 surgeries to determine the factors influencing persistent epithelial defects. Having SJS and a very low tear meniscus were the prognostic factors for persistent epithelial defects ($P = 0.0204$ and $P = 0.0388$, respectively). Thus, it is likely that both the disease category and dryness of the eye influenced the prognosis.

Long-term ocular surface appearance was examined in 17 of the 72 patients with a follow-up of more than 3 years.[34] No further surgery was carried out in these patients. The ocular surface in each case became stable from 6 months after COMET, with a gradual reduction in corneal neovascularization,[34] as others have reported in similar studies.[40] Moreover, postoperative invasion of conjunctival tissue and symblepharon formation was inhibited significantly for more than 3 years.[34] Deep lamellar or penetrating keratoplasty was performed for the patients with corneal stromal opacity after the stabilization of the ocular surface (as the second step of a 2-step surgical combination), in most cases from 24 weeks after COMET.

After COMET, upper or lower eyelid cicatricial entropion with various degrees of tarsal-plate atrophy sometimes was found. In cases with an eyelid abnormality, eyelid surgery was performed to correct entropion, trichiasis, or lagophthalmos. Eyelid condition is an important factor for maintaining ocular surface stability, as well as for avoiding complications such as infection or persistent epithelial defects.

In conclusion, the findings of this retrospective study showed that long-term visual improvement can be obtained in end-stage severe OSDs with complete LSCD and that COMET offered substantial visual improvement even for patients with severe tear deficiency. The findings also showed that patients with corneal blindness resulting from severe OSDs such as SJS benefited from critical improvement of visual acuity.

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Footnotes and Financial Disclosures

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Expression of prostaglandin E receptor subtype EP4 in conjunctival epithelium of patients with ocular surface disorders: case-control study

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ABSTRACT

Objectives: To confirm the downregulation of PTGER4 mRNA in the conjunctiva of Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) and ocular cicatricial pemphigoid (OCP) patients and to examine the expression of its EP4 protein in the conjunctival epithelium of patients with various ocular surface disorders.

Design: Case-control study.

Setting and participants: We performed quantitative reverse transcription-PCR (RT-PCR) analysis of *PTGER4* mRNA in conjunctival tissue sections from patients with SJS/TEN and OCP to confirm the downregulation of *PTGER4* mRNA expression. We also analysed EP4 immunohistologically in other ocular surface disorders. Conjunctival tissues were obtained from patients undergoing surgical reconstruction of the ocular surface due to chemical eye burns, subacute SJS/TEN or chronic SJS/TEN, chronic OCP, severe graft versus host disease (GVHD) and from patients with Mooren's ulcers treated by resection of the inflammatory conjunctiva.

Primary and secondary outcome measures: The expression of *PTGER4* mRNA and EP4 protein assessed by quantitative RT-PCR assay and immunohistological methods.

Results: *PTGER4* mRNA was significantly lower in conjunctival tissues from SJS and OCP patients than in the control conjunctivochalasis samples. EP4 protein was detected in conjunctival epithelium from patients with chemical eye burn and in control conjunctival epithelium from patients with conjunctivochalasis. Its expression varied in conjunctival epithelium from patients with Mooren's ulcer. We did not detect EP4 immunoreactivity in conjunctival epithelium from patients with subacute SJS/TEN, severe GVHD, chronic SJS/TEN or OCP.

Conclusions: The strong downregulation of EP4 expression in conjunctival epithelium from patients with OCP or SJS/TEN may be attributable to ocular surface inflammation.

ARTICLE SUMMARY

Article focus

- We previously reported that EP4 protein was down-regulated in devastating ocular surface inflammatory disorders such as chronic Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) and chronic ocular cicatricial pemphigoid (OCP). Article focus of this study are to confirm the downregulation of *PTGER4* mRNA, which protein is EP4, in the conjunctiva of SJS/TEN and OCP patients and to examine the expression of its EP4 protein in the conjunctival epithelium of patients with other various ocular surface disorders in addition chronic SJS/TEN and OCP.

Key messages

- EP4 is expressed not only in normal conjunctival epithelium but also in conjunctival epithelium from patients with chemical eye burns and some patients with Mooren's ulcer. On the contrary, it is strongly downregulated in conjunctival epithelium from patients with OCP and chronic SJS/TEN and subacute SJS/TEN.

Strengths and limitations of this study

- The function of EP4 in conjunctival epithelial cells is not elucidated.

INTRODUCTION

The prostanoids PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2 and TXA_2 are lipid mediators that form in response to various stimuli. They are released extracellularly immediately after their synthesis and they act by binding to a G protein-coupled rhodopsin-type receptor on the surface of target cells.[1] PGE_2 is produced during inflammatory responses and it suppresses the production of cytokines and chemokines induced by lipopolysaccharide-stimulated macrophages[2 3] and dendritic cells.[4] Elsewhere we reported that PGE_2 modulates the expression of polyI:C-induced proinflammatory genes in human conjunctival epithelial cells.[5]

There are four PGE receptor subtypes, EP1, EP2, EP3 and EP4. The intestinal epithelium has been reported to express EP4 mRNA,[6] and intestinal homeostasis was

maintained and the immune response downregulated by EP4.[7] The ocular surface is also one of the mucosa that is in contact with commensal bacteria like the intestine. Therefore, we focused on the expression of EP4 in human conjunctival epithelium and the difference of its expression between various ocular surface diseases.

We documented that while normal human conjunctival epithelium expressed EP4 protein, it was down-regulated in devastating ocular surface inflammatory disorders such as chronic Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) and chronic ocular cicatricial pemphigoid (OCP).[8] Here we examined the mRNA expression of *PTGER4*, which is the gene of EP4 protein, in the conjunctiva of SJS/TEN and OCP patients in the chronic stage to confirm that *PTGER4* mRNA EP4 is down-regulated in their conjunctiva. We also examined the expression of *PTGER4* mRNA protein in the conjunctival epithelium of patients with various ocular surface disorders such as chemical eye burn, Mooren's ulcer, severe graft versus host disease (GVHD) and of patients in the subacute stage of SJS/TEN.

MATERIALS AND METHODS

Human conjunctival tissues

This study was approved by the Institutional Review Board of Kyoto Prefectural University of Medicine, Kyoto, Japan. All experiments were conducted in accordance with the principles set forth in the Helsinki Declaration.

For quantitative reverse transcription-PCR (RT-PCR) the controls were nearly normal conjunctival tissues obtained at surgery for conjunctivochalasis, a disease in which the conjunctiva relaxes due to aging, resulting in a foreign body sensation on the ocular surface. We also prepared human conjunctival tissues from samples obtained during surgery to reconstruct the ocular surface in four patients in the chronic stage of SJS/TEN and four patients in the chronic stage of OCP.

The controls for immunohistochemical analyses were nearly normal conjunctival tissues obtained during surgery for conjunctivochalasis. We also prepared human conjunctival tissues from samples obtained during surgery to reconstruct the ocular surface in three patients with chemical (alkali) eye burn (two in the chronic stage and one in the subacute stage), two patients with subacute SJS/TEN, one patient with severe GVHD and from four patients with Mooren's ulcer undergoing resection of inflammatory conjunctiva. SJS/TEN, OCP, Mooren's ulcer, chemical burn and GVHD are all ocular surface inflammatory diseases with persistent inflammation on the ocular surface not only in the acute stage but also in the chronic stage.

Quantitative RT-PCR

Total RNA was isolated from conjunctival tissue sections using the RNeasy mini kit (Qiagen, Valencia, California, USA) according to the manufacturer's instructions. The RT reaction was with the SuperScript preamplification kit (Invitrogen, Carlsbad, California, USA). Quantitative RT-PCR was on an ABI-prism 7700 instrument (Applied Biosystems, Foster City, California, USA). The probes for human PTGER4 and human GAPDH were from Applied Biosystems. For cDNA amplification we performed PCR in a 25 µl total volume that contained a 1 µl cDNA template in 2×TaqMan universal PCR master mix (Applied Biosystems) at 50°C for 2 min and 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. The results were analysed with sequence detection software (Applied Biosystems). The quantification data were normalised to the expression of the housekeeping gene GAPDH.

Immunohistochemistry

For EP4 staining we used rabbit polyclonal antibody to EP4 (Cayman Chemical Co, Ann Arbor, Michigan, USA). The secondary antibody (Biotin-SP-conjugated AffiniPure $F(ab')_2$ fragment donkey antirabbit IgG (H+L), 1 : 500 dilution; Jackson Immuno Research, Baltimore, Maryland, USA) was applied for 30 min. The VECTASTAIN ABC reagent (Vector Laboratories, Inc, Burlingame, California, USA) was used for increased sensitivity with peroxidase substrate solution (DAB substrate kit; Vector) as a chromogenic substrate.

Data analysis

Data were expressed as the mean±SEM and evaluated by the Student's t test using the Microsoft Excel software program.

RESULTS

We previously documented that EP4 protein expression was down-regulated in conjunctival epithelium of devastating ocular surface inflammatory disorders such as chronic SJS/TEN and chronic OCP.[8] In this study, to confirm the down-regulation of EP4 in the ocular surface of SJS/TEN and OCP patients we examined the expression of *PTGER4* mRNA in control conjunctival tissues from six conjunctival chalasis patients and in conjunctival tissues from four SJS/TEN patients and four OCP patients. Representative findings of EP4 immunoreactivity in each of these groups are shown in figure 1A. Although EP4 protein was detected in the control tissues, conjunctival epithelium from SJS patients and OCP patients did not manifest EP4 immunoreactivity. *PTGER4* mRNA was significantly lower in conjunctival tissues from SJS/TEN and OCP patients than in the control conjunctivochalasis samples (figure 1B).

Moreover, we examined the expression of EP4 protein in the conjunctival epithelium of patients with other various ocular surface disorders. EP4 protein was detected in nearly normal conjunctival epithelium from patients with conjunctivochalasis (figure 2A) and in conjunctival tissues from three patients with chemical eye burn (figure 2B). Its expression varied in conjunctival epithelium from four patients with Mooren's ulcer (figure 2C): in one patient is was similar to the control,

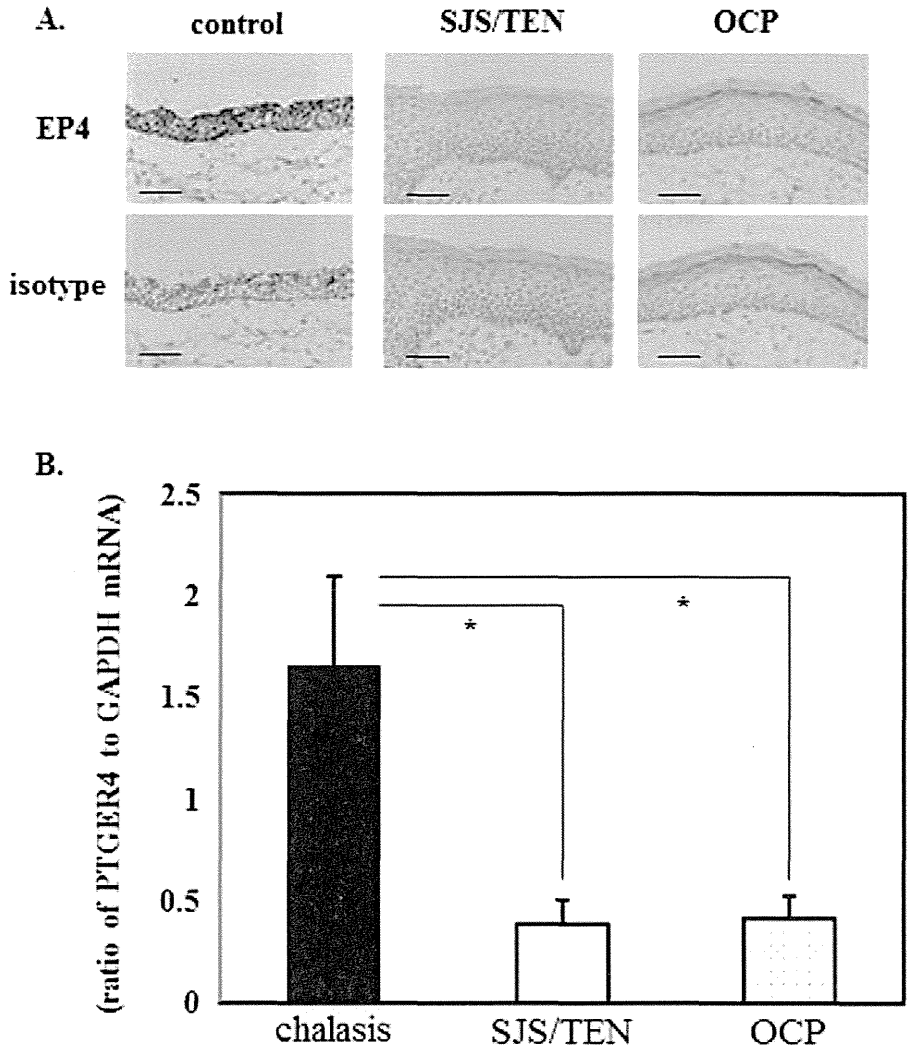

Figure 1 The expression of *PTGER4* mRNA in conjunctival tissues from patients with Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN), ocular cicatricial pemphigoid (OCP) and the controls. (A) Representative findings of EP4 immunoreactivity in each group (control, SJS/TEN, OCP). (B) Expression of *PTGER4* mRNA in human conjunctival tissues (*p<0.05).

in two it was slightly lower than in the control and in the remaining patient it was not detected. There was no EP4 immunoreactivity in conjunctival epithelium from two patients with subacute SJS/TEN (figure 2D), a patient with severe GVHD (figure 2E) as same as patients with chronic SJS/TEN or OCP.[8]

We found that, as in normal human conjunctival epithelium, EP4 is expressed in conjunctival epithelium from patients with chemical eye burn. On the other hand, EP4 immunoreactivity was not detected in conjunctival epithelium from patients with SJS/TEN, OCP or severe GVHD. We did not detect EP4 protein in cells infiltrating subconjunctival tissues in any of the human conjunctival tissues we examined.

DISCUSSION

Elsewhere we reported the expression of EP4 in normal human conjunctival epithelium and its down-regulation in conjunctival epithelium from patients with SJS/TEN and OCP.[8] Here we confirmed that in conjunctival tissues from SJS/TEN and OCP patients its mRNA expression was significantly down-regulated, and we also document that EP4 is expressed normally in conjunctival epithelium from patients with severe chemical eye burn which, like SJS/TEN and OCP, is a devastating ocular surface disorder.

On the ocular surface of patients with severe chemical eye burn, conjunctival invasion into the cornea may occur due to the stem cell deficiency of corneal epithelial cells. This results in devastating ocular surface disorders similar to OCP and SJS/TEN. However, in the conjunctiva of patients with severe chemical eye burns, EP4 expression was not down-regulated.

In patients with Mooren's ulcer, an ocular surface inflammatory disease, the expression of EP4 protein varied; in some patients it was down-regulated. In patients in the subacute stage of SJS/TEN with ocular surface inflammation, the expression of EP4 protein was remarkably down-regulated.

Our results suggest that it is possible that EP4 in conjunctival epithelium might contribute the ocular surface homeostasis, while the EP4 may not necessarily be down-regulated in all devastating ocular surface disorders.

Kabashima *et al*[7] reported that in mice, EP4 deficiency impaired mucosal barrier function and induced

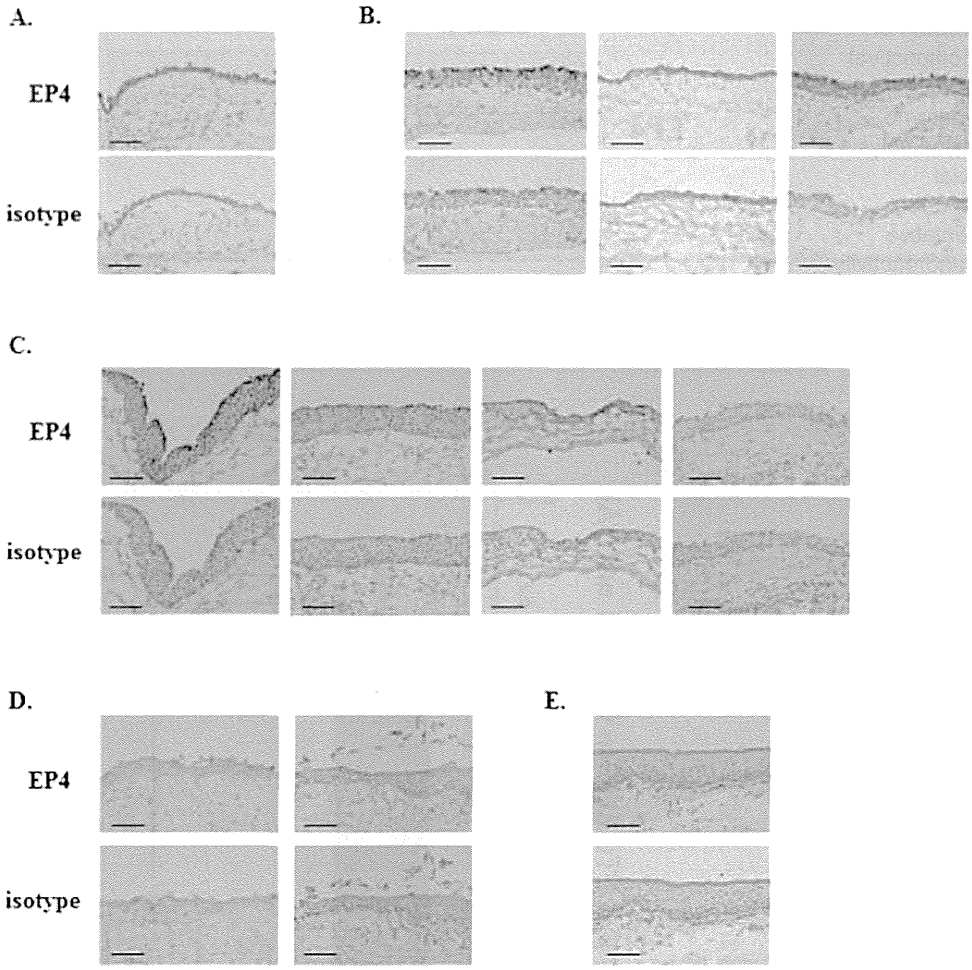

Figure 2 Immunohistological analysis of prostaglandin E receptor subtype EP4 in conjunctival epithelium of patients with ocular surface diseases. (A) Nearly normal conjunctival tissues from patients with conjunctivochalasis. (B) Conjunctival tissues from patients with chemical eye burn requiring ocular surface reconstruction. (C) Inflammatory conjunctival tissues from patients with active Mooren's ulcer requiring resection of the inflammatory conjunctiva. (D) Conjunctival tissues from Stevens-Johnson syndrome/toxic epidermal necrolysis patients in the subacute stage. (E) Conjunctival tissues from a patient with severe graft versus host disease. Each scale bar represents 100 μm.

the aggregation of lymphocytes and neutrophils in the colon, and that the administration of an EP4-selective agonist to wild-type mice ameliorated severe colitis. In mice treated with an EP4-selective antagonist the recovery from colitis was suppressed, leading them to conclude that EP4 maintains intestinal homeostasis by preserving mucosal integrity and down-regulating the immune response. On the other hand, Yao *et al*[9] found that PGE_2 acting on its receptor EP4 on T cells and dendritic cells not only facilitated T helper 1 (T_H1) cell differentiation but also amplified interleukin-23-mediated T_H17-cell expansion in vitro. The administration of an EP4-selective antagonist to mice with experimental autoimmune encephalomyelitis or contact hypersensitivity decreased the accumulation of both T_H1 and T_H17 cells in regional lymph nodes and suppressed disease progression. Based on these observations they concluded that PGE_2-EP4 signalling promotes immune inflammation.

In human conjunctival tissues EP4 protein was expressed in epithelial cells but not in cells infiltrating subconjunctival tissues. We posit that the down-regulation of EP4 in conjunctival epithelium is associated with the ocular surface inflammation seen in patients with OCP, SJS/TEN and Mooren's ulcer.

On the other hand, elsewhere we reported that although EP3 and EP2 agonists suppressed the production of CCL5, CXCL11 and CCL20 in response to polyI:C stimulation, these chemokines were not suppressed by the EP4 agonist in human conjunctival epithelial cells.[5] Studies are underway in our laboratory to elucidate the function of EP4 in conjunctival epithelial cells.

In summary, EP4 is expressed not only in normal conjunctival epithelium but also in conjunctival epithelium from patients with chemical eye burns and some patients with Mooren's ulcer. On the other hand, it is strongly down-regulated in conjunctival epithelium from patients with OCP and chronic SJS/TEN and subacute SJS/TEN.