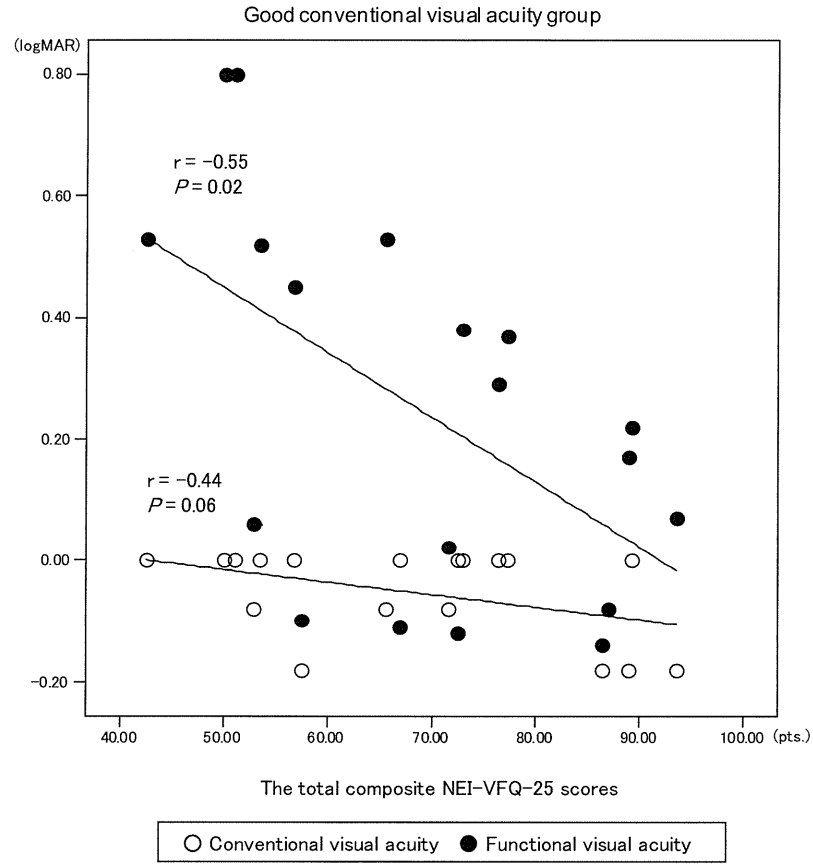


FIGURE 7. Correlations between visual function and the total composite NEI VFQ-25 scores in the good conventional visual acuity group of Stevens-Johnson syndrome patients. logMAR = logarithm of minimal angle of resolution.

observed between the composite NEI VFQ-25 scores and logMAR Landolt functional visual acuities in the good conventional visual acuity group (r = 0.55, P = .02), while no correlation was observed between the composite NEI VFQ-25 scores and logMAR Landolt conventional visual acuities in this group (r = 0.44, P = .06) (Figure 7).

• CORRELATION BETWEEN NEI VFQ-25 SCORES AND CLINICAL FINDINGS: Table 5 shows the correlation between ocular complications and the total composite NEI VFQ-25 scores in SJS patients with aqueous tear deficiency and SS patients. Strong significant correlations were observed between total ocular surface grading score and the composite NEI VFQ-25 scores in SJS patients with aqueous tear deficiency and SS patients (Table 5), and similarly in SJS patients without aqueous tear deficiency (r = 0.51, P = .002) (data not shown).

DISCUSSION

SEVERE OCULAR SURFACE DISEASE ASSOCIATED WITH SJS has been reported to cause visual deterioration. However, quantifying visual acuity in SJS patients has not been assessed, although an interest in the quantitative interpretation of visual function has been rising over the last few years, especially in the fields of refractive surgery, cataract, and dry eyes, through analyses by contrast sensitivity, contrast visual acuity, and wavefront analysis.[9–19] In this report, we measured the functional visual acuity in addition to conventional visual acuity testing and evaluated the relations between visual functions, ocular surface clinical findings, and the vision-related quality of life in SJS patients. We chose functional visual acuity testing for the assessment of the visual function, which has been shown to be efficient in the detection of "masked impairment of visual function" in dry eye patients, since SJS is known to be associated with severe dry eyes.[20–24]

Visual function testing revealed several interesting findings. First, visual acuities measured by conventional Landolt visual acuity testing were low in the SJS patients as compared with SS patients and normal subjects. When we focused on the visual function of the SS patients and normal subjects, the functional visual acuity scores in the SS patients were significantly lower than in the normal subjects, although there were no differences in the conventional visual acuities. In addition, the mean visual maintenance ratios in the SJS patients were significantly lower than in the SS patients, indicating that ability to

maintain the best visual acuity in SJS patients had deteriorated more than in the SS patients.

The functional visual acuity examination has been shown to be useful for the assessment of visual function related to dry eyes in our previous reports.[20–22] A previous report has also suggested the possibility that the functional visual acuity examination might reflect the effect of ocular surface findings and dry eye states on visual functions.[8] In this report, we analyzed the relation of ocular surface findings with visual function and quality of life in detail by grading the severity of ocular surface findings. The clinical severity scores of the examined ocular surface findings were much higher in the SJS patients. We analyzed whether visual disturbance and quality of life were similarly affected in SJS and SS patients without corneal complications or only with minimal corneal complications. Interestingly, we observed that visual function and quality of life were deteriorated in SJS patients with minimal corneal complications compared with SS patients. Moreover, we noted more visual dysfunction and declined quality of life in SJS patients with similar aqueous tear deficiency compared to SS patients. According to the multiple linear regression analysis, neovascularization, opacification, and keratinization involving the optical axis appeared to have a significant effect on the logMAR conventional visual acuities. SPK, symblepharon, and conjunctivalization also had a significant effect on the logMAR functional visual acuities.

Our findings that a stronger correlation existed between ocular surface grading score and logMAR functional visual acuity compared to logMAR conventional visual acuity suggest that functional visual acuity testing can indeed reflect the effect of clinical complications of ocular surface disease on visual function in SJS. In the correlations between visual function and ocular surface grading scores in the good, intermediate, and poor conventional visual acuity groups in SJS patients, a strong positive significant correlation was observed between total ocular surface grading scores and logMAR Landolt functional visual acuities in the good conventional visual acuity group and intermediate conventional visual acuity group, while no correlation was observed between total ocular surface grading scores and logMAR Landolt conventional visual acuities in these groups. These results suggest that functional visual acuity reflects the effect of ocular surface complications on visual function more sensitively in SJS patients with good and intermediate conventional visual acuities. The strong correlation of logMAR functional visual acuity with ocular surface grading score also suggests that functional visual acuity may be detecting the effect of ocular surface disease severity on other visual functions such as contrast, glare, or higher-order aberrations (compared to conventional visual acuity testing), which needs to be investigated in future studies employing the above-mentioned methodologies in conjunction with functional visual acuity testing.

The mean of all VFQ-25 subscale scores was remarkably worse in the SJS patients compared to normal subjects. Likewise, the mean of all subscale scores in SS patients was significantly lower than in the normal subjects. When analyzed in detail, only the subscale scores of "general health" and "ocular pain" were worse, without marked changes in other subscale scores. In SJS patients, as compared with normal subjects, all VFQ-25 subscale scores, especially "ocular pain," "near activities," "distance activities," "mental health," "role difficulties," and "driving," were very low. These findings suggest that SJS patients suffer from an actual limitation of vision-related daily activity rather than a sense of decreased visual performance and health decline.

A strong negative correlation was observed in the relation between logMAR conventional visual acuities and the VFQ-25 composite scores in this study, with a strong correlation detectable for the relation between the VFQ-25 composite scores and the logMAR functional visual acuities.

We had noteworthy observations that patients with SJS had significantly worse dry eye and visual symptom scores compared to SS patients. We believe these observations owe to the presence of a higher incidence of ocular surface complications in SJS such as symblepharon, corneal opacification, and SPK.

One of the weak points of the current study is that SJS patients, SS patients, and normal subjects were not age-matched. However, it was actually difficult to recruit subjects with age matching in the current study. In fact, the age of onset of SS is usually beyond middle age, while individuals have a risk to be involved with SJS at any age. Moreover, recruitment of elderly individuals with normal tear functions as normal control subjects is another challenging task. It should be noted that the VFQ-25 subscale scores might have been affected by sex and age differences. Another weakness was the lack of definitive diagnosis of SJS/TEN by skin biopsy. We diagnosed SJS or TEN on the history of the presence of cryptogenic fever and acute inflammation of mucosal membranes, most commonly after taking cold remedies, antibiotics, or anti-inflammatory drugs, and on the presence of the chronic ocular surface complications.

Overall, although standard visual acuity testing is a good measurement of one aspect of visual function, the functional visual acuity examination provided other important and detailed information on visual functions related with clinical findings and vision-related quality of life. In conclusion, SJS patients with good or intermediate visual acuity scores measured by conventional visual acuity testing were found to suffer from lower vision-related quality of life, as assessed by functional visual acuity testing and VFQ scores.

ALL AUTHORS HAVE COMPLETED AND SUBMITTED THE ICMJE FORM FOR DISCLOSURE OF POTENTIAL CONFLICTS OF Interest. None of the authors received lecture fees or equity payments from Nidek. Drs Kazuo Tsubota and Minako Kaido both hold patent rights for the method and the apparatus for the measurement of functional visual acuity (US patent no: 7470026). All study centers received and shared an official grant from the Japanese Ministry of Health and Welfare, Tokyo, Japan during the conduct of the study. Involved in conception and design (M.K., C.S., S.K., K.T.); analysis and interpretation (M.K., C.S., K.T.); writing the article (M.K.); critial revision of the article (M.Y., C.S., S.K., J.S., Y.T., Y.H., T.C., K.T.); final approval of the article (M.K., M.Y., C.S., S.K., J.S., Y.T., Y.H., T.C., K.T.); data collection (M.K., M.Y., C.S., J.S., Y.T., Y.H., T.C.); provision of materials, patients, or resources (M.K., M.Y., C.S., J.S., Y.T., Y.H., T.C.); statistical expertise (M.K.); obtaining funding (M.K., M.Y., C.S.); lierature search (M.K., C.S.); and administrative, technical, or logistical support (C.S., S.K., K.T.). Ethics committee approvals for the examination procedures and study protocol were obtained at each center for this prospective study (IRB approval number: 17–129, Keio University School of Medicine, 20. 6, 2006). Written informed consent was obtained from each patient to participate in this study.

REFERENCES

1. Arsujo OE, Floweres FP. Steven-Johnson syndrome. *J Emerg Med* 1984;2(2):129–135.
2. Tsubota K, Toda I, Saito H, Shinozaki N, Shimazaki J. Reconstruction of the corneal epithelium by limbal allograft transplantation for severe ocular surface disorders. *Ophthalmology* 1995;102(10):1486–1496.
3. Power WJ, Ghoraishi M, Merayo-Llovers J, Neves RA, Foster CS. Analysis of the acute ophthalmic manifestations of the erythema multiforme/Stevens-Johnson syndrome/toxic epidermal necrolysis disease spectum. *Ophthalmology* 1995; 102(11):1669–1676.
4. Puangsricharern V, Tseng SCG. Cytologic evidence of corneal disease with limbal stem cell deficiency. *Ophthalmology* 1995;102(19):1476–1485.
5. Tsubota K, Satake Y, Kaido M, et al. Treatment of severe ocular-surface disorders with corneal epithelial stem-cell transplantation. *N Engl J Med* 1999;340(22):1697–1703.
6. Kaido M, Goto E, Dogru M, Tsubota K. Punctal occlusion in the management of chronic Stevens-Johnson syndrome. *Ophthalmology* 2004;111(5):895–900.
7. Kaido M, Dogru M, Yamada M, et al. Functional visual acuity in Stevens-Johnson syndrome. *Am J Ophthalmol* 2006; 142(6):917–922.
8. Sotozono C, Ueta M, Koizumi N, et al. Diagnosis and treatment of Stevens-Johnson syndrome and toxic epidermal necrolysis with ocular complications. *Ophthalmology* 2009; 116(4):685–690.
9. Rieger G. The importance of the precorneal tear film for the quality of topical imaging. *Br J Ophthalmol* 1992;76(3):157–158.
10. Niesen U, Businger U, Hartmann P, Senn P, Schipper I. Glare sensitivity and visual acuity after excimer laser photorefractive for myopia. *Br J Ophthalmol* 1997;81(2):136–140.
11. Ghaith AA, Daniel J, Stulting RD, Thompson KP, Lynn M. Contrast sensitivity and glare disability after radial keratotomy and photorefractive keratectomy. *Arch Ophthalmol* 1998; 116(1):12–18.
12. Roland M, Iester M, Maci A, Calabria G. Low spatial-contrast sensitivity in dry eyes. *Cornea* 1998;17(4):376–379.
13. Liu Z, Pflugfelder SC. Corneal surface regularity and the effect of artificial tears in aqueous tear deficiency. *Ophthalmology* 1999;106(5):939–943.
14. Nakamura K, Bissen-Miyajima H, Toda I, Hori Y, Tsubota K. Effect of laser in situ keratomileusis correction on contrast visual acuity. *J Cataract Refract Surg* 2001;27(3):357–361.
15. Huang FC, Tseng SH, Shih MH, Chen FK. Effect of artificial tears on corneal surface regularity, contrast sensitivity, and glare disability in dry eyes. *Ophthalmology* 2002;109(10): 1934–1940.
16. Bron AJ, Tifffany JM, Gouveia SM, Yokoi N, Voon LW. Functional aspects of the tear film lipid layer. *Exp Eye Res* 2004;78(3):347–360.
17. Kojima T, Ishida R, Dogru M, et al. A new noninvasive tear stability analysis system for the assessment of dry eyes. *Invest Ophthalmol Vis Sci* 2004;45(5):1369–1374.
18. Puell MC, Benitez-del-Castillo JM, Martinez-de-la-Casa J, et al. Contrast sensitivity and disability glare in patients with dry eye. *Acta Ophthalmol Scand* 2006;84(4):527–531.
19. Koh S, Maeda N, Hirohara Y, et al. Serial measurements of high-order aberrations after blinking in patients with dry eye. *Invest Ophthalmol Vis Sci* 2008;49(1):133–138.
20. Goto E, Yagi Y, Matsumoto Y, Tsubota K. Impaired functional visual acuity of dry eye patients. *Am J Ophthalmol* 2002;133(2):181–186.
21. Goto E, Yagi Y, Kaido M, Matsumoto Y, Konomi K, Tsubota K. Improved functional visual acuity after punctal occlusion in dry eye patients. *Am J Ophthalmol* 2003;135(5):704–705.
22. Ishida R, Kojima T, Dogru M, et al. The application of a new continuous functional visual acuity measurement system in dry eye syndrome. *Am J Opthalmol* 2005;139(2):253–258.
23. Goto E, Ishida R, Kido M, et al. Optical aberrations and visual disturbances associated with dry eye. *Ocul Surf* 2006; 4(4):207–213.
24. Kaido M, Ishida R, Dogru M, Tamaoki T, Ysubota K. Efficacy of punctum plug treatment in short break-up time dry eye. *Optom Vis Sci* 2008;85(8):758–763.
25. Fox RI, Robinson CA, Curd JG, Kozin F, Howell FV. Sjogren's syndrome: proposed criteria for classification. *Arthritis Rheum* 1986;29(5):577–585.
26. Sotozono C, Ang LP, Koizumi N, et al. New grading system for the evaluation of chronic ocular manifestations in patients with Stevens-Johnson syndrome. *Ophthalmology* 2007; 114(7):1294–1302.
27. Van Bijsterveld OP. Diagnostic tests in the Sicca syndrome. *Arch Ophthalmol* 1969;82(1):10–14.
28. Suzukamo Y, Oshika T, Yuzawa M, et al. Psychometric properties of the 25-item National Eye Institute Visual Function Questionnaire (NEI VFQ-25), Japanese version. *Health Qual Life Outcome* 2005;3:65.
29. Miyata K, Amano S, Sawa M, Nishida T. A novel grading method for superficial punctuate keratopathy magnitude and its correlation with corneal epithelial permeability. *Arch Ophthalmol* 2003;121(11):1537–1539.
30. Bron AJ, Benjamin L, Snibson GR. Meibomian gland diseases. Classification and grading of lid changes. *Eye* 1991; 5(Pt 4)395–411.

Biosketch

Minako Kaido graduated from the Medical University of Occupational and Environmental Health, Fukuoka, Japan in 1991. She joined Dr Tsubota's dry eye and cornea team at Tokyo Dental College Ichikawa Hospital in 1995 and has been a pivotal member of Dr Tsubota's team at Keio University School of Medicine, Tokyo Japan since 2004. She received her PhD degree in 2012. Dr Kaido's work is focused on the treatment of dry eyes and functional visual acuity technology.

BMJ open accessible medical research

Vancomycin Ophthalmic Ointment 1% for methicillin-resistant *Staphylococcus aureus* or methicillin-resistant *Staphylococcus epidermidis* infections: a case series

Chie Sotozono,[1] Masahiko Fukuda,[2] Masao Ohishi,[3] Keiko Yano,[4] Hideki Origasa,[5] Yoshinori Saiki,[6] Yoshikazu Shimomura,[2] Shigeru Kinoshita[1]

To cite: Sotozono C, Fukuda M, Ohishi M, *et al*. Vancomycin Ophthalmic Ointment 1% for methicillin-resistant *Staphylococcus aureus* or methicillin-resistant *Staphylococcus epidermidis* infections: a case series. *BMJ Open* 2013;**3**:e001206. doi:10.1136/bmjopen-2012-001206

► Prepublication history for this paper are available online. To view these files please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2012-001206).

Received 11 June 2012
Revised 29 October 2012
Accepted 14 December 2012

For numbered affiliations see end of article.

Correspondence to
Chie Sotozono;
csotozon@koto.kpu-m.ac.jp

ABSTRACT

Objectives: To investigate the efficacy and safety of Vancomycin Ophthalmic Ointment 1% (Toa Pharmaceutical Co., Ltd, Toyama, Japan) in patients with external ocular infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) or methicillin-resistant *Staphylococcus epidermidis* (MRSE).

Design: A case series.

Setting: This study was a multicentre, open-label, uncontrolled study in Japan approved as orphan drug status.

Participants: Patients with MRSA or MRSE external ocular infections unresponsive to the treatment of fluoroquinolone eye drops.

Interventions: Vancomycin Ophthalmic Ointment 1% was administered four times daily.

Primary and secondary outcome measures: The subjective and objective clinical scores and bacterial cultures were collected at days 0 (baseline), 3, 7 and 14. The primary outcome was clinical response evaluation (efficacy rate) determined as *complete response*, *partial response*, *no response* and *worsening*. Secondary outcome was the eradication of the bacteria. Safety was assessed by adverse events including cases in which neither MRSA nor MRSE was detected.

Results: Twenty-five cases with MRSA (20) or MRSE (5) infections were enrolled. Of these 25 cases, 4 discontinued the treatment due to the negative results for bacterial culture during screening or at baseline. Of the 21 cases with conjunctivitis (14), blepharitis (3), meibomitis (1), dacryocystitis (2) or keratitis (1), 14 (66.7%) cases were evaluated as being excellently (*complete response*, 2 cases) or well (*partial response*, 12 cases) treated. The eradication rates were 68.4% in MRSA (13 of 19 cases) and 100% in MRSE (2 of 2 cases). Ten adverse events occurred in 7 (28.0%) of 25 cases at the local administration site.

Conclusions: Vancomycin Ophthalmic Ointment 1% was considered to be useful for the treatment of intractable ocular MRSA/MRSE infections.

ARTICLE SUMMARY

Article focus

- Ophthalmic solution prepared by in-house prescription from bulk powder with saline is unstable and acidic.
- Since vancomycin exerts its actions time dependently, an ophthalmic ointment with high tissue retentivity is well suited for clinical use.
- The aim of this study was to examine the effects of Vancomycin Ophthalmic Ointment 1% for the treatment of external ocular methicillin-resistant *Staphylococcus aureus* (MRSA) or methicillin-resistant *Staphylococcus epidermidis* (MRSE) infections.

Key messages

- Vancomycin Ophthalmic Ointment 1% is useful for the treatment of external ocular MRSA or MRSE infections.

Strengths and limitations of this study

- Although a randomised control trial is ideal, this is an open-label, uncontrolled study.
- Due to strict inclusion criteria, the number of patients enrolled is small. Most of the cases were chronic and/or prolonged mild infections in elderly patients.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) was first reported in 1960, the same year that methicillin was developed,[1] and it is still a bacteria that is frequently detected in hospitals worldwide.[2] In the field of ophthalmology, ocular infections such as dacryocystitis, conjunctivitis and keratitis are often reported,[3–7] and infectious keratitis and endophthalmitis caused by MRSA are increasing problems throughout the world.[8–10] In recent years, the number of multidrug-resistant MRSA strains showing resist-

ance to other antibiotics such as aminoglycosides, minocyclin and fluoroquinolones has been on the rise.[11–15] *Staphylococcus epidermidis* has developed the same bacterial resistance as *S aureus* and has now been termed as methicillin-resistant *S epidermidis* (MRSE). Moreover, previous reports have shown that MRSE can cause ophthalmic infections and blindness.[4 16]

Vancomycin, a glycopeptide antibiotic, is known to be effective for treating MRSA infections. Since its injection formulation was first approved for the indication of infectious disease due to Gram-positive bacteria in 1958 in the USA, vancomycin has become an approved antibiotic throughout the world and is highly valued particularly for the treatment of MRSA infections.[9 15] In the therapy of ocular infections, a topical application of vancomycin solutions prepared by in-house prescription is often used.[16 17] However, vancomycin is unstable in an aqueous solution. In addition, vancomycin solutions prepared by in-house prescription using saline are acidic, and the irritation of the solutions to tissues causes patient compliance problems.[17]

We first prepared vancomycin ophthalmic ointments for the treatment of destructive MRSA keratitis postlamellar keratoplasty and found that the infectious keratitis healed dramatically.[4 18] Considering the fact that vancomycin is a drug that exerts its actions time dependently,[19] an ophthalmic ointment with high tissue retentivity, is well suited for clinical use. Indeed, vancomycin ophthalmic ointments remained at least 3 h after administration in a 5-year-old boy with severe MRSA keratitis. It has been suggested that vancomycin ophthalmic ointments remain longer on the ocular surface compared with vancomycin solutions.[18] However, those ointments have proved to be difficult to prepare, and a commercially made product with long-term stability that can be distributed at an effective concentration to the site of an infection has been in demand.

Vancomycin Ophthalmic Ointment 1% (Toa Pharmaceutical Co., Ltd, Toyama, Japan) was developed for the treatment of MRSA/MRSE ocular infections.[20] In 2001, it was designated as an orphan drug for the treatment of 'ocular infections, such as blepharitis, conjunctivitis and keratitis caused by MRSA and MRSE' (Grant No. 13–152, dated 23 April 2001). Thereafter, a phase I study confirmed the safety and tolerability of vancomycin ophthalmic ointment in healthy adult volunteers. In this study, we investigated the efficacy and safety of Vancomycin Ophthalmic Ointment 1% in patients with external ocular infections caused by MRSA or MRSE.

MATERIALS AND METHODS

Study design

This study was a multicentre, open-label, uncontrolled study approved as orphan drug status. The study protocol was designed to evaluate the efficacy and safety of Vancomycin Ophthalmic Ointment 1% in patients with MRSA or MRSE external ocular infections. The study included a 3-day (or more) screening period with the treatment of fluoroquinolone eye drops, and a 14-day treatment period during which patients received Vancomycin Ophthalmic Ointment 1% (four times daily; figure 1). It was approved by the institutional review board at each study site. The study was carried out in accordance with the tenets set forth in the Declaration of Helsinki and in compliance with the 'Good Clinical Practice (GCP)' stipulated by the Ministry of Health, Labour and Welfare of Japan. Written informed consent was obtained from each patient at the respective institution before the initiation of the study protocol.

Screening and eligibility

The subjects involved in this study were patients with external ocular infections caused by MRSA or MRSE who were diagnosed with conjunctivitis, blepharitis, hordeolum, meibomitis, dacryocystitis and keratitis after presentation at 1 of 20 medical institutions in Japan between February 2006 and February 2007. Patient inclusion and exclusion criteria are shown in box 1. Only the patients who met all of the inclusion criteria were enrolled in this study.

Dosage regimen of the study drug

Vancomycin Ophthalmic Ointment 1% (containing 10 mg (potency) of vancomycin hydrochloride per gram) was administered at a dose of around 1 cm (approximately 50 mg) four times (morning, noon, evening and before bedtime) daily. The study treatment was started in the morning. The maximum treatment period was 14 days, and the treatment was terminated before 14 days in cases with diminishing subjective and objective findings of ocular infection.

Evaluation methods

Efficacy

The results of the bacteriological evaluation and clinical symptom assessment at days 3, 7 and 14 after the study

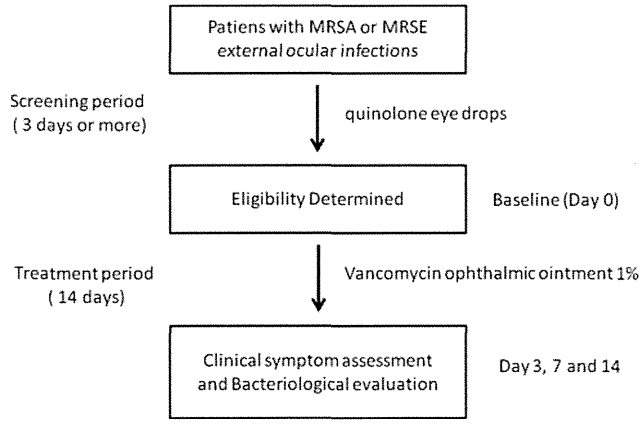


Figure 1 Study design. External ocular infections caused by methicillin-resistant *Staphylococcus aureus* or methicillin-resistant *Staphylococcus epidermidis* and cases in which fluoroquinolone eye drops showed no clinical effect were enrolled.

Box 1 Inclusion/exclusion criteria.

*Inclusion criteria**

- Age: 20 years or older and 90 years or younger (at the time of informed consent)
- Ocular infections due to MRSA or MRSE including the following target diseases: conjunctivitis, blepharitis, hordeolum, meibomianitis, dacryocystitis, keratitis and corneal ulcer
- Patients whose symptoms did not improve after local treatment with a fluoroquinolone antibacterial agent for the eyes for 3 days or more

*Exclusion criteria**

- Prior episode of hypersensitivity to vancomycin hydrochloride
- Prior episode of hypersensitivity to teicoplanin, peptide antibiotics or aminoglycoside antibiotics
- Patients who were on vancomycin hydrochloride or drugs of the same class and with the same effect (arbekacin sulfate and teicoplanin)
- Patients with a clinically significant disease of the autoimmune, cardiovascular, haematological, nervous, endocrine, hepatic, renal or digestive system
- Pregnant women, women of childbearing potential and lactating women

MRSA, methicillin-resistant *Staphylococcus aureus*; MRSE, methicillin-resistant *Staphylococcus epidermidis*.
* Pertains to study eyes, except where otherwise noted.

treatment initiation, and at study treatment completion or discontinuation, were evaluated and judged in comparison with those at the study treatment initiation (day 0; baseline). In cases with bilateral infection, either the severely affected eye or the right eye was evaluated.

In the clinical symptom assessment, symptoms and findings were scored according to the evaluation criteria, and the course of clinical symptoms at screening, at day 0 (baseline) and at days 3, 7 and 14 after the study treatment initiation, and study treatment completion or discontinuation were evaluated. Eye discharge, eye pain, foreign body sensation, photophobia and lacrimation as symptoms and redness (hyperaemia) and oedema (swelling), swelling of the eyelid, lacrimal-sac fluid reflux and keratitis as objective findings were classified into four levels and recorded as follows: notably severe (+++), 3 points; marked (++), 2 points; obvious (+),1 point; and none (−), 0 points.

In the bacteriological evaluation, samples for bacterial culture and identification were collected with sterile swabs from the eyes of patients at screening, at day 0 (baseline) and at days 3, 7 and 14 after the study treatment initiation, and at study treatment completion or discontinuation. These samples were inoculated into aerobic media, and the antibiotic sensitivity of the isolated bacterial strains was tested at the central laboratory for microbial testing (Research Foundation for Microbial Diseases of Osaka University). The effect towards negative conversion at days 3, 7 and 14 was calculated and evaluated.

The efficacy was determined as *complete response* (eradication of detected bacteria (estimated causative bacteria, hereinafter referred to as 'the bacteria') within 4 days and the disappearance of main symptoms within 1 week), *partial response* (1) eradication of the bacteria within 1 week and the disappearance of main symptoms within 2 weeks, (2) eradication of the bacteria within 4 days and a symptom score changed to ≥1/4 to ≤1/2 within 1 week or (3) no eradication of the bacteria but a symptom score changed to ≤1/3 within 1 week), *no response* (efficacy not corresponding to partial response or better) and *worsening* (deterioration of the main symptoms or symptom score compared with those at baseline).

Safety evaluation

All adverse drug reactions (ADRs) were recorded, and the frequency and incidence of the ADRs were then evaluated.

Analysis methods

Efficacy

The main efficacy analysis population was defined as a 'full analysis set (FAS)' not including patients with major GCP violations. Analyses in a 'per protocol set (PPS)', the population meeting the protocol criteria, were also performed. The results of the bacteriological evaluation and clinical symptom assessment were classified into five levels (complete response, partial response, no response, worsening and indeterminate) and a frequency table was then prepared. In addition, the percentage of patients with complete response and a partial response was calculated as an 'efficacy rate' that was evaluated by a one-sample exact test (two-sided significance level of 0.05: null hypothesis, efficacy rate: 10%) based on a binomial distribution. The 95% CIs for the efficacy rates were also calculated. In the bacteriological evaluation, the percentages of patients with eradication of MRSA or MRSE at the treatment completion or discontinuation were calculated as eradication rates.

Safety

In a 'safety population (SP)', patients who received at least one dose of the study drug and excluding those with major GCP violations, the frequency (number of patients with ADRs, number of ADRs and incidence) was tabulated by a system organ. Causal relationship, severity and outcomes in each ADR were judged by the attending physician.

RESULTS

Disposition of patients

In regard to the analysis populations, 25 patients, not including a patient with a major GCP violation, were adopted to the SP. Of the 25 patients in the SP, 4 patients

Table 1 Frequency tabulation of patient background characteristics: FAS, PPS and SP

Analysis population Item	FAS Number of patients (%)	PPS Number of patients (%)	SP Number of patients (%)
Number of patients	21 (100.0)	18 (100.0)	25 (100.0)
Sex			
Male	8 (38.1)	8 (44.4)	9 (36.0)
Female	13 (61.9)	10 (55.6)	16 (64.0)
Age (years)			
20≤ to <40	1 (4.8)	1 (5.6)	1 (4.0)
40≤ to <60	1 (4.8)	1 (5.6)	2 (8.0)
60≤ to <75	7 (33.3)	6 (33.3)	8 (32.0)
75≤ to ≤90	12 (57.1)	10 (55.6)	14 (56.0)
Bacterial strain			
MRSA	19 (90.5)	16 (88.9)	20 (80.0)
MRSE	2 (9.5)	2 (11.1)	5 (20.0)
Diagnosis (target disease)			
Blepharitis	3 (14.3)	3 (16.7)	3 (12.0)
Hordeolum	0 (0.0)	0 (0.0)	0 (0.0)
Meibomianitis	1 (4.8)	1 (5.6)	1 (4.0)
Conjunctivitis	14 (66.7)	11 (61.1)	16 (64.0)
Dacryocystitis	2 (9.5)	2 (11.1)	2 (8.0)
Keratitis	1 (4.8)	1 (5.6)	3 (12.0)
Severity			
Mild	19 (90.5)	16 (88.9)	21 (84.0)
Moderate	2 (9.5)	2 (11.1)	4 (16.0)
Severe	0 (0.0)	0 (0.0)	0 (0.0)

FAS, full analysis set; PPS, per protocol set; SP, safety population.

with treatment discontinuation due to negative results for bacterial culture during screening or at baseline were excluded, and 21 patients were included in the FAS. Of the 21 patients adopted into the FAS, 3 patients with protocol deviations were excluded and 18 patients were included in the PPS (table 1). As to the demographic characteristics of the patients, the mean age was 72.1±14.0 years (hereinafter: mean±SD).

Efficacy

In the clinical response evaluation (efficacy rate) defined as the primary endpoint, the efficacy rate was 66.7% in both the FAS and PPS. It was significantly higher in both populations as compared with the efficacy rate of 10% specified in the null hypothesis ($p<0.001$). The 95% CIs for the efficacy rate were 43–85.4% in the FAS and 41–86.7% in the PPS. In the evaluation by bacterial strain, the efficacy rates for MRSA were 63.2% in the FAS and 62.5% in the PPS. The efficacy rates for MRSE were 100% in both the FAS and PPS. In the evaluation by disease, conjunctivitis was most frequent, and the efficacy rates were 71.4% in the FAS and 72.7% in the PPS (table 2). In the bacteriological evaluation, the eradication rates were 68.4% (13 of 19 cases) for MRSA and 100% (2 of 2 cases) for MRSE in the FAS (figure 2).

Safety

Ten ADRs occurred in seven (28%) patients, and all the ADRs occurred at the local administration site. The main ADRs were eyelid oedema in three (12%) patients and conjunctival hyperaemia in three (12%) patients. Eyelid oedema, increased eye discharge and swelling of the face were moderate, and the other events such as conjunctival hyperaemia, abnormal sensation in the eye

Table 2 Clinical response evaluation by disease (full analysis set, FAS)

Target disease	Number of patients	Clinical response					Efficacy rate (%)
		Complete response	Partial response	No response	Worsening	Indeterminate	
Conjunctivitis	14	2 (14.3%)	8 (57.1%)	3 (21.4%)	0	1 (7.1%)	71.4
Blepharitis	3	0	2 (66.7%)	1 (33.3%)	0	0	66.7
Meibomianitis	1	0	1 100.0%)	0	0	0	100.0
Dacryocystitis	2	0	1 (50.0%)	1 (50.0%)	0	0	50.0
Keratitis	1	0	0	1 (100.0%)	0	0	0.0

Efficacy rate: (number of patients with 'complete response' or 'partial response'/number of patients studied)×100.

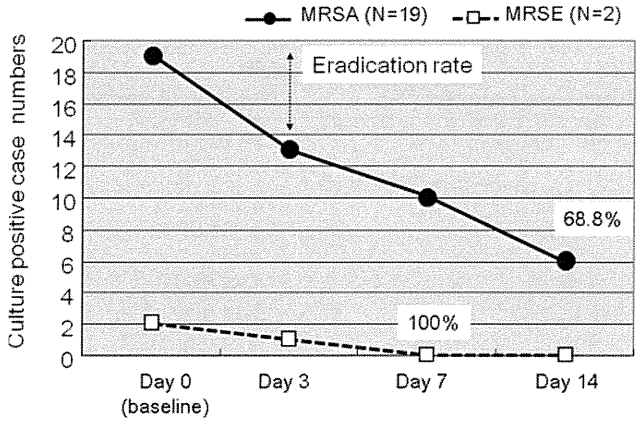


Figure 2 Bacteriological evaluation at 3, 7 and 14 days after initiation of treatment.

and pruritus at the application site were mild. Treatment was discontinued only in one patient with atopic dermatitis who developed swelling of the face and bilateral swelling of the eyelid. All the ADRs were confirmed to have resolved after the study completion.

DISCUSSION

In recent reports on drug-susceptibility of detected bacteria in the field of ophthalmology, the resistance rates of MRSA to ophthalmic antibiotics such as levofloxacin, cefmenoxime and erythromycin have risen. In contrast, the susceptibility rate of MRSA to vancomycin is reportedly still 100%.[2 11 13–15] Physicians in the clinical setting use ophthalmic solutions prepared by in-house prescription from bulk powder for injection, and their efficacy for MRSA or MRSE ocular infections has been previously reported.[4 16 17] Nonetheless, vancomycin for local ophthalmic use has yet to become available on the open market.

Since vancomycin exerts its actions time dependently,[19] an ophthalmic ointment with high tissue retentivity is well suited for clinical use. Vancomycin Ophthalmic Ointment 1% is a product with good stability achieved by creating an ophthalmic ointment in which vancomycin is dispersed in an oily base.[20 21] This case series showed that Vancomycin Ophthalmic Ointment 1% is useful for the treatment of external ocular MRSA or MRSE infections.

In this study, the subjects were defined as patients in whom MRSA or MRSE was detected in a bacterial test, and moreover, whose symptoms did not improve after local treatment with fluoroquinolone eye drops. Due to such strict inclusion criteria, the number of patients enrolled is small. It was difficult to obtain participants in whom acutely severe infections occurred. In most of the hospitals involved in this study, vancomycin solutions prepared by in-house prescription had already been used for sight-threatening severe infections such as severe MRSA keratitis. Most of the cases in this study were chronic and/or prolonged mild infections in elderly patients.

Of the total 25 patients, 10 ADRs occurred in 7 (28%) patients, and all the ADRs occurred at the local administration site. The main ADRs were eyelid oedema in three (12%) patients and conjunctival hyperaemia in three (12%) patients. All of the ADRs were confirmed to have resolved after the study completion. In terms of the systemic distribution following the administration of vancomycin ophthalmic ointment, plasma concentrations after administration were below the detection limit in all subjects in the phase I study. Vancomycin ophthalmic ointment was presumably a product that would be quite unlikely to cause systemic ADRs based on its pharmacokinetics.

The proportion of MRSA in conjunctival bacterial flora is reportedly high in elderly individuals and in patients with atopic dermatitis or neonates.[22–24] Postoperative endophthalmitis or keratitis can occur in these MRSA carriers, and the application of vancomycin in conjunctival MRSA carriers might be effective in preventing MRSA infections.

There has been concern about the growing resistance of *S aureus* to vancomycin.[25] Particular attention should be paid to not facilitate the growth of bacterial resistance to vancomycin. MRSA isolated from ocular infections is often susceptible to chloramphenicol, fourth-generation fluoroquinolones and other antibiotics.[15 26] Thus, it is preferable to use Vancomycin Ophthalmic Ointment 1% only for a short period of time and only for patients who specifically require this new drug.

Author affiliations
[1]Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan
[2]Department of Ophthalmology, Kinki University Faculty of Medicine, Osaka, Japan
[3]Shiranekensei Hospital, Niigata, Japan
[4]Yata Eye Clinic, Shizuoka, Japan
[5]Division of Biostatistics and Clinical Epidemiology, University of Toyama School of Medicine, Toyama, Japan
[6]Toa Pharmaceuticals Co, Ltd, Toyama, Japan.

Acknowledgements We would like to thank Toa Pharmaceutical Co, Ltd, Toyama, Japan, and all of the following doctors and institutes who participated in the study: Dr Atsushi Moriguchi at the Hanwa the Second Hospital, Senboku Kinsyu Medical Corporation, Dr Hiroshi Shioda from Tokushima University Hospital, Dr Yoshitsugu Inoue at Tottori University Hospital, Dr Toshihiko Uno at Ehime University Hospital, Dr Tomoko Goto at Takanoko Hospital, Dr Shoichi Sawaguchi at the University Hospital, University of the Ryukyu, Dr Kenji Matsuo at Tomakomai City Hospital, Dr Masaaki Ohashi at Tokyo Metropolitan Geriatric Medical Center, Dr Kazuya Inamochi at Asahi Central Hospital, Dr Kaori Hirai at the Hoshi General Hospital Foundation, Dr Shigeki Okamoto at Kozuka Eye Clinic, Dr Shusaku Ishikawa at Chibana Clinic, Dr Toshio Kodama at Matsuyama Red Cross Hospital, Dr Momoyo Shinjo at Oroku Hospital, Dr Kazunori Miyata at Miyata Eye Hospital, Dr Yoshitsugu Tagawa at Hokkaido University Hospital, Dr Tomohiro Iida at Fukushima Medical University Hospital, Dr Shigeto Kumakura at Tokyo Medical University Hospital. We also wish to thank Mr John Bush at Kyoto Prefectural University of Medicine for his assistance with the preparation of the manuscript.

Funding This study was supported by Toa Pharmaceuticals Co. Ltd in study design, conducting the study and data collection management.

Competing interests YS is an employee of Toa Pharmaceuticals Co Ltd.

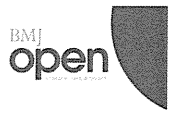
Patient consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

REFERENCES

1. Jevons MP, Coe AW, Parker MT. Methicillin resistance in staphylococci. *Lancet* 1963;1:904–7.
2. Maple PA, Hamilton-Miller JM, Brumfitt W. World-wide antibiotic resistance in methicillin-resistant Staphylococcus aureus. *Lancet* 1989;1:537–40.
3. Brennen C, Muder RR. Conjunctivitis associated with methicillin-resistant Staphylococcus aureus in a long-term-care facility. *Am J Med* 1990;88:14N–7N.
4. Sotozono C, Inagaki K, Fujita A, *et al.* Methicillin-resistant Staphylococcus aureus and methicillin-resistant Staphylococcus epidermidis infections in the cornea. *Cornea* 2002;21:S94–101.
5. Forster W, Becker K, Hungermann D, *et al.* Methicillin-resistant Staphylococcus aureus keratitis after excimer laser photorefractive keratectomy1. *J Cataract Refract Surg* 2002;28:722–4.
6. Shanmuganathan VA, Armstrong M, Buller A, *et al.* External ocular infections due to methicillin-resistant Staphylococcus aureus (MRSA). *Eye* 2005;19:284–91.
7. Sahu DN, Thomson S, Salam A, *et al.* Neonatal methicillin-resistant Staphylococcus aureus conjunctivitis. *Br J Ophthalmol* 2006;90:794–5.
8. Wroblewski KJ, Pasternak JF, Bower KS, *et al.* Infectious keratitis after photorefractive keratectomy in the United States army and navy. *Ophthalmology* 2006;113:520–5.
9. Solomon R, Donnenfeld ED, Perry HD, *et al.* Methicillin-resistant Staphylococcus aureus infectious keratitis following refractive surgery. *Am J Ophthalmol* 2007;143:629–34.
10. Shimada H, Nakashizuka H, Hattori T, *et al.* Incidence of endophthalmitis after 20- and 25-gauge vitrectomy causes and prevention. *Ophthalmology* 2008;115:2215–20.
11. Maffett M, O'Day DM. Ciprofloxacin-resistant bacterial keratitis. *Am J Ophthalmol* 1993;115:545–6.
12. Cimolai N. Ciprofloxacin and multiresistant staphylococci. *Lancet* 1997;349:1030.
13. Marangon FB, Miller D, Muallem MS, *et al.* Ciprofloxacin and levofloxacin resistance among methicillin-sensitive Staphylococcus aureus isolates from keratitis and conjunctivitis. *Am J Ophthalmol* 2004;137:453–8.
14. Cavuoto K, Zutshi D, Karp CL, *et al.* Update on bacterial conjunctivitis in South Florida. *Ophthalmology* 2008; 115:51–6.
15. Elsahn AF, Yildiz EH, Jungkind DL, *et al.* In vitro susceptibility patterns of methicillin-resistant Staphylococcus aureus and coagulase-negative staphylococcus corneal isolates to antibiotics. *Cornea* 2010;29:1131–5.
16. Goodman DF, Gottsch JD. Methicillin-resistant staphylococcus epidermidis keratitis treated with vancomycin. *Arch Ophthalmol* 1988;106:1570–1.
17. Fleischer AB, Hoover DL, Khan JA, *et al.* Topical vancomycin formulation for methicillin-resistant Staphylococcus epidermidis blepharoconjunctivitis. *Am J Ophthalmol* 1986;101:283–7.
18. Fujita A, Sotozono C, Inatomi T, *et al.* Severe MRSA infection of the eye treated with vamcomycin ointment. *Atarashii Ganka* 2000; 17:93–5.
19. Chua K, Howden BP. Treating Gram-positive infections: vancomycin update and the whys, wherefores and evidence base for continuous infusion of anti-Gram-positive antibiotics. *Curr Opin Infect Dis* 2009;22:525–34.
20. Fukuda M, Hanazome I, Sasaki K. The intraocular dynamics of vancomycin hydrochloride ophthalmic ointment (TN-011) in rabbits. *J Infect Chemother* 2003;9:93–6.
21. Eguchi H, Shiota H, Oguro S, *et al.* The inhibitory effect of vancomycin ointment on the manifestation of MRSA keratitis in rabbits. *J Infect Chemother* 2009;15:279–83.
22. Kato T, Hayasaka S. Methicillin-resistant Staphylococcus aureus and methicillin-resistant coagulase-negative staphylococci from conjunctivas of preoperative patients. *Jpn J Ophthalmol* 1998;42:461–5.
23. Nakata K, Inoue Y, Harada J, *et al.* A high incidence of Staphylococcus aureus colonization in the external eyes of patients with atopic dermatitis. *Ophthalmology* 2000;107:2167–71.
24. Hori Y, Nakazawa T, Maeda N, *et al.* Susceptibility comparisons of normal preoperative conjunctival bacteria to fluoroquinolones. *J Cataract Refract Surg* 2009;35:475–9.
25. Hiramatsu K, Aritaka N, Hanaki H, *et al.* Dissemination in Japanese hospitals of strains of Staphylococcus aureus heterogeneously resistant to vancomycin. *Lancet* 1997;350:1670–3.
26. Fukuda M, Ohashi H, Matsumoto C, *et al.* Methicillin-resistant Staphylococcus aureus and methicillin-resistant coagulase-negative Staphylococcus ocular surface infection efficacy of chloramphenicol eye drops. *Cornea* 2002;21:S86–9.



Vancomycin Ophthalmic Ointment 1% for methicillin-resistant *Staphylococcus aureus* or methicillin-resistant *Staphylococcus epidermidis* infections: a case series

Chie Sotozono, Masahiko Fukuda, Masao Ohishi, et al.

BMJ Open 2013 3:
doi: 10.1136/bmjopen-2012-001206

Visual Improvement after Cultivated Oral Mucosal Epithelial Transplantation

Chie Sotozono, MD, PhD,[1] Tsutomu Inatomi, MD, PhD,[1] Takahiro Nakamura, MD, PhD,[1] Noriko Koizumi, MD, PhD,[1] Norihiko Yokoi, MD, PhD,[1] Mayumi Ueta, MD, PhD,[1] Kotone Matsuyama, BS,[2] Keiko Miyakoda, MS, MPH,[2] Hideaki Kaneda, MD, PhD,[2] Masanori Fukushima, MD, PhD,[2] Shigeru Kinoshita, MD, PhD[1]

Purpose: To report the effectiveness, disease-specific outcomes, and safety of cultivated oral mucosal epithelial sheet transplantation (COMET), with the primary objective of visual improvement.

Design: Noncomparative, retrospective, interventional case series.

Participants: This study involved 46 eyes in 40 patients with complete limbal stem cell deficiency (LSCD) who underwent COMET for visual improvement. These LSCD disorders fell into the following 4 categories: Stevens-Johnson syndrome (SJS; 21 eyes), ocular cicatricial pemphigoid (OCP; 10 eyes), thermal or chemical injury (7 eyes), or other diseases (8 eyes).

Methods: Best-corrected visual acuity (BCVA) and ocular surface grading score were examined before surgery; at the 4th, 12th, and 24th postoperative week; and at the last follow-up. Data on COMET-related adverse events and postoperative management were collected. The outcomes in each disease category were evaluated separately.

Main Outcome Measures: The primary outcome was the change in median logarithm of the minimum angle of resolution (logMAR) BCVA at the 24th postoperative week. The secondary outcome was the ocular surface grading score.

Results: Median logMAR BCVA at baseline was 2.40 (range, 1.10 to 3.00). In SJS, logMAR BCVA improved significantly during the 24 weeks after surgery. In contrast, the BCVA in OCP was improved significantly only at the 4th postoperative week. In 6 of the 7 thermal or chemical injury cases, logMAR BCVA improved after planned penetrating keratoplasty or deep lamellar keratoplasty. Grading scores of ocular surface abnormalities improved in all categories. Of 31 patients with vision loss (logMAR BCVA, >2) at baseline, COMET produced improvement (logMAR BCVA, ≤2) in 15 patients (48%). Visual improvement was maintained with long-term follow-up (median, 28.7 months). Multivariate stepwise logistic regression analysis showed that corneal neovascularization and symblepharon were correlated significantly with logMAR BCVA improvement at the 24th postoperative week ($P = 0.0023$ and $P = 0.0173$, respectively). Although postoperative persistent epithelial defects and slight to moderate corneal infection occurred in the eyes of 16 and 2 patients, respectively, all were treated successfully with no eye perforation.

Conclusions: Long-term visual improvement was achievable in cases of complete LSCD. Cultivated oral mucosal epithelial sheet transplantation offered substantial visual improvement even for patients with end-stage severe ocular surface disorders accompanying severe tear deficiency. Patients with corneal blindness such as SJS benefited from critical improvement of visual acuity.

Financial Disclosure(s): The author(s) have no proprietary or commercial interest in anymaterials discussed in this article. *Ophthalmology 2013;120:193–200 © 2013 by the American Academy of Ophthalmology.*

Corneal renewal and repair are mediated by corneal epithelial stem cells situated mainly in the limbus, the narrow region between the cornea and the bulbar conjunctiva.[1] Damage or depletion of the corneal epithelial stem cells, known as limbal stem cell deficiency (LSCD), leads to conjunctival invasion that results in vascularization and scarring of the cornea with an associated profound loss of vision.[1] Limbal stem cell deficiency can be caused by Stevens-Johnson syndrome (SJS), ocular cicatricial pemphigoid (OCP), and thermal or chemical injury, which are all characterized by the loss of corneal epithelial stem cells. Such LSCD may cause severe ocular surface diseases (OSDs) in which cicatrization resulting from conjunctival fibrosis, symblepharon, and severe dry eye greatly disrupt visual function and can progress gradually with chronic inflammation.[2–4] To date, few effective medical or surgical treatments for severe OSDs have been available.[5–15]

Since 1998, the authors have used amniotic membrane transplantation to treat severe OSDs. Amniotic membrane exhibits an anti-inflammatory effect and also acts as a substrate for epithelialization.[16] The results of previous studies have shown that amniotic membrane transplantation alone[17,18] or amniotic membrane transplantation combined with limbal transplantation[6,19,20] promoted epithelialization, reduced pain, reconstructed the fornix, and minimized inflammation of the ocular surface to a remarkable degree in

ISSN 0161-6420/13/$–see front matter
http://dx.doi.org/10.1016/j.ophtha.2012.07.053

patients with severe OSDs. Based on these promising results, novel methods have been developed for the cultivation of allogeneic corneal[7,8,21] or autologous oral mucosal[22–25] epithelial cells on a denuded amniotic membrane. Immunologic rejection and increased risk of infection or systemic adverse effects associated with the long-term immunosuppressive therapy accompanying allograft transplantation[6] encouraged changing to autologous cultivated oral mucosal epithelial transplantation (COMET) in patients with severe OSDs in 2002.[10,11,23,26]

To clarify the effectiveness, disease-specific outcomes, and safety of COMET, all of the clinical data from all 72 patients that the authors treated with COMET since 2002 were analyzed. The objective of this present study was to summarize the long-term clinical outcomes of 40 of those 72 patients who underwent COMET with the primary objective of visual improvement between June 2002 and December 2008.

Patients and Methods

Patients

Autologous COMET was performed on consecutive patients who were diagnosed with total LSCD based on the complete disappearance of the palisades of Vogt and 360° of conjunctivalization.[1] The COMET treatment protocol was approved by the ethical review board of Kyoto Prefectural University of Medicine, Kyoto, Japan, in 2002. The final decision to perform COMET was made by the university's team of corneal specialists. Before the surgery, written informed consent was obtained from all patients in accordance with the tenets of the Declaration of Helsinki for research involving human subjects. The current retrospective study used an itemized data collection form, and the medical records of all patients who underwent COMET between June 2002 and December 2008 were examined retrospectively. This retrospective study protocol was approved by the ethical review board of Kyoto Prefectural University of Medicine in 2009. In this study, 40 of the 72 patients who underwent COMET were analyzed with the primary objective of visual improvement.

Cell Culture

All of the COMET sheets were prepared at the good manufacturing practices–graded Cell Processing Center at Kyoto Prefectural University of Medicine as previously described.[23,26] Autologous oral mucosal epithelial cells were obtained from a 6-mm–diameter biopsy specimen obtained from each patient's buccal mucosa, and the cells then were cultured on an amniotic membrane spread on the bottom of a culture insert and were cocultured with mitomycin C-inactivated 3T3 fibroblasts (NIH-3T34; RIKEN Cell Bank, Tsukuba, Japan). The cultured cells were submerged in medium for approximately 1 week and then were exposed to air by lowering the medium level (airlifting) for 1 to 2 days. All amniotic membrane was obtained from caesarean sections according to the preparation method described previously.[23] Although fetal bovine serum initially was used as a culture medium, autologous serum was used in later cultures to reduce the risk of transmitting nonhuman pathogens.[26]

Transplantation and Postoperative Management

The surgical procedure (see the Supplemental Video, available at http://aaojournal.org) and postoperative management have been described previously.[24,25] In patients with severe symblepharon or a large area of bare sclera exposed during surgery, amniotic membrane was transplanted onto the bare sclera to reconstruct conjunctival fornices.[18] In patients with a cataract, phacoemulsification and aspiration plus intraocular lens implantation were performed simultaneously with COMET. No penetrating keratoplasty or deep lamellar keratoplasty was performed simultaneously with COMET. For patients with severe corneal stromal opacity, a 2-step surgical approach was planned, with the first step being COMET and the second step being either penetrating or deep lamellar keratoplasty.[25]

Systemic corticosteroid (betamethasone, 1 mg/day) and cyclosporine (2 to 3 mg/kg daily) were administered to prevent postoperative inflammation and immunologic response and then were tapered, depending on the clinical findings. Dexamethasone (0.1%) and antibiotic eye drops were instilled 4 times daily. Dry-eye patients were administered artificial tears. A therapeutic soft contact lens was used for at least 1 month to protect transplanted epithelium from mechanical ablation.

Postoperative Follow-up and Outcomes

Best-corrected visual acuity (BCVA) was converted to the logarithm of the minimum angle of resolution (logMAR). Ocular surface conditions including corneal appearance (epithelial defects, clinical conjunctivalization, neovascularization, opacification, keratinization, and symblepharon) were graded by at least 2 ophthalmologists (C.S., T.I., and T.N.) on a scale from 0 to 3 according to their severity, in accordance with a previously reported grading system.[27] Severe OSDs are characterized by an associated loss of conjunctival stem cells, and the severity of conjunctival involvement affects the visual prognosis. Therefore, findings on upper and lower fornix shortening were added to evaluate the grade of conjunctival appearance. Fornix shortening was graded from 0 to 3 based on the following clinical features: normal depth (grade 0), shortened by less than one quarter (grade 1), shortened by one quarter to one half (grade 2), and shortened by more than one half (grade 3). Upper and lower fornix shortenings were graded separately. The sum of each grading score was defined as the ocular surface grading score (maximum, 24).

Each patients logMAR BCVA, ocular surface grading score, and data on adverse events related to COMET or postoperative management were collected from the medical records at these specific time points: before surgery; at the 4th, 12th, and 24th postoperative weeks; and at the last follow-up examination. The primary outcome was the change in logMAR BCVA at the 24th postoperative week. Because other ocular diseases can affect this visual outcome, a secondary outcome, the ocular surface grading score, also was defined.

Statistical Analysis

The change in BCVA and 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 for other diseases. Multivariate stepwise logistic regression analysis was used to determine the factors influencing visual improvement.

This study defined the critical visual improvement rate as the proportion of patients in whom BCVA at the 24th postoperative week had improved to at least 0.01, as a percentage of the patients with a BCVA of less than 0.01 at baseline. Patients with a visual acuity of 0.01 or more can read and walk using vision aids. Thus, an improvement to at least 0.01 indicates a capacity for independence in daily life. If data were missing from the 24th postoperative week, data from follow-up at the last visit were substituted.

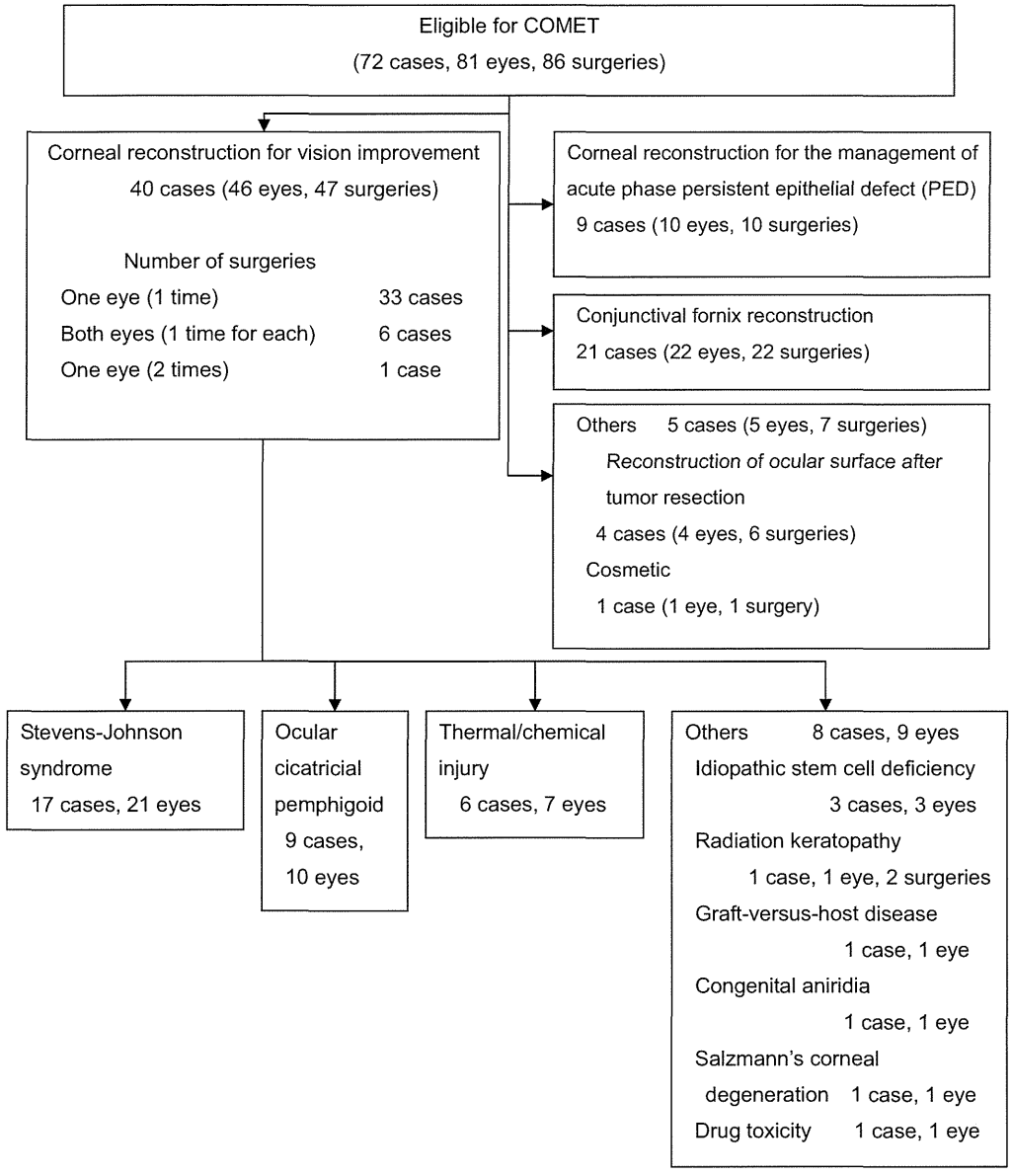


Figure 1. Diagram showing flow of study. Seventy-two patients (81 eyes) underwent cultivated oral mucosal epithelial sheet transplantation (COMET) between June 2002 and December 2008, and 40 patients (46 eyes) were analyzed for visual improvement in this study. Both corneal reconstruction and conjunctival 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:

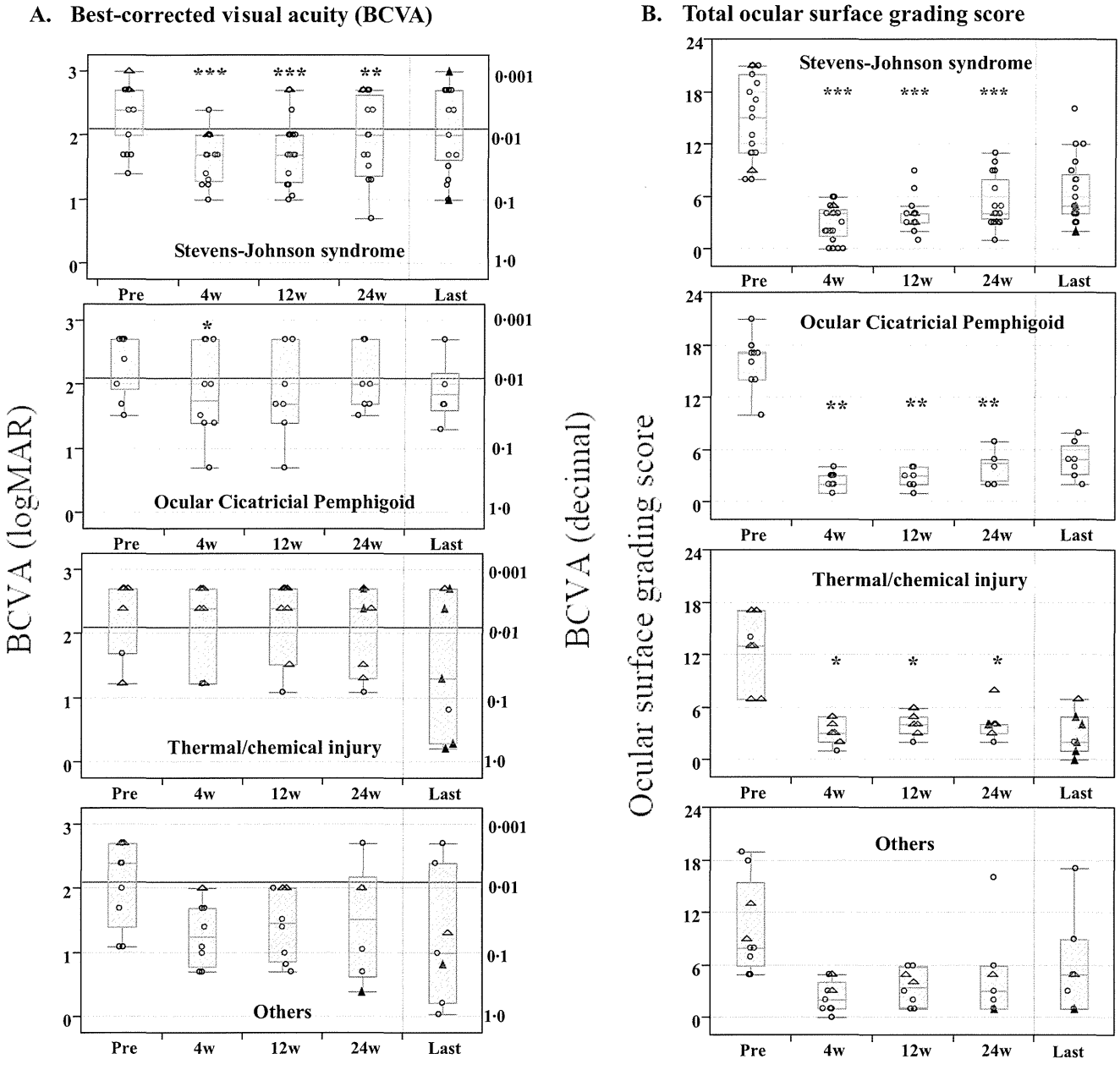


Figure 2. Graphs showing preoperative (Pre) and postoperative clinical outcomes. **A**, Best-corrected visual acuity (BCVA). The BCVA values for each patient are shown grouped according to the cause of corneal dysfunction: Stevens-Johnson syndrome (SJS), ocular cicatricial pemphigoid (OCP), thermal or 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.

Acknowledgments. The authors thank Dr. Shin Kawamata of the Foundation for Biological Research and Innovation and Dr. Kazunori Takeda, Dr. Hiroaki Kato, Dr. Hiroshi Tanaka, Dr. Yuji Yamamoto, and Dr. Takahiro Yamawaki of Kyoto Prefectural University of Medicine for data collection.

References

1. Kinoshita S, Adachi W, Sotozono C, et al. Characteristics of the human ocular surface epithelium. Prog Retin Eye Res 2001;20:639–73.
2. Tugal-Tutkun I, Akova YA, Foster CS. Penetrating keratoplasty in cicatrizing conjunctival diseases. Ophthalmology 1995;102:576–85.
3. Kawasaki S, Nishida K, Sotozono C, et al. Conjunctival inflammation in the chronic phase of Stevens-Johnson syndrome. Br J Ophthalmol 2000;84:1191–3.
4. Wall V, Yen MT, Yang MC, et al. Management of the late ocular sequelae of Stevens-Johnson syndrome. Ocul Surf 2003;1:192–201.
5. Kenyon KR, Tseng SC. Limbal autograft transplantation for ocular surface disorders. Ophthalmology 1989;96:709–22.
6. Tsubota K, Satake Y, Kaido M, et al. Treatment of severe ocular-surface disorders with corneal epithelial stem-cell transplantation. N Engl J Med 1999;340:1697–703.
7. Koizumi N, Inatomi T, Suzuki T, et al. Cultivated corneal epithelial transplantation for ocular surface reconstruction in acute phase of Stevens-Johnson syndrome. Arch Ophthalmol 2001;119:298–300.
8. Koizumi N, Inatomi T, Suzuki T, et al. Cultivated corneal epithelial stem cell transplantation in ocular surface disorders. Ophthalmology 2001;108:1569–74.
9. Nakamura T, Inatomi T, Sotozono C, et al. Successful primary culture and autologous transplantation of corneal limbal epithelial cells from minimal biopsy for unilateral severe ocular surface disease. Acta Ophthalmol Scand 2004;82:468–71.
10. Kinoshita S, Koizumi N, Nakamura T. Transplantable cultivated mucosal epithelial sheet for ocular surface reconstruction. Exp Eye Res 2004;78:483–91.
11. Kinoshita S, Koizumi N, Sotozono C, et al. Concept and clinical application of cultivated epithelial transplantation for ocular surface disorders. Ocul Surf 2004;2:1–33.
12. Nishida K, Yamato M, Hayashida Y, et al. Corneal reconstruction with tissue-engineered cell sheets composed of autologous oral mucosal epithelium. N Engl J Med 2004;351:1187–96.

13. Nakamura T, Inatomi T, Sotozono C, et al. Transplantation of autologous serum-derived cultivated corneal epithelial equivalents for the treatment of severe ocular surface disease. Ophthalmology 2006;113:1765–72.
14. Ang LP, Sotozono C, Koizumi N, et al. A comparison between cultivated and conventional limbal stem cell transplantation for Stevens-Johnson syndrome. Am J Ophthalmol 2007;143: 178–80.
15. Rama P, Matuska S, Paganoni G, et al. Limbal stem-cell therapy and long-term corneal regeneration. N Engl J Med 2010;363:147–55.
16. Tseng SC, Espana EM, Kawakita T, et al. How does amniotic membrane work? Ocul Surf 2004;2:177–87.
17. Tseng SC, Prabhasawat P, Lee SH. Amniotic membrane transplantation for conjunctival surface reconstruction. Am J Ophthalmol 1997;124:765–74.
18. Solomon A, Espana EM, Tseng SC. Amniotic membrane transplantation for reconstruction of the conjunctival fornices. Ophthalmology 2003;110:93–100.
19. Tsubota K, Satake Y, Ohyama M, et al. Surgical reconstruction of the ocular surface in advanced ocular cicatricial pemphigoid and Stevens-Johnson syndrome. Am J Ophthalmol 1996;122:38–52.
20. Tsai RJ, Li LM, Chen JK. Reconstruction of damaged corneas by transplantation of autologous limbal epithelial cells. N Engl J Med 2000;343:86–93.
21. Koizumi N, Inatomi T, Quantock AJ, et al. Amniotic membrane as a substrate for cultivating limbal corneal epithelial cells for autologous transplantation in rabbits. Cornea 2000;19:65–71.
22. Nakamura T, Endo K, Cooper LJ, et al. The successful culture and autologous transplantation of rabbit oral mucosal epithelial cells on amniotic membrane. Invest Ophthalmol Vis Sci 2003;44:106–16.
23. Nakamura T, Inatomi T, Sotozono C, et al. Transplantation of cultivated autologous oral mucosal epithelial cells in patients with severe ocular surface disorders. Br J Ophthalmol 2004; 88:1280–4.
24. Inatomi T, Nakamura T, Koizumi N, et al. Midterm results on ocular surface reconstruction using cultivated autologous oral mucosal epithelial transplantation. Am J Ophthalmol 2006; 141:267–75.
25. Inatomi T, Nakamura T, Kojyo M, et al. Ocular surface reconstruction with combination of cultivated autologous oral mucosal epithelial transplantation and penetrating keratoplasty. Am J Ophthalmol 2006;142:757–64.
26. Ang LP, Nakamura T, Inatomi T, et al. Autologous serum-derived cultivated oral epithelial transplants for severe ocular surface disease. Arch Ophthalmol 2006;124:1543–51.
27. Sotozono C, Ang LP, Koizumi N, et al. New grading system for the evaluation of chronic ocular manifestations in patients with Stevens-Johnson syndrome. Ophthalmology 2007;114: 1294–302.
28. Sotozono C, Inagaki K, Fujita A, et al. Methicillin-resistant *Staphylococcus aureus* and methicillin-resistant *Staphylococcus epidermidis* infections in the cornea. Cornea 2002; 21:S94–101.
29. Samson CM, Nduaguba C, Baltatzis S, Foster CS. Limbal stem cell transplantation in chronic inflammatory eye disease. Ophthalmology 2002;109:862–8.
30. Solomon A, Ellies P, Anderson DF, et al. Long-term outcome of keratolimbal allograft with or without penetrating keratoplasty for total limbal stem cell deficiency. Ophthalmology 2002;109:1159–66.
31. Sotozono C, Ueta M, Koizumi N, et al. Diagnosis and treatment of Stevens-Johnson syndrome and toxic epidermal necrolysis with ocular complications. Ophthalmology 2009;116:685–90.
32. Khan B, Dudenhoefer EJ, Dohlman CH. Keratoprosthesis: an update. Curr Opin Ophthalmol 2001;12:282–7.
33. Liu C, Okera S, Tandon R, et al. Visual rehabilitation in end-stage inflammatory ocular surface disease with the osteo-odonto-keratoprosthesis: results from the UK. Br J Ophthalmol 2008;92:1211–7.
34. Nakamura T, Takeda K, Inatomi T, et al. Long-term results of autologous cultivated oral mucosal epithelial transplantation in the scar phase of severe ocular surface disorders. Br J Ophthalmol 2011;95:942–6.
35. Tanioka H, Kawasaki S, Sotozono C, et al. The relationship between preoperative clinical scores and immunohistological evaluation of surgically resected tissues in chronic severe ocular surface diseases. Jpn J Ophthalmol 2010;54:66–73.
36. Shimazaki J, Shimmura S, Fujishima H, Tsubota K. Association of preoperative tear function with surgical outcome in severe Stevens-Johnson syndrome. Ophthalmology 2000;107:1518–23.
37. Turgeon PW, Nauheim RC, Roat MI, et al. Indications for keratoepithelioplasty. Arch Ophthalmol 1990;108:233–6.
38. Ma DH, Kuo MT, Tsai YJ, et al. Transplantation of cultivated oral mucosal epithelial cells for severe corneal burn. Eye (Lond) 2009;23:1442–50.
39. Shimazaki J, Aiba M, Goto E, et al. Transplantation of human limbal epithelium cultivated on amniotic membrane for the treatment of severe ocular surface disorders. Ophthalmology 2002;109:1285–90.
40. Satake Y, Higa K, Tsubota K, Shimazaki J. Long-term outcome of cultivated oral mucosal epithelial sheet transplantation in treatment of total limbal stem cell deficiency. Ophthalmology 2011;118:1524–30.

Footnotes and Financial Disclosures

Originally received: March 10, 2012.
Final revision: July 17, 2012.
Accepted: July 18, 2012.
Available online: October 16, 2012. Manuscript no. 2012-353.

[1] Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan.

[2] Translational Research Informatics Center, Foundation for Biomedical Research and Innovation, Kobe, Japan.

Presented at: American Academy of Ophthalmology Annual Meeting, October 2011, Orlando, Florida.

Financial Disclosure(s):
The author(s) have no proprietary or commercial interest in any materials discussed in this article.

Supported in part by a Grant-in-aid for Scientific Research from the Japanese Ministry of Health, Labor and Welfare, Tokyo, Japan; and a Research Grant of the Coordination, Support and Training Program for Translational Research from the Japanese Ministry of Education, Culture, Sports, Science and Technology, Tokyo, Japan.

Correspondence:
Chie Sotozono, MD, PhD, Department of Ophthalmology, Kyoto Prefectural University of Medicine, 465 Kajii-cho, Hirokoji-agaru, Kawaramachi-dori, Kamigyo-ku, Kyoto 602-0841, Japan. E-mail: csotozon@koto.kpu-m.ac.jp.

皮膚病診療：35(1)；35～38, 2013

◇サルコイドーシス——2013<臨床例>——④

フェニトインによる薬疹後に帯状疱疹を生じ，サルコイドーシスを続発した例

稲岡　峰幸*　　狩野　葉子**　　塩原　哲夫**

Key words
サルコイドーシス，帯状疱疹，フェニトイン

症例のポイント

・フェニトインによる薬疹を生じた15カ月後にフェニトイン再投与による薬疹が発症し，1カ月後に帯状疱疹が出現，その後，サルコイドーシスを続発した例を経験した．
・水痘-帯状疱疹ウイルス(以下，VZV)は肉芽腫反応を誘発しやすく，帯状疱疹後に肉芽腫を生じた例は多数報告されている．
・自験例では再活性化したVZVがサルコイドーシス発症の誘因となった可能性が示唆された．

症例　74歳，女．

初診　2009年8月．

家族歴　特記すべきことなし．

既往歴　73歳，変形性頸椎症．

現病歴　2009年7月，右肋間帯状疱疹罹患2日後に急性硬膜下血腫を発症し当院脳外科に入院し，フェニトイン内服開始．内服13日目に全身に紅斑が出現し，内服23日目に38℃台の発熱を認めたため，当科受診．薬疹の疑いでフェニトインを中止したが，改善傾向なく25日目に当科に入院した．

現症　顔面にはびまん性に紅斑が認められ，軀幹・四肢では大豆大までの浮腫性紅斑が多発融合していた(**図1**)．右前胸部に小豆大の瘢痕が集簇し，頸部リンパ節は拇指頭大に腫脹していた．

臨床検査成績

第13病日；WBC 6,400/μl(Band 3.0%〈正常値<0.0〉, Neut 63.0%〈42.0-62.0〉, Eos 16.5%〈1.0-5.0〉, Mono 3.0%, Lym 14.0% (25.0-45.0), BUN 30.4 mg/dl, Cr 2.4mg/dl(0.2-0.8), AST 46 IU/l, ALT 26 IU/l(3-30), γ-GTP 74 IU/l(4-50), LDH 641 IU/l(118-226), CRP 6.3 mg/dl(0.0-0.4), IgG 1,406 mg/dl. 胸部X線では異常所見はみられなかった．第14病日;薬剤リンパ球刺激試験：フェニトイン 147%(180%以上が陽性)，第60病日；フェニトイン 398%.

ウイルス学的検索

第13病日；HHV-6 IgG 10倍(10倍未満陰性)，VZV-IgG 280(<2.0), HSV-IgG 57.2(<2.0)，全血中HHV-6 DNA 20copy/10^6WBC未満(20copy/10^6WBC未満 陰性)．第26病日；HHV-6 IgG 10倍, VZV-IgG 169, HSV-IgG 70.3.

病理組織学的所見

腹部の紅斑を，第13病日に生検した．表皮では表皮突起の消失がみられ，真皮上層から深層の血管周囲にリンパ球主体の炎症細胞浸潤が認められた(**図2**)．明らかな肉芽腫の形成はみられなかった．

*Inaoka, Miyuki(科長)　阿伎留医療センター皮膚科(〒197-0834 あきる野市引田78－1)
**Kano, Yoko(教授)／Shiohara, Tetsuo(教授)　杏林大学医学部付属病院皮膚科(〒181-8611 三鷹市新川6－20－2)