Table 4 Condition of the tongue in subjects

Subjects	n	Normal (%)	Atrophy of surface of the tongue (%)	Groove on the tongue (%)	Shiny tongue (%)	Strawberry tongue (%)
SS-DE[a]	18	27.8	16.7	33.3	16.7	5.6
Non-SS-DE[b]	18	44.8	27.8	27.8	0	0
CP[c]	21	85.7	4.8	9.5	0	0
C[d]	37	83.8	13.5	2.7	0	0

[a]SS-DE, Sjögren's syndrome with dry eye; [b]non-SS-DE, dry eye not associated with Sjögren's syndrome; [c]SJS-DE, Stevens–Johnson syndrome with dry eye; [d]C, healthy volunteers as a control.

Table 5 Angular cheilitis in subjects

Subjects	n	Normal (%)	Slight angular cheilitis (%)	Heavy angular cheilitis or slight angular cheilitis at both corners of the mouth (%)
SS-DE[a]	18	55.6	16.7	27.8
Non-SS-DE[b]	18	77.8	22.2	0
CP[c]	21	100	0	0
C[d]	37	94.5	2.6	0

[a]SS-DE, Sjögren's syndrome with dry eye; [b]non-SS-DE, dry eye not associated with Sjögren's syndrome; [c]CP, control patients; [d]C, healthy volunteers as a control.

Relationship between subjective symptoms of dry mouth and salivary flow rates

The patients with dry eye complained of a sensation of dry mouth than the control subjects more frequently (47.5% *vs* 4.9%; $P < 0.05$) (Table 6). Their stimulated whole and parotid salivary flow rates were lower than those of patients who complained of only an occasional sensation of a dry mouth or no symptoms of dry mouth ($P < 0.01$) (Table 7).

Table 6 Sensation of dry mouth in subject groups

Sensation of dry mouth	n	Frequent (%)	Occassional (%)	No symptoms (%)	
Dry eye patients*	141	47.52	27.66	18.44	]**
Controls	82	4.88	8.54	86.59	]**

*Dry eye patients SS-DE, non-SS-DE, and SJS-DE.
**$P < 0.05$.

Discussion

This study was conducted to compare the association of oral signs and symptoms in dry eye patients. When stimulated whole and parotid saliva was considered, lower secretion rates were observed in SS-DE. Atkinson *et al* (1990) reported that the stimulated flows of submandibular/sublingual glands were also below normal in 56 of 64 patients. In our study, patients with non-SS-DE also suffered from the sensation of a dry mouth, and the flow rates of their stimulated whole and parotid saliva were lower than those of the control groups. This study also showed that the salivary flow rates in

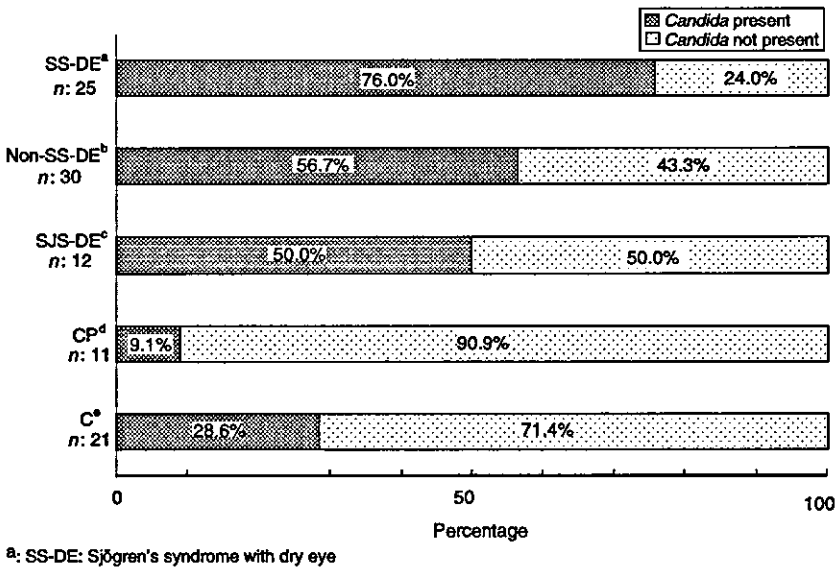

Figure 2 Condition of buccal mucosa in subject groups: SS-DE, Sjögren's syndrome with dry eye; non-SS-DE, dry eye not associated with Sjögren's syndrome; CP, control patients; C, healthy volunteer controls

Table 7 Realtionship between sensation of dry mouth and salivary flow rates in dry eye patients

Salivary flow rates in dry eye patients	*Sensation of dry mouth*		
	Frequent	*Occassional*	*No symptoms*
Stimulated whole salivary flow rate (ml min^{-1})	0.45 ± 0.48 ($n = 43$)	0.60 ± 0.48 ($n = 37$)	1.08 ± 0.67 ($n = 45$)
Stimulated whole salivary flow rate [ml (5 min^{-1})]	2.18 ± 2.03 ($n = 41$)**	2.56 ± 1.98 ($n = 28$)**	3.86 ± 2.14 ($n = 38$)**

Values are give as mean ± s.d.
**$P < 0.01$.

stimulated whole and parotid saliva in SJS-DE were lower than those in the control groups. In fact, SJS patients complained of a sensation of dry mouth and stomatitis. It might be inferred from these data that the salivary gland and oral mucosa were also damaged by an aberrant reaction. Although the ocular and skin manifestations in SJS are often reported, little information is available about the relationship of oral symptoms and salivary flow.

Dry mouth results not only from SS, but also from medical treatments such as medication, radiation, and chemotherapy, which all decrease salivary flow. Multiple medications in older patients make them more susceptible to dry mouth. Sreebny and Schwartz (1986) described how increased drug intake was positively correlated with age. Further, the total number of drugs taken was positively correlated with the prevalence of xerostomia. Wijers *et al* (2002) demonstrated that of 39 patients treated with radiation therapy between 1965 and 1995, 64% experienced a moderate to severe degree of permanent xerostomia. With respect to age and salivary flow, resting saliva was negatively correlated with age among females (Heintze, Birkhed and Björn, 1983) and stimulated saliva exhibited no diminution with increased age (Baum, 1981).

It is generally accepted that inadequate salivation is commonly associated with increased dental caries (Papas *et al*, 1993), complaints of frequent sores on the tongue, cheeks, and lips, and the growth of *Candida*. Our studies showed that dry eye patients exhibited a high DMFT index, high detection rate of oral *Candida*, and changes in oral soft tissues. The DMFT index of 27–61-year-old SS patients reported by Christensen *et al* (2001) was similar to that found in the present study. Furthermore, patients with SJS-DE suffering from dry eye also had a higher prevalence of decayed and missing teeth. These results suggest that an insufficient amount of saliva can induce dental caries. It may be difficult for such patients to visit a dental clinic alone because of the loss of their eyesight, which could therefore also affect their oral care.

Rhodus *et al* (1997) stated that 80% of all SS patients were positive for *C. albicans* but that the amount of *C. albicans* was not related to the salivary flow rate. However, other researchers have reported that the prevalence of *C. albicans* was significantly higher at almost all sites in SS patients and that there was an approximately inverse relationship between the *Candida* population and the rate of salivary flow (Tapper-Jones, Aldred and Walker, 1980; Torres *et al*, 2002). Our study also demonstrated that over half of dry eye patients had *Candida* (76% of SS-DE, 56.7% of non-SS-DE, and 50.0% of SJS-DE) but the presence of *Candida* was not correlated with a decrease in either of whole and parotid salivary flow rates.

Soft tissue changes were more prevalent in dry eye patients. These finding are not related to the volume of the stimulated salivary flow rates. Regarding oral mucosal pathology, Sweeney *et al* (1994) demonstrated that 24% of geriatric patients had denture stomatitis, 16% had angular cheilitis, and 41% had atrophic glossitis. In that study, patients with mucosal disease had significantly lower serum iron concentrations in their blood. Other researchers showed that the prevalence of *Candida*, fissured tongue, irritation fibroma, and traumatic ulcers were seen more frequently in patients with insulin-dependent diabetes (Guggenheimer *et al*, 2000).

Of the dry eye patients in this study, the volume of both stimulated whole saliva and parotid saliva were low which accounted for over half those having the sensation of dry mouth. However, 18% of dry eye patients whose volume of saliva was similar to the control group did not have the sensation of oral dryness. These results suggest that the association with the sensation of dry mouth was relatively consistent with the objective evaluation about the volume of saliva in dry eye patients.

In conclusion, a sensation of oral dryness frequently occurs in all types of dry eye patients and that the decreased salivary flow rates, changes in oral soft tissues, dental caries, and high prevalence of *Candida* are relevant in dry eye patients. When dry eye patients are treated, their oral conditions should be closely examined.

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Autologous Serum Application in the Treatment of Neurotrophic Keratopathy

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Objective: To evaluate the effect of autologous serum application for epithelial disorders in neurotrophic keratopathy (NK).

Design: Retrospective, noncomparative case series.

Participants: Fourteen eyes of 11 patients with NK seen at Tokyo Dental College, Ichikawa General Hospital, Department of Ophthalmology, were studied.

Intervention: Twenty percent topical autologous serum eye drops were applied 5 to 10 times daily until resolution of the NK. Patients underwent routine ophthalmic examinations, including slit-lamp examination, corneal fluorescein dye testing, Cochet-Bonnet corneal sensitivity (Luneau, France), and best-corrected visual acuity (BCVA) measurements before and at the end of the treatment. Moreover, serum samples from 10 healthy volunteers were studied for the levels of substance P (SP), insulinlike growth factor (IGF-1), and nerve growth factor (NGF) by using radioimmunoassay and enzyme-linked immunosorbent assay techniques. Tear samples from 3 healthy subjects also were analyzed for NGF and IGF-1 levels by the same techniques.

Main Outcome Measures: The changes in corneal disease state, corneal sensitivity, and BCVA with treatment were evaluated. The levels of neural healing factors like SP, IGF-1, and NGF in serum as well as NGF and IGF-1 in tears of healthy subjects also were examined.

Results: The epithelial disorders healed completely in all eyes within 6 to 32 days (mean, 17.1±8.0 days), with a decrease in corneal scarring. The mean pretreatment corneal sensitivity was 11.8±11.6 mm, which increased to 30.0±22.9 mm after treatment at the last follow-up. Five eyes attained normal corneal sensitivity with treatment. The BCVA improved by >2 Landolt lines in 78.6% of the eyes. The mean concentrations of SP in diluted and undiluted serum were 31.4±8.4 pg/ml and 157.0±42.1 pg/ml, respectively. The mean respective concentrations of IGF-1 in diluted and undiluted serum were 31.4±14.8 ng/ml and 157.0±73.9 ng/ml. The mean concentrations for NGF were 93.6±63.5 pg/ml and 468.3±317.4 pg/ml in serum samples with and without dilution, respectively. The mean concentration of NGF in tears was found to be 54 pg/ml. Insulinlike growth factor 1 was not detected in tears in this study.

Conclusions: Autologous serum harbors neurotrophic factors. Autologous serum treatment may provide neural healers to the compromised ocular surface and seems promising for the restoration of the ocular surface epithelial integrity in patients with NK. *Ophthalmology 2004;111:1115–1120 © 2004 by the American Academy of Ophthalmology.*

Numerous ocular and systemic diseases, including viral keratitis, chemical or thermal burns of the ocular surface, fifth nerve palsy after removal of acoustic neuroma or radiation, drug toxicity, corneal surgery, multiple sclerosis, and diabetes, may lead to neurotrophic keratopathy (NK), which presents with a broad spectrum of corneal findings, including superficial punctate keratitis, epithelial defects, ulceration, and perforation.[1]

The treatment of NK still poses a challenge to many ophthalmologists. Current therapeutic modalities include the use of bandage contact lenses, topical artificial tears, sodium hyaluronate,[2] fibronectin,[3,4] substance P (SP), insulinlike growth factor 1 (IGF-1),[5,6] or nerve growth factor (NGF) eye drops.[7,8] Reported surgical treatment options include applications of cyanoacrylate glue,[9] conjunctival flap,[10] tarsorrhaphy, and amniotic membrane transplantation.[11] Recently, autologous serum eye drops have been reported to contain various growth factors and have been used successfully to treat persistent epithelial defects and dry eyes in patients with Sjögren's syndrome.[12,13] With the

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belief that serum may contain neurotrophic factors and neurotransmitters, we investigated the levels of SP, IGF-1, and NGF in serum as well as IGF-1 and NGF levels in tears of healthy subjects and also evaluated the effects of topical application of autologous serum in the management of epithelial disorders in NK.

Materials and Methods

Subjects and Examinations

The charts for 14 eyes of 11 patients (5 females and 6 males) with NK who did not respond to previous conventional treatment with therapeutic soft contact lenses, artificial preservative-free tears, and sodium hyaluronate eye drops for 10 weeks and who did not choose to undergo a tarsorrhaphy procedure were reviewed in this study. The mean age of the patients was 60.7±17.7 years (range, 21-91 years). All patients had established low corneal sensitivity as measured by a Cochet-Bonnet esthesiometer (Luneau, France). Subjects underwent ophthalmic examinations, including best-corrected Landolt visual acuity measurements, biomicroscopy, anterior segment photography, corneal sensitivity measurements, and corneal fluorescein staining before and after treatment with autologous serum eye drops. At ocular examination, particular attention was paid to lids, lid margins, tarsal and bulbar conjunctiva, and cornea. Informed consent about the procedures was obtained from the patients.

Measurement of corneal sensitivity was performed using a Cochet-Bonnet esthesiometer. The measurements were begun with the nylon filament fully extended. The tip of the nylon filament was applied perpendicularly to the surface of the cornea, making certain not to touch the eyelashes, and was pushed until the fiber's first visible bending. The length of the fiber was decreased gradually until a blink reflex was observed. The length was recorded in millimeters. Measurements were taken from the central, superior, inferior, nasal, and temporal areas of the cornea, and the mean of the measurements was recorded as the corneal sensitivity reading of that eye. A corneal sensitivity measurement of less than 50 mm was regarded as low corneal sensitivity in this study.

Fluorescein vital staining was performed using a fixed concentration (1%) and 2-μl volume of dye to obtain stable results. The dye was applied to the conjunctival fornices with a micropipette and the patients were asked to blink several times. Using this examination, we classified the eyes with NK according to the Mackie classification (Table 1). Briefly, corneal epithelial disorders were recorded as stage 1 NK with superficial punctate keratitis, stage 2 disease with epithelial defects, and stage 3 disease with corneal ulcers.

Preparation of Autologous Serum

Twenty milliliters of blood from each patient was procured by venopuncture and centrifuged for 10 minutes at 3000 rpm. The serum was separated carefully in a sterile manner under clean bench conditions and was diluted by sterile saline solution to 20%. The final preparation was allocated into 5-ml bottles. Patients were instructed to keep their current bottles in a dark, cool place under refrigeration at 4° C and the remaining bottles in a freezer at −20° C until required.

Application of Autologous Serum

Serum drops were applied 5 to 10 times daily as required in addition to preservative-free artificial tears, 0.1% hyaluronic acid (Santen Pharmaceutical Co, Osaka, Japan), and levofloxacin eye drops (Santen Pharmaceutical Co., Osaka, Japan). All topical medications were discontinued with the resolution of the corneal lesions except autologous serum eye drops, which were instilled continuously 3 to 4 times daily afterward in patients with low corneal sensitivity.

Table 1. Clinical Staging of Neurotrophic Keratopathy

Stage 1
Rose bengal staining of the palpebral conjunctiva
Decreased breakup time
Increased viscosity of tear mucus
Punctate corneal keratopathy
Dellen
Small facets of drying epithelium (Gaule spots)
Superficial vascularization
Stromal scarring
Epithelial hyperplasia and irregularity
Hyperplastic precorneal membrane
Stage 2
Epithelial deficit, usually in the superior half of the cornea
Surrounding rim of loose epithelium
Stromal edema
Anterior chamber inflammatory reaction
Edges of the defect becoming smooth and rolled with time
Stage 3
Corneal ulcer
Stromal melting
Perforation

Measurement of Substance P, Insulinlike Growth Factor 1, and Nerve Growth Factor in Serum and of Nerve Growth Factor in Tears

The concentrations of SP and IGF-1 in stored serum samples obtained from healthy volunteers (n = 10) were measured within 3 months of preservation at −80° C. The concentrations of SP and IGF-1 in serum were measured by radioimmunoassay methods as previously reported.[14,15] Insulinlike growth factor radioimmunoassay kits were obtained from Daiichi Radioisotope Laboratories (Tokyo, Japan). The NGF in stored serum samples obtained from healthy volunteers (n = 12) was measured within 1 month of preservation at −80° C. The concentrations of NGF were measured by enzyme-linked immunosorbent assay (ELISA) methods as described elsewhere.[16] Using the same method, the concentrations of NGF and IGF-1 in tears obtained from healthy volunteers (n = 3) were measured within 1 month of preservation at −80° C. One hundred microliters of tear samples were obtained by micropipettes from the subjects for the assays. Insulinlike growth factor ELISA kits were obtained from R&D Sys (Minneapolis, MN). The ELISA kits for NGF were obtained from Roche Molecular Biochemicals (Mannheim, Germany).

Statistical Analysis

Variance analyses was performed by the paired *t* test to detect statistical significance related to best-corrected visual acuity (BCVA) and corneal sensitivity measurements. The probability level for statistical significance was set at 5% in this study.

Results

Clinical Features

The neurotrophic status was the result of diabetes mellitus in 7 eyes, herpes zoster ophthalmicus in 3 eyes, herpes simplex kera-

Table 2. Clinical Features of Neurotrophic Keratopathy

Patient No.	Age (yrs)	Gender	Laterality	Cause	Eye History	Systemic Disease	Stage of Neurotrophic Keratopathy	Defect Size (mm)	Ulcer Depth (%)	Epithelial Healing (days)	Follow-up (mos)
1	60	F	RE	Diabetes	DE	DM, SS	1	NA	NA	20	28
			LE	Diabetes	DE	DM, SS	3	3 × 3	50	32	28
2	21	F	RE	Diabetes	AAU	DM, SS	2	4 × 7	NA	21	24
			LE	Diabetes	AAU	DM, SS	2	3 × 3	NA	21	24
3	48	M	RE	Diabetes		DM	3	3 × 3	90	17	4
			LE	Diabetes		DM	3	2 × 3	70	17	4
4	91	M	LE	Diabetes	IOL	DM	2	3 × 2	NA	7	6
5	70	F	RE	Herpes zoster			1	NA	NA	30	18
6	75	M	RE	Herpes zoster	DE		2	1 × 1	NA	14	24
7	57	M	LE	Herpes zoster	DE		2	3 × 3	NA	9	36
8	64	F	LE	Herpes simplex	Iritis	DM	2	2 × 2	NA	24	10
9	78	M	RE	Herpes simplex	IOL, Gla		2	4 × 4	NA	7	29
10	48	M	RE	Keratoplasty	KC		2	1 × 1	NA	14	4
11	56	F	RE	Neurosurgery		DM, AN	2	3 × 3	NA	6	13

AAU = acute anterior uveitis; AN = acoustic neuroma; DE = dry eye; DM = diabetes mellitus; F = female; Gla = glaucoma; IOL = intraocular lens; KC = keratoconus; LE = left eye; M = male; NA = not applicable; RE = right eye; SS = Sjögren's syndrome.

titis in 2 eyes, and removal of acoustic neuroma with neuroparalysis in 1 eye. After partial penetrating keratoplasty for keratoconus, NK developed in 1 other eye (Table 2). Associated systemic and ophthalmic diseases also are shown in Table 2. The mean follow-up period was 15.6±10.8 months (range, 4–36 months). There were no systemic or local side effects that emerged from autologous serum treatment.

Slit-Lamp Findings

Slit-lamp biomicroscopy of the eyelid margins and conjunctiva did not reveal any coexistent blepharitis, meibomian gland disorder, or conjunctivitis.

Two eyes had stage 1 NK with extensive superficial punctate keratitis, 9 eyes had stage 2 disease with epithelial defects, and 3 eyes had stage 3 disease with corneal ulcers without signs of active inflammation before treatment. The epithelial disorders healed completely in all eyes within 6 to 32 days (mean, 17.1±8.0 days), with a decrease in corneal scarring and no corneal neovascularization. Figure 1 shows the healing of stage 2 NK, whereas Figure 2 shows healing of stage 3 NK in a patient with diabetes after serum treatment. There were no recurrences during the follow-up period, except 1 patient with diabetic keratopathy who relapsed twice but responded to our protocol with autologous serum eye drops each time.

Corneal Sensitivity

The mean pretreatment corneal sensitivity was 11.8±11.6 mm, which increased to 29.0±22.9 mm after treatment at the last follow-up ($P<0.005$). Nine eyes (64.2%) had improvement of corneal sensitivity, with 5 eyes attaining normal corneal sensitivity with treatment (Fig 3). Three eyes with diabetes, and eyes with corneal hypoesthesia after penetrating keratoplasty and acoustic neuroma removal, did not have any improvement of corneal sensitivity.

Best-Corrected Visual Acuity

As shown in Figure 4, the BCVA significantly improved with treatment ($P<0.005$). The BCVA improved by >1 Landolt lines in 85.8% of eyes (12 eyes). An improvement of more than 2 lines was observed in 78.6% of eyes (11 eyes). The mean pretreatment log visual acuity was +1.00±1.19 and the mean value after treatment was +0.25±0.48.

Analysis of Serum and Tears

The mean concentrations of SP in diluted and undiluted serum were 31.4±8.4 pg/ml and 157.0±42.1 pg/ml, respectively. Likewise, the mean respective concentrations of IGF-1 in diluted and undiluted serum were 31.4±14.8 ng/ml and 157.0±73.9 ng/ml. The mean concentrations for NGF were 93.6±63.5 pg/ml and 468.3±317.4 pg/ml in serum samples with and without dilution, respectively. The mean concentration of NGF in tears was found to be 54 pg/ml. In this study, IGF-1 was not detected in tears.

Discussion

Although several medical and surgical treatments have been reported for the treatment of NK, its management is still a problem for the ophthalmologist. Conventional medical therapeutic modalities, including preservative-free artificial tears, sodium hyaluronate eye drops, patching, and soft contact lens applications, are useful, but they are not thoroughly effective in each case. Recent experimental and clinical research into the use of neuropeptides and growth factors has opened new perspectives for the cure and prevention of NK. Indeed, SP[17,18] and calcitonin gene-related peptide[19] have been shown to induce the proliferation of corneal epithelial cells in vitro. The depletion of these substances induced by mechanical or chemical injury on the cornea was observed to be associated with impairment of corneal sensitivity.[20,21] Recent reports point to complete recovery of NK in patients receiving combined SP and IGF-1 eyedrops, suggesting that their combined use stimulates corneal epithelial migration and the expression of integrin a5 and b1, which are essential for the attachment of epithelial cells to the extracellular matrix protein.[5,6,22,23]

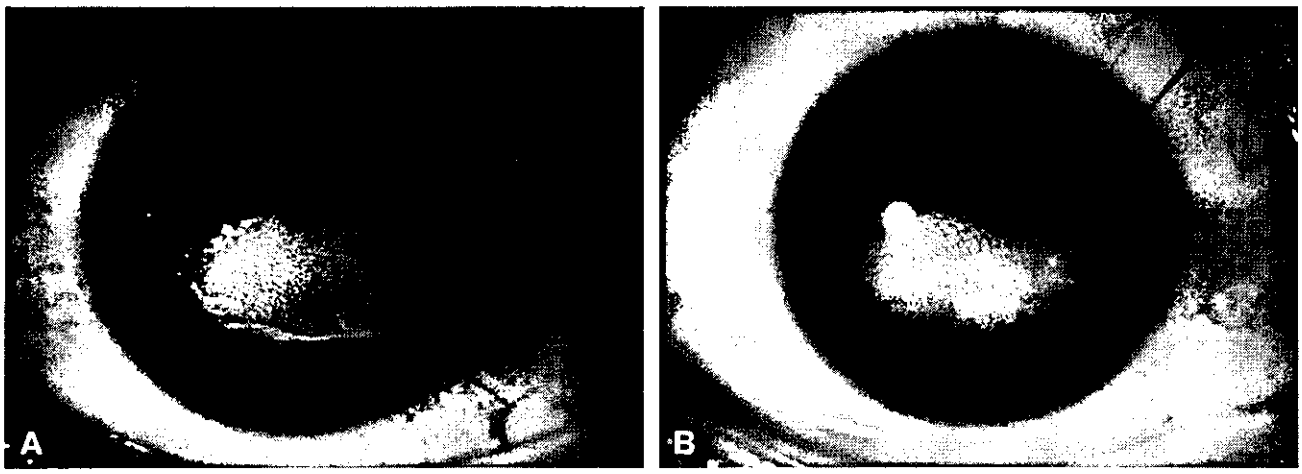

Figure 1. A, Diabetic stage 2 neurotrophic keratopathy before treatment by topical application of autologous serum. Note the central corneal epithelial defect and haze. **B,** The corneal epithelial defect healed within 21 days with a notable decrease in corneal haze.

Other reports state the efficacy of the novel use of NGF, the best known neurotrophin, in resurfacing corneal ulcers resulting from NK.[7,8] It is well known that NGF induces neurite sprouting by neural cells and restores the function of injured neurons. Nerve growth factor also has been shown to induce production of SP and calcitonin gene-related peptide in the central and peripheral nervous system.[24–26] The biologic effects of NGF on the ocular surface are known to be mediated by specific receptors localized on corneal and conjunctival epithelial cells and immune cells.[27]

We previously reported that autologous serum eye drops are beneficial for the treatment of ocular surface disorders such as persistent epithelial defects, superior limbic keratoconjunctivitis, and dry eyes.[12,13,28] Autologous serum is believed to facilitate epithelialization resulting from induction of cellular migration and adhesion because it harbors epidermal growth factor, transforming growth factor-β, hepatocyte growth factor, fibronectin, and vitamin A.[29] We believed that autologous serum could harbor and provide neurotrophins and nerve-healing factors to a compromised ocular surface with NK. An investigation into the levels of NGF, SP, and IGF-1 in serum of healthy subjects showed that neural and epithelial healers such as NGF, IGF-1, and SP were present in diluted and undiluted serum. A PubMed and MEDLINE search revealed that NGF, IGF-1, and SP levels have not been reported in autologous serum eye drops. We also provided information on the tear NGF levels for the first time. We could not detect IGF-1 in tears, which might have been the result of either the scarcity of IGF-1 in tears or the low sensitivity of the measurement procedure used. Our previous investigations showed that the tear SP level measured by ELISA was 70.9 ± 34.8 pg/ml.[30] These findings confirm that autologous serum not only contains NGF and SP in concentrations several times higher than in tears, but also harbors IGF-1. It is our belief that autologous serum helped in the healing of NK by providing lubrication as well as substrates for epithelialization and nerve healing. An increased nerve trophism on the ocular surface in patients with corneal sensitivity recovery might have helped the overall healing process.

Five eyes attained normal corneal sensitivity with treatment, and 4 eyes had partial improvement. It is also possible

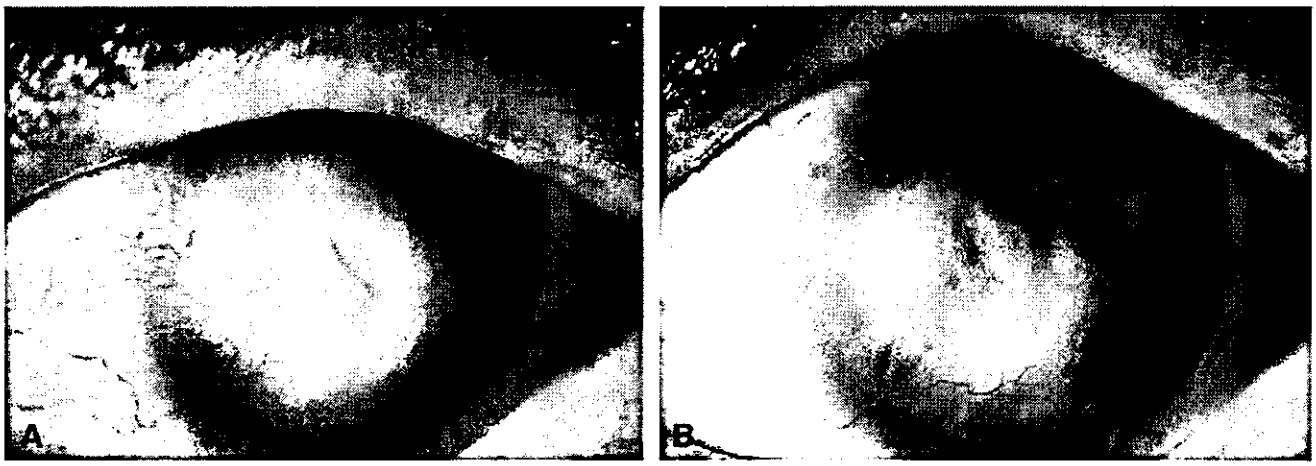

Figure 2. A, Stage 3 neurotrophic keratopathy in a patient with diabetes before treatment. Note the central corneal ulcer with infiltration and neovascularization. **B,** The corneal ulcer healed in 17 days with a considerable decrease in central corneal haze.

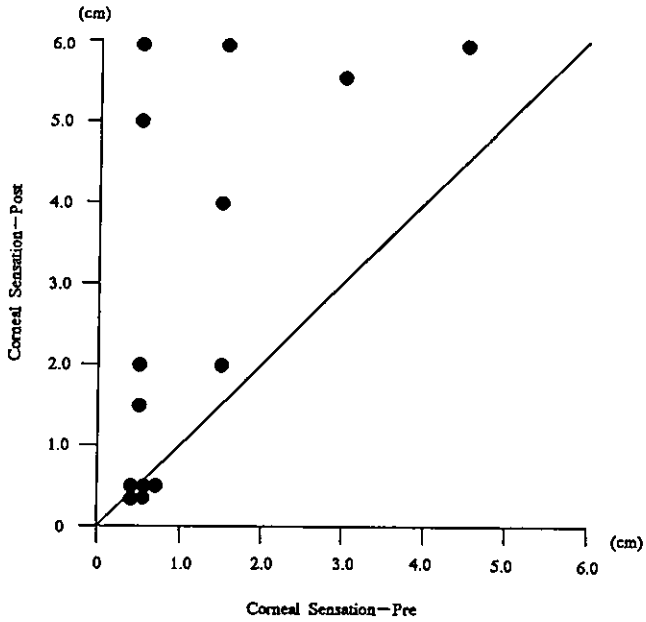

Figure 3. Changes in corneal sensation before and after treatment

to note further recovery of corneal sensitivity in the eye with corneal hypoesthesia developing after partial penetrating keratoplasty that had an overall follow-up of 4 months because it has been documented that it takes between 18 to 24 months for the central area of a corneal graft to be reinnervated.[31,32]

We believe that there should be a threshold corneal sensitivity limit that maintains the ocular surface epithelial milieu, epithelial functions, and integrity and that below this limit, the epithelium disintegrates. Thus, we were surprised to see that the corneal epithelial disease healed and remained healed, even in patients with very low corneal sensitivity who did not show improvement with treatment. We think that this outcome was the result of a continuous

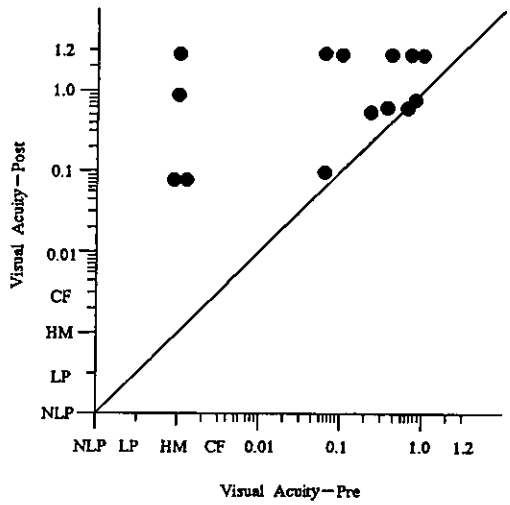

Figure 4. Changes in visual acuity before and after treatment. CF = counting fingers; HM = hand movements; LP = light perception; NLP = no LP.

supply of neurotrophic and epithelium-maintaining factors by the autologous serum eye drops.

We also noted that autologous serum treatment was associated with an improvement of BCVA. The epithelial defects themselves or the peripheral haze associated with the defects involved the central cornea in our cases. Increased corneal surface regularity with a decrease in the intensity of corneal scarring should have contributed to the increase in visual acuity. It will be interesting to conduct comparative-controlled studies evaluating the visual and healing effects of different treatment protocols, such as topical nerve growth factor, autologous serum, tarsorrhaphy, bandage contact lenses, and amniotic membrane transplantation, in the future.

The main disadvantage of treatment with serum drops is the inconvenience of preparing and storing autologous serum and the potential for bacterial contamination, requiring tedious handling and preparation under clean bench conditions.

In conclusion, we noted that autologous serum harbors neurotrophic factors such as NGF, IGF-1, and SP. Autologous serum treatment seems promising for the restoration of the ocular surface epithelial integrity in patients with NK and was associated with visual recovery as well. We tried to provide some understanding of the disease process in NK and the clinical outcome with autologous serum treatment, and we emphasized the unresolved issues. We strongly believe that further work must be initiated and continued along these lines.

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Therapeutic Effect of Cevimeline on Dry Eye in Patients with Sjögren's Syndrome: A Randomized, Double-blind Clinical Study

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• PURPOSE: Sjögren's syndrome (SS) is a systemic autoimmune disease characterized by salivary and lacrimal glandular destruction leading to symptoms of dry mouth and dry eye. Dryness can also occur in the absence of glandular destruction. Patients with SS have autoantibodies that bind to muscarinic acetylcholine receptors in the exocrine glands. Recently, a muscarinic acetylcholine receptor agonist, cevimeline, has been approved for use against symptoms of dry mouth in patients with SS. In this study, the efficacy of cevimeline in improving symptoms of dry eye was examined.

• DESIGN: Prospective, randomized, double-blind, multicenter clinical study.

• METHODS: Sixty patients were randomly assigned to three groups—placebo; cevimeline, 20 mg three times daily; or cevimeline, 30 mg three times daily—and received treatment for 4 weeks. Patients were evaluated before treatment, at week 2, at the end of treatment, and at the end of a 2- to 4-week follow-up period.

• RESULTS: Compared with the placebo, statistically significant differences were seen with cevimeline, 20 mg three times daily, in subjective symptoms, tear dynamics, condition of the corneoconjunctival epithelium, and global improvement rating. No difference was found among the three groups regarding the safe use of the drug.

• CONCLUSIONS: These results indicate that cevimeline, 20 mg three times daily, is safe and effective in improving symptoms of dry eye in patients with SS. Additional studies, with larger patient populations, are needed to further assess the effectiveness of cevimeline for dry eye. (Am J Ophthalmol 2004;138:6–17. © 2004 by Elsevier Inc. All rights reserved.)

Biosketch and/or additional material at www.ajo.com

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SJOGREN'S SYNDROME (SS) IS A SYSTEMIC AUTOIMmune disease of unknown etiology characterized by progressive lymphocytic and plasma cell infiltration of the salivary and lacrimal glands.[1,2] The infiltrating lymphocytes play a significant role in glandular destruction, which leads to symptoms of dry mouth and dry eye.[2] However, glandular destruction appears not to be the sole cause of dryness in patients with SS.[3,4] Exocrine protein secretion is mainly controlled by acetylcholine acting through muscarinic acetylcholine receptors.[5] Antibodies directed against these muscarinic acetylcholine receptors have been implicated in the pathophysiology of dryness.[4] Studies have shown that patients with SS have autoantibodies in their sera that bind to the M3 muscarinic acetylcholine receptor.[5]

Dry eye is a syndrome comprising various pathologic conditions and related diseases.[6] A variety of definitions and diagnostic criteria have been used at different institutions. In Japan, dry eye is now defined as a corneoconjunctival epithelial disturbance caused by qualitative or quantitative abnormality of tears (layers).[6,7] Dryness of the eyes results in part from decreased tear flow. Symptoms related to dryness, such as discomfort, irritation, and pain, result from increased friction of the upper eyelid's movement across the eyeball.[2,8] Treatment for ocular dryness has for the most part been restricted to symptom relief and includes the use of artificial tears (with or without preservatives) and sodium hyaluronate eyedrops[9] or special glasses that prevent moisture loss.[10] In patients with SS who have severe dryness of the eyes, quantitative tear replacement and moisture retention using artificial tears

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and sodium hyaluronate eyedrops produce only a slight improvement of corneoconjunctival epithelial damage and symptoms related to ocular dryness. Hence, it is also necessary to perform treatment with proteins such as growth factors and cytokines to maintain a normal ocular surface.[11] Punctum occlusion, including insertion of punctum plugs,[12] to retain the existing small volume of tears and treatment with autologous serum eyedrops prepared from serum (containing similar active proteins to tears, although in different proportions)[13] have been reported, but these approaches cannot achieve complete relief because of differences in normal lacrimal secretion. Antibiotics, antiallergic agents, or anti-inflammatory agents can also be used if infection, allergy, or inflammation is present.[2,14,15]

Recently, the U.S. Food and Drug Administration approved cevimeline (Evoxac, Daiichi Pharmaceutical Co., Montvale, New Jersey, USA) as an oral agent for the treatment of symptoms of dry mouth in patients with SS. Cevimeline binds to muscarinic acetylcholine receptors in exocrine glands, specifically to those of the M3 subtype.[16] Randomized, double-blind, controlled clinical studies have shown that cevimeline increases salivary secretion and improves subjective symptoms of xerostomia in patients with SS.[17]

Patients in clinical studies of cevimeline who received the active medication also reported an improvement in their dry eye symptoms by piocarpine[18] and cevimeline.[19] An increase in lacrimal flow was observed as well,[19] however, other finer details of improvement of dry eye in SS is still unclear. We undertook this randomized study to further evaluate the efficacy of cevimeline in improving dry eye symptoms in patients with SS and to determine the necessary dosage. Three groups of patients receiving the placebo or cevimeline 20 mg or 30 mg three times daily were examined in a double-blind comparative study for the effect of treatment on dry eye associated with SS.

DESIGN

THIS WAS A PROSPECTIVE, RANDOMIZED, DOUBLE-BLIND, multicenter clinical study.

METHODS

PATIENTS WERE ENROLLED IN THIS STUDY IF THEY HAD confirmed or suspected SS. The diagnosis was based on the criteria established by the Research Committee on Sjögren's Syndrome of the Japanese Ministry of Health and Welfare (1978).[20] Enrolled patients suffered from dry eye. Study patients were 20 years of age or older and were treated on an inpatient or outpatient basis at three institutions: Tokyo Dental College, Ichikawa General Hospital, Chiba, Japan; Tokyo Women's Medical University, Tokyo, Japan; and Osaka Seamen's Insurance Hospital, Osaka, Japan. Subinvestigators gave patients adequate information about the trial using documents for explanation and obtained patients' voluntary written agreement to participate in this trial. Institutional Review Board approval was not required for this study.

Patients were excluded from the study if they had obviously decreased drainage through the lacrimal punctum after measurement by tear clearance test one, including those with punctal occlussion,[21] large variations in the results of tear dynamics or keratoconjunctival epithelium examinations within the 6-month period before the start of this trial, glaucoma or ocular hypertension, infectious external ophthalmopathy (active bacterial/viral infection), diabetic keratopathy, neurotrophic keratopathy, allergic conjunctivitis, marked salivary gland swelling and pain, drug allergies, or complications involving one of the following: pancreatitis, intestinal hypersensitivity disorders, peptic ulcer, asthma, angina, myocardial infarction, severe hepatic dysfunction, renal dysfunction, or pancreatic dysfunction. Also excluded were patients who had used cholinergic or anticholinergic agents, patients who were participating in other clinical trials, and women who were, might be, or wanted to become pregnant or were lactating.

The following information was gathered for each patient before treatment and recorded: gender, age, body weight, medical history, diagnosis and its rationale, duration of disease, complications, prior therapy, histologic results of lacrimal or salivary gland biopsy, and serology (IgG, IgM, IgA, IgE, RF, RAHA, antinuclear antibody, anti-RNP antibody, anti-SSA antibody, anti-SSB antibody, RAST, EBNA, VCA-IgG, VCA-IgK, EBDA-IgG, EBDA-IgM, and EBDA-IgA).

• RANDOMIZATION AND DOSING: The study medication was prepared in white capsules containing either placebo, 20 mg of cevimeline, or 30 mg of cevimeline and was randomly distributed to each group of six patients at a ratio of 1:1:1. A controller was selected who ensured that the three test medications were indistinguishable. The controller randomly assigned patients to a test group. A key code was prepared and sealed to be opened after completion of the study. An emergency key code was also prepared and sealed. Patients received the study medication orally, three times a day, usually after meals, for a total daily dose of 0, 60, or 90 mg of cevimeline. The study period consisted of 4 weeks of treatment with the test drug followed by a 2- to 4-week follow-up period. Patients were evaluated at the start of treatment, at week 2, at the end of treatment, and at the end of the follow-up period.

• CONCOMITANT DRUGS: The following drugs were in principle not prescribed throughout the study period: ophthalmic solutions containing a β-blocker; parotin (a salivary gland hormonal agent); anetholtrithion (a chola-

gogue); bromhexine hydrochloride (an expectorant); bakumondo-to (Kampo [Japanese herbal] medicine); koujin powder (a herbal medicine); and other drugs that could affect tear and saliva secretion.

In the event that it was essential to use any of the above medications to treat a patient's disease and complications, the patient was allowed to receive the concomitant drug if the dosage of the concomitant drug was not changed during the course of the study. The type, dosage, and method of administration of the concomitant drugs were recorded on the Case Record Form. The use of ophthalmic solutions was not allowed for one 1 before patient evaluation.

• TREATMENT COMPLIANCE: Unused study medication was recovered at the end of the study period. Compliance with the protocol was confirmed and graded according to the following criteria:

(1) Patient took the test drug according to instructions (more than 90% of drug taken).

(2) Patient sometimes forgot to take the test drug (between 75% and 90% of drug taken).

(3) Patient took approximately half the test drug (between 40% and 75% of drug taken).

(4) Patient took relatively little of the test drug (less than 40% of drug taken).

(5) As part of the study design, patients complying less than 75% were to be excluded. There was a high compliance rate overall.

• PATIENT EVALUATIONS AND MEASUREMENT OF EFFICACY: In general, patient examinations were conducted at week 2 and at the end of the dosing period, 1 to 2 hours after the test medication was administered. The order of the examinations were (1) rose bengal and fluorescein staining using 1-μl solution; (2) Schirmer testing and tear clearance over 20 minutes after the staining.

• IMPROVEMENT OF CORNEOCONJUNCTIVAL EPITHELIUM: The following tests were performed to assess improvement of the corneoconjunctival epithelium.

Rose bengal/fluorescein staining: The corneoconjunctival epithelium was stained with 2 μl of a solution containing 1% fluorescein and 1% rose bengal in a physiologic saline solution. After the patient had blinked and rolled the eyes several times, the degree of staining was determined according to the methods described below and recorded in the following order.[22,23]

Scoring of rose bengal staining: The corneoconjunctival epithelium was divided into seven parts (Figure 1A). The degree of rose bengal staining (0–3 points) of each part was scored and the total score was calculated for evaluation.[22]

Scoring of fluorescein staining: The degree of fluorescein staining of the corneal upper part, corneal center part, and corneal lower part (Figure 1B) was scored under a cobalt blue light according to the grades described above, and the total score was obtained for evaluation.[24]

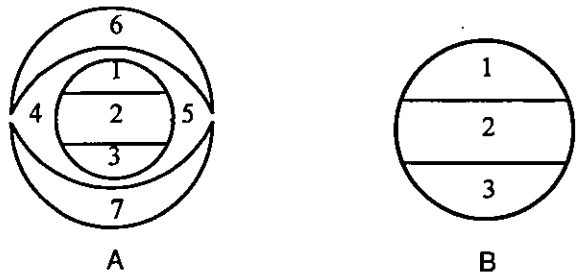

FIGURE 1. (A) Diagram of rose bengal staining. 1 upper part of cornea. 2 central part of cornea. 3 lower part of cornea. 4 nasal bulbar conjunctiva 5 temporal bulbar conjunctiva. 6 upper eye lid conjunctiva. 7 lower eye lid conjunctiva. (B) Diagram of fluorescein staining. 1 upper part of cornea. 2 central part of cornea. 3 lower part of cornea.

Tear break-up time (BUT): The time of tear break-up was counted in seconds from the last blink to the appearance of the first dry spot using the rose bengal/fluorescecein solution, described above, and a cobalt blue light.[25]

Conjunctival congestion: Bulbar conjunctival congestion was judged by an investigator according to the following grades: 0 point = no congestion; 1 point = slight congestion (affecting a part of the conjunctiva); 2 points = moderate congestion (more than half of the conjunctiva); 3 points = severe congestion (entire conjunctiva).

Impression cytology: A cellulose acetate filter paper was placed on the conjunctiva and then removed after pressing several times. The paper was stained with hematoxylin to evaluate presence or absence and density of goblet cells, nuclear histologic changes, and appearance of keratinization in the epithelial cells. Detailed information about the use of impression cytology to evaluate dry eye states has been published elsewhere.[26]

• TEAR DYNAMICS: The following tests were performed to evaluate tear dynamics.

Tear clearance test and Schirmer test with anesthesia:[21] An ophthalmic solution (10 μl) containing 0.1% oxybuprocaine and 0.5% fluorescein was placed in both of the patients' eyes. After 5 minutes, the Schirmer test with anesthesia was done. The dilution rate of fluorescein in the conjunctival sac was measured by the Schirmer test strip with the colorimetric standard chart to determine the degree of dye clearance. The length (in millimeters) of the colored water-absorbed part of the Schirmer test paper was measured.

Tear function index (TFI): The TFI was obtained by multiplying the value obtained in the Schirmer test by the value in the clearance test.[27]

Cotton thread method: A crimped end of a piece of phenol red-impregnated fine cotton thread was placed between the eyelid and globe. The amount of wetting,

which turned yellow to red, was measured in length (millimeters) over 15 seconds.[28]

• VISUAL ANALOG SCALE OF SYMPTOMS: Patients evaluated their symptoms of ocular fatigue, ocular dryness, and eye soreness on a 100-point visual analog scale (VAS) ranging from no symptoms to severe symptoms. Evaluations were performed at the initiation of treatment, at week 2, at the end of treatment, and at the end of the follow-up period.

• USE OF ARTIFICIAL TEARS: Patients were asked four times during the study period—at the start of treatment, at week 2, at the end of treatment, and at the end of the follow-up period—the number of times per day they used ophthalmic solutions. The average number of instillations per day from the last evaluation to the current one was recorded on the Case Record Form.

• OPHTHALMIC AND CLINICAL LABORATORY TESTS: Visual acuity, intraocular pressure, ocular fundus, and pupil size were examined at the initiation and termination of treatment. The following clinical laboratory values were obtained at the initiation and termination of treatment: red blood cell count, hemoglobin, hematocrit value, white blood cell count, platelet count, total protein, albumin, total bilirubin, total cholesterol, neutral fat, urea nitrogen, creatinine, amylase, amylase fraction, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, alkaline phosphatase, leucine aminopeptidase, γ-glutamyl transpeptidase, sodium, potassium, and chlorine.

• DETERMINATION OF ADVERSE EVENTS: The relationship between abnormal parameters of ophthalmic and clinical laboratory tests and the test drug was judged according to the following categories: unrelated, probably unrelated, indefinite, probably related, and clearly related.

After the initiation of treatment, any symptom observed was recorded, with the date of appearance and disappearance, the severity, the action taken, and the outcome. The severity of the symptoms was evaluated as mild (no action taken), moderate (countermeasures were needed), or severe (treatment had to be stopped).

• DETERMINATION OF CLINICAL OUTCOME: Assessment of drug efficacy was performed at the termination of the follow-up period.

Subjective symptoms: Based on the results of four parameters, including three ophthalmic symptoms (ocular fatigue, ocular dryness, and eye soreness) and the number of instillations of ophthalmic solution, the subjective symptoms were judged according to the following rating scale: improved (two or more of four parameters improved); unchanged (other than "improved" or "aggravated"); aggravated (two or more of four parameters were aggravated).

Tear dynamics: Based on the results of four parameters (tear clearance test one, Schirmer test with anesthesia, TFI, and cotton thread test), tear dynamics were judged according to the following rating scale: improved (two or more of four parameters improved); unchanged (other than "improved" or "aggravated"); aggravated (two or more of four parameters were aggravated).

Condition of corneoconjunctival epithelium: Based on the results of five essential parameters (rose bengal staining, fluorescein staining, BUT, conjunctival congestion, and impression cytology), the condition of the corneoconjunctival epithelium was judged according to the following rating scale: improved (two or more of five parameters improved); unchanged (other than "improved" or "aggravated"); aggravated (two or more of five parameters were aggravated).

Overall improvement rating: Based on the results of the three assessments described above, the overall improvement rating was judged according to the following scale: markedly improved (subjective symptoms, tear dynamics, and condition of corneoconjunctival epithelium were improved); moderately improved (subjective symptoms and tear dynamics improved); slightly improved (either subjective symptoms or tear dynamics improved); unchanged (none of the parameters were changed); aggravated (either subjective symptoms or tear dynamics were aggravated).

Safety: Adverse reactions and abnormal changes in clinical laboratory test results were collectively evaluated, and safety was judged according to the following rating scale: safe (no adverse reaction occurred); almost safe (treatment could continue without any action); questionable (countermeasures were needed); not safe (treatment was stopped or should have been stopped).

Efficacy: Overall improvement rating and safety were collectively evaluated, and the efficacy of treatment was judged according to the following rating scale: very useful; useful; slightly useful; indefinite; undesirable[17] (Table 1).

• ANALYTICAL METHODS: In patient demographics, order data, classification data, and metrical data were analyzed using Kruscal-Wallis test,[2] and analysis of variance, respectively. A P value less than .05 was considered significant. Correction would be made when a deviation was observed in the major patient demographics.

Tear dynamics, particularly TFI, and subjective symptoms were compared among the three groups as major parameters. Similar comparisons were made with respect to overall improvement rate, safety, and efficacy as well. For timeline data of laboratory tests, pre- and post-treatment values were compared. For any data measured in both eyes, only those data were used that were measured in the eye that had the lower value when the TFI was calculated for both eyes. The Tukey-Kramer multicomparison test,[2] paired t test, and analysis of variance were used in consideration of characteristics of data, and a P value less than .05 (2-tailed) was considered significant.

TABLE 1. Determination of Efficacy Ratings

		Overall Improvement Rating				
		Markedly Improved	Moderately Improved	Slightly Improved	Unchanged	Aggravated
Safety rating	Safe	Very useful	Useful	Slightly useful	Indefinite	Undesirable
	Almost safe	Useful	Useful/Slightly useful	Slightly useful/Indefinite	Indefinite/Undesirable	Undesirable
	Questionable	Useful/Slightly useful	Slightly useful/Indefinite	Indefinite/Undesirable	Undesirable	Undesirable
	Not safe	Indefinite	Undesirable	Undesirable	Undesirable	Undesirable

TABLE 2. Patient Demographics and Baselines

		Placebo	Cevimeline, 20 mg tid	Cevimeline, 30 mg tid
Gender	M	1	1	1
	F	17	18	14
Age (years)		55.5	56.9	49.5
Status	I	0	0	0
	O	17	19	15
	B	1	0	0
Diagnosis*	+/+	12	17	12
	+/−	6	2	3
Duration of disease (years)	<1	0	3	1
	1–3	5	4	6
	3–5	6	6	2
	5–10	2	2	4
	>10	5	4	2
RB score (BL) ± SD		5.6 ± 3.9	7.4 ± 3.7	7.5 ± 3.9
FL score (BL) ± SD		3.9 ± 2.1	4.9 ± 2.1	4.7 ± 2.0
BUT (BL) ± SD (sec)		1.7 ± 1.0	1.7 ± 2.4	1.3 ± 2.1
SA (BL) ± SD (mm)		2.3 ± 2.0	1.9 ± 2.7	2.5 ± 1.6
TFI (BL)		1.3	0.8	1.2
Ocular fatigue ± SD (mm) (BL)		51.8 ± 34.9	54.6 ± 33.6	67.4 ± 25.6
Dry feeling of eyes ± SD (mm) (BL)		54.6 ± 26.3	57.2 ± 33.2	59.1 ± 28.9
Pain or discomfort in eyes ± SD (mm) (BL)		56.9 ± 29.2	62.9 ± 29.6	76.0 ± 20.0
Number of instillations ± SD (BL)		11.9 ± 13.8	17.0 ± 13.3	10.7 ± 8.7

B = both in- and out-patient; BL = base line, before week 0; BUT = tear break-up time; F = female; FL = fluorescein; I = inpatient; M = male; O = outpatient; RB = rose bengal; SA = Schirmer with anesthesia; TFI = tear function index.

*+/+ indicates a definite diagnosis; +/− indicates a suspected diagnosis.

RESULTS

• PATIENT DEMOGRAPHICS: Sixty patients were included in this study: 20 receiving placebo, 21 receiving 20 mg three times daily of cevimeline, and 19 receiving 30 mg of cevimeline three times daily. Improvement ratings were determined for 52 patients, because 7 patients did not complete the study, and 1 patient did not return to the hospital after signing an informed consent form.

As is shown in Table 2, the majority of the patients were female, corresponding to the demographics of SS, which primarily affects women.[2] The mean age of the trial participants was 54 years. There was a significant difference ($P = .128$; 95% confidence interval [CI], −14.95 to 0.09) between the ages of the patients receiving 20 mg of cevimeline three times daily (57 years) and those receiving 30 mg three times daily (49 years). However, when improvement ratings were compared between groups after all the patients were grouped by 10-year age intervals, a significant difference was not found. Therefore, the data obtained were subjected to analyses without correction. The majority of the patients had a definite diagnosis of SS and were treated on an outpatient basis (Table 2).

The goal was to enroll 80 patients. In an early phase 2 study of cevimeline in Japan for the treatment of xerostomia in patients with SS, the volume of saliva secreted and subjective symptoms with increasing dosages of 10, 20, and 30 mg three times daily were compared. Subjective symp-

TABLE 3. Improvement Rating for Individual Assessment Items After Four Weeks' Treatment

	Placebo n (%)		Cevimeline, 20 mg tid, n (%) (*P* value compared with placebo)		Cevimeline, 30 mg tid, n (%) (*P* value compared with placebo)	
	I	U/A	I	U/A	I	U/A
Subjective symptoms	3 (16.7)	15 (83.3)	9 (47.4) (.46)	10 (52.6)	7 (46.7) (.062)	8 (53.3)
Corneoconjunctival epithelium	6 (33.3)	12 (66.7)	8 (42.1) (.582)	11 (57.9)	10 (66.7) (.056)	5 (33.3)
Tear dynamics*	2 (12.5)	14 (87.5)	8 (47.1) (.031)	9 (52.9)	4 (26.7) (.318)	11 (73.3)
Overall improvement rating	0 (0.0)	18 (100)	6 (31.6) (.009)	13 (68.4)	2 (13.3) (.110)	13 (86.7)

I = remarkably improved, moderately improved; U/A = slightly improved, unchanged, or aggravated.

*Because tear dynamics could not be measured in four of the completed cases, the percentages are based on smaller total numbers than for the other parameters.

toms, including "dry eyes," improved in 27.5% of patients with the first course of treatment (10 mg three times daily) and in 66.7% at the completion of the third course (20 or 30 mg three times daily). Applying these results to this study, with the assumption that the placebo group and the 20 mg three times daily cevimeline group would show 25% effectiveness ratio or the 30 mg three times daily cevimeline group would show 65% effectiveness ratio, at a level of significance (2-sided) of $P < .05$ and with power ($1-\beta$) of 0.8, a sample size of more than 23 cases per group was planned. However, because of difficulty in the enrollment of patients, the study was closed when 60 patients were enrolled over a 3-year period.

The baseline of rose bengal score, fluorescein score, BUT, Schirmer with anesthesia, TFI ocular fatigue (VAS), dry feeling of eyes (VAS), pain or discomfort in eyes (VAS) and numbers of instillations are shown in Table 2 with patient demographics. Baselines of rose bengal and fluorescein scores are significantly higher than cevimeline 20 mg three times daily and cevimeline 30 mg three times daily than the placebo. Schirmer with anesthesia and TFI of the placebo show significantly lower data than the 20 mg three times daily at the baseline. Regarding pain or discomfort of the eyes, the data in the placebo is shown to be significantly lower than the 20 mg and 30 mg.

• IMPROVEMENT RATES FOR INDIVIDUAL ASSESSMENTS: Compared with the placebo group, the groups receiving 20 mg three times daily and 30 mg three times daily of cevimeline showed a trend toward improvement in subjective symptoms, tear dynamics, condition of the corneoconjunctival epithelium, and the global improvement rating (Table 3). For the patients receiving cevimeline, 20 mg three times daily, vs the placebo group, statistically significant differences were observed in the ratings of subjective symptoms ($P = .046$), tear dynamics ($P = .031$), and global improvement ($P = .009$).

• CONDITION OF CORNEACONJUNCTIVAL EPITHELIUM: In the rose bengal score, there was a significant difference at week 2 in the group receiving cevimeline, 20 mg three times daily ($P = .018$; 95% CI, −1.71–−.19), and at week 2 and week 4 (the termination of treatment) in the group treated with cevimeline, 30 mg three times daily; ($P = .001$ and .007, respectively; 95% CIs, −3.18–−0.95 and −2.81–0.53)(Table 4, Figure 2).

In the fluorescein score, there was a significant difference at week 2 in the group receiving cevimeline, 20 mg three times daily ($P = .019$; 95% CI, −1.53–−0.16) (Table 4, Figure 3).

Results of BUT revealed a significant difference at week 2 in both treatment groups: $P = .035$ (95% CI, 0.05–1.28) for cevimeline, 20 mg three times daily, and $P = .008$ (95% CI, 0.34–1.80) for cevimeline, 30 mg three times daily (Table 4, Figure 4).

The TFI was obtained by multiplying the value in the Schirmer test by the dilution rate in the clearance test. Table 5 represents the difference in TFI logarithmically converted between pre- and post-treatment values. There was a significant difference at week 2 in the group treated with cevimeline, 20 mg three times daily ($P = .025$; 95% CI, 0.05–0.58).

• CHANGES IN SUBJECTIVE SYMPTOMS: Patient assessments of subjective symptoms, such as ocular fatigue, dry feeling of the eyes, and pain or discomfort in the eyes, were measured before and after treatment and expressed as the difference between those measurements. Greater improvement of symptoms is indicated by a larger difference, that is, a higher minus value.

In the assessment of ocular fatigue, compared with baseline, a significant difference was found for cevimeline, 20 mg three times daily ($P = .004$; 95% CI, −14.44–−3.34), and cevimeline, 30 mg three times daily ($P = .008$; 95% CI, −18.52–−3.35). A significant difference was also observed for the group receiving cevimeline, 20

TABLE 4. Condition of Corneoconjunctival Epithelium*

	Placebo, Mean ± SD (n)	Cevimeline, 20 mg tid, Mean ± SD (n) (*P* value)	Cevimeline, 30 mg tid, Mean ± SD (n) (*P* value)
Rose bengal score (Fig. 3)			
Week 2	−0.6 ± 2.25 (18)	−0.9 ± 1.58 (19)(.018)†	−2.1 ± 2.02 (15)(.001)†
Week 4	−1.3 ± 3.93 (16)	−0.3 ± 2.47 (19)(.585)	−1.7 ± 2.06 (15)(.007)†
End of follow-up period	−0.1 ± 2.42 (18)	−0.5 ± 2.04 (19)(.324)	−1.2 ± 2.78 (15)(.117)
Fluorescein score			
Week 2	−0.3 ± 2.24 (18)	−0.8 ± 1.42 (19)(.019)†	−0.3 ± 1.40 (15)(.371)
Week 4	−0.4 ± 1.86 (16)	−0.5 ± 2.27 (19)(.375)	−0.9 ± 1.85 (15)(.091)
End of follow-up period	−0.3 ± 1.85 (18)	−0.6 ± 2.46 (19)(.318)	−0.5 ± 1.36 (15)(.204)
Tear break-up time (Fig. 4)			
Week 2	0.6 ± 1.50 (18)	0.7 ± 1.24 (18)(.035)†	1.1 ± 1.27 (14)(.008)†
Week 4	0.4 ± 1.15 (16)	0.8 ± 1.65 (19)(.052)	1.1 ± 2.02 (13)(.079)
End of follow-up period	0.2 ± 0.92 (18)	−0.1 ± 2.36 (18)(.922)	0.4 ± 1.78 (14)(.466)
Conjunctival congestion			
Week 2	0.1 ± 0.24 (17)	−0.1 ± 0.34 (16)(.164)	−0.1 ± 0.27 (14)(.336)
Week 4	0.1 ± 0.36 (14)	−0.2 ± 0.40 (16)(.083)	−0.1 ± 0.29 (12)(.339)
End of follow-up period	0.0 ± 0.00 (16)	−0.2 ± 0.41 (15)(.082)	−0.1 ± 0.41 (12)(.339)
Impression cytology			
Week 2		Not examined	
Week 4	−0.2 ± 1.82 (13)	−0.3 ± 1.63 (15)(.442)	0.3 ± 1.27 (11)(.493)
End of follow-up period	1.7 ± 2.08 (3)	0.2 ± 1.92 (5)(.828)	0.7 ± 2.07 (6)(.465)

*Each point minus baseline.
SD = standard deviation.
P value (comared with baseline).
†Statistically significant, $P < .05$.

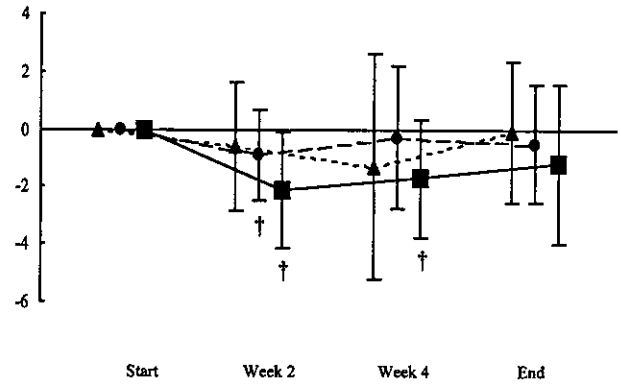

FIGURE 2. Rose bengal score. In the rose bengal test, there was a significant difference at week 2 in the group receiving cevimeline, 20 mg three times daily, and at week 2 and week 4 (the termination of treatment) in the group treated with cevimeline, 30 mg three times daily. (filled triangle) Placebo; (filled circle) 20 mg three times daily; (filled square) 30 mg three times daily. †Statistically significant, P < .05.

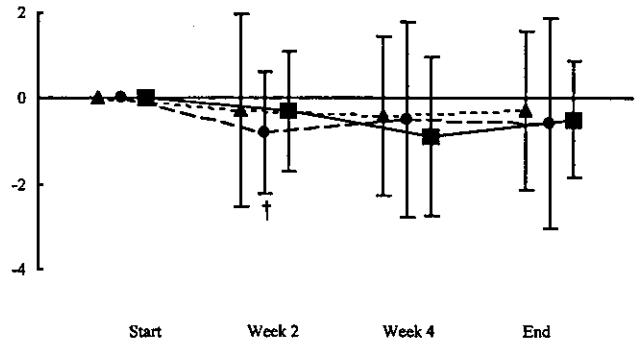

FIGURE 3. Fluorescein score. In the fluorescein score, there was a significant difference at week 2 in the group receiving cevimeline, 20 mg three times daily. (filled triangle) Placebo; (filled circle) 20 mg three times daily; (filled square) 30 mg three times daily. †Statistically significant, P < .05.

mg three times daily, at week 4 ($P = .003$; 95% CI, −15.77–−3.90) and at the end of the follow-up period ($P = .003$; 95% CI, −21.57–−5.13), as well as for the group treated with cevimeline, 30 mg three times daily, at week 4 ($P = .012$; 95% CI, −24.80–−3.74)(Figure 5).

In the assessment of dry feeling of the eyes, the groups treated with cevimeline, 20 and 30 mg three times daily, both showed a significant difference between pre- and post-treatment assessments at week 2 ($P = .015$ and .003, respectively; 95% CIs, −15.39–−1.94, and −20.72–−5.14) and week 4 ($P = .012$ and .003, respectively; 95% CIs, −21.53–−3.02, and −27.22–−6.91). There was also a significant difference at the end of the follow-up period

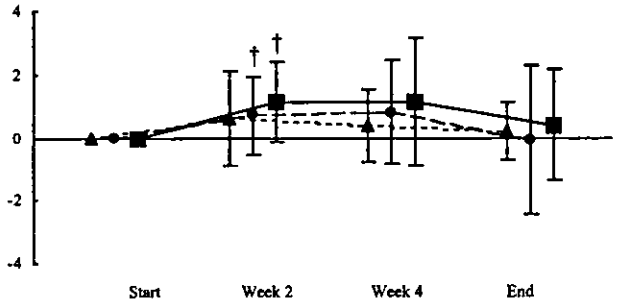

FIGURE 4. Break-up time of tear film. Results of break-up time of tear film revealed a significant difference at week 2 in both treatment groups for cevimeline, 20 mg three times daily, and for cevimeline, 30 mg three times daily. (filled triangle) Placebo; (filled circle) 20 mg three times daily; (filled square) 30 mg three times daily. †Statistically significant, $P < .05$.

for the group that received cevimeline, 20 mg three times daily ($P = .005$; 95% CI, −21.22–−4.55)(Figure 6).

In the assessment of pain or discomfort in the eyes, there were significant differences at week 2, week 4, and termination of the follow-up period in the groups treated with cevimeline, 20 mg three times daily ($P = .023$, .025, and .012, respectively; 95% CIs, −26.65–−2.29, −30.55 to −2.40, and −25.06–−3.53), and cevimeline, 30 mg three times daily ($P = .011$, .004, and .015, respectively; 95% CIs, −23.34–−3.60, −30.39–−6.95, and −22.47–−2.96) (Figure 7).

• **EFFICACY:** Efficacy results are shown in Table 6. Compared with the placebo, the groups treated with cevimeline, 20 and 30 mg three times daily, both showed a trend toward improved efficacy. The efficacy ratings were "useful" or "remarkably useful" for 0%, 23.8%, and 11.8% of patients treated with the placebo, cevimeline, 20 mg three times daily, and cevimeline, 30 mg three times daily, respectively. There was a high compliance rate. All of the 52 patients completing the study were compliant with using 75% or more of the drug or placebo.

• **SAFETY:** Safety results are represented in Table 7. In the group treated with cevimeline, 30 mg three times daily, there was a slightly lower rating of "safe" compared with the other two groups, but the difference was not statistically significant.

Table 8 indicates adverse events recorded during the study period. More patients experienced adverse events in the groups treated with cevimeline, 20 and 30 mg three times daily, than in the placebo group. Gastrointestinal symptoms, including nausea and diarrhea and increased sweating, were the main adverse events observed. All of these symptoms, however, were judged to be mild or moderate and disappeared during treatment or when treatment was discontinued.

DISCUSSION

THIS DOUBLE-BLIND COMPARATIVE STUDY WAS PERformed to determine the optimum dosage of cevimeline for the treatment of dry eye in patients with SS. Cevimeline at a dosage of 20 mg three times daily significantly improved tear dynamics, subjective symptoms, and overall improvement rating compared with the placebo. The dosage of 20 mg three times daily also achieved a significantly higher efficacy rating compared with the placebo. These results indicate that for dry eye 20 mg three times daily is an effective dosage of cevimeline.

Ocular symptoms and BUT show a tendency of a dose response, and fluorescein score and BUT show a tendency of a dose-response at week 4, as shown in Figures 3, 4, 5, and 6. But tear dynamics and rose bengal scores did not show a dose-response. There are a few possibilities for this explanation; (1) variations of the baseline value, (2) plateau of dose response for tear secretion, (3) limitation of measuring tear flow by Schirmer with anesthesia. The patients were randomly enrolled and baselines of each group show several significant differences (Table 2). These data are evaluated as baselines, and if there are some differences, the variances would be minimal. However, in this study, from these baseline results of ocular surface scores and tear dynamics, the small differences of severity of dry eye exist. This might be one of the reasons that there is no existence of dose-response in this study. Petrone and associates reported this improvement of lacrimal flow. Lacrimal flow change did not similarly show a dose-response between pre- and post-dosing. This lack of dose-response of tear dynamics might be a plateau of dose-response for tear secretion, or there might be limitations of measuring tear flow by Schirmer with anesthesia. Also, there is a possibility of some differences that some interaction between antimuscarinic receptor antibodies and the drug, or its interaction with the receptor might exist between the salivary and lacrimal glands. Furthermore, some of these variances may be because of the small size of the patient population studied; the variability is disturbing and questions efficacy. Further research is necessary to solve this point.

For the treatment of dry eyes in patients with SS, quantitative tear replacement using preservative-free artificial tears, closure of the punctum and canaliculi to retain tears, or autologous serum eyedrops are usually employed. However, such methods cannot completely restore tear production. In this study, a statistically significant improvement in subjective symptoms and the condition of the corneoconjunctival epithelium was observed, whereas an increase in tear secretion could not be confirmed. These results might indicate that although cevimeline would induce tear secretion, this increase in secretion could not be detected by the tests used here, namely the Schirmer test and the clearance test. Similar results were obtained in the study of topical cyclosporine.[29] In evaluating drugs

TABLE 5. Modified Schirmer 1* and Tear function Index (TFI)*

	Placebo, Mean ± SD(n) or Mean	Cevimeline, 20 mg tid, Mean ± SD(n) or Mean	Cevimeline, 30 mg tid, Mean ± SD(n) or Mean
Schirmer test with anesthesia (mm)			
Week 2	1.8 ± 4.59 (16)	2.7 ± 7.38 (18)	0.1 ± 2.47 (15)
Week 4	0.8 ± 4.06 (16)	0.7 ± 3.03 (18)	−0.1 ± 1.70 (14)
End of follow-up period	1.4 ± 6.75 (18)	0.9 ± 2.22 (19)	0.4 ± 2.36 (15)
Tear function index			
Week 2	0.0	0.3†	−0.1
Week 4	−0.1	0.1	−0.1
End of follow-up period	−0.1	0.3	0.0

*Each point minus baseline.
†Statistically significant, $P = .025$; 95% CI, 0.05–0.58.

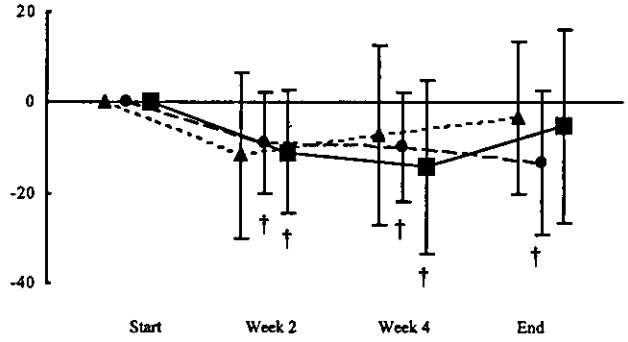

FIGURE 5. Ocular fatigue. In the assessment of ocular fatigue, compared with baseline, a significant difference was found for cevimeline, 20 mg three times daily, and cevimeline, 30 mg three times daily A significant difference was also observed for the group receiving cevimeline, 20 mg three times daily, at week 4 and at the end of the follow-up period, as well as for the group treated with cevimeline, 30 mg three times daily, at week four. (filled triangle) Placebo; (filled circle) 20 mg three times daily; (filled square) 30 mg three times daily. †Statistically significant, $P < .05$.

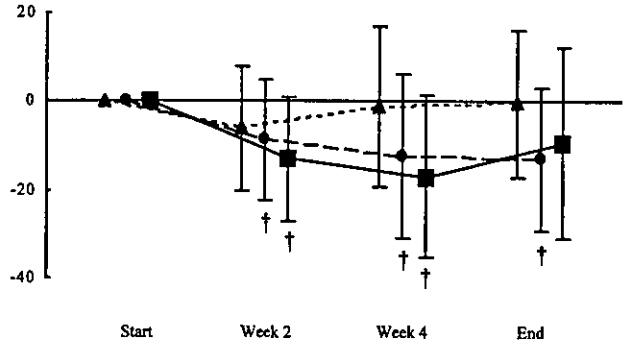

FIGURE 6. Dry feeling of eyes. In the assessment of dry feeling of the eyes, the groups treated with cevimeline, 20 and 30 mg three times daily, both showed a significant difference between pre- and post-treatment assessments at week 2 and week 4. There was also a significant difference at the end of the follow-up period for the group that received cevimeline, 20 mg three times daily. (filled triangle) Placebo; (filled circle) 20 mg three times daily; (filled square) 30 mg three times daily. †Statistically significant, $P < .05$.

such as cevimeline that improve the symptoms of dry eye, the rating of subjective symptoms as well as the condition of the corneoconjunctival epithelium would be appropriate as the major evaluation variable rather than objective measurement of tear secretion.

There was no significant difference in safety among the three treatment groups; however, more adverse events were observed in patients treated with the test drug compared with the placebo. The most common adverse events were gastrointestinal symptoms, including diarrhea and nausea and increased sweating. The adverse events were all considered to be mild or moderate and disappeared during treatment or when treatment was discontinued.

Both improvement of rose bengal and fluorescein scores were relatively small. As part of the criteria when setting up the protocol, "slightly useful" was predetermined to be grouped as "not useful." The improvement rate would have been higher if patients answering "slightly usesful" had been included.

The initial study design called for 80 patients; however, it took 3 years to recruit 60 patients. One of the reasons that the statistical significance of differences among the three groups was not clear for several of the parameters tested could be the insufficient number of patients available for evaluation and analysis.

Among the oral agents for treatment of severe dry eyes, there have been few reports showing an improvement in corneoconjunctival epithelial damage. In the present study, corneoconjunctival epithelial damage was improved, although there was no marked change of tear dynamics. These apparently contradictory results may have

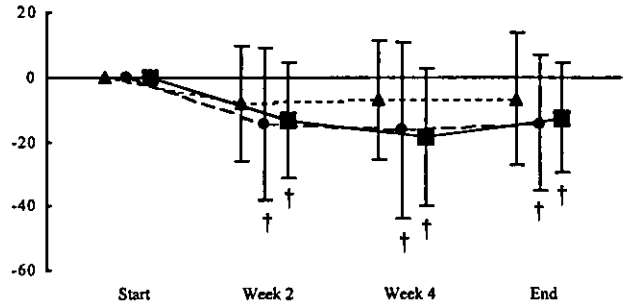

FIGURE 7. Pain or discomfort in eyes. In the assessment of pain or discomfort in the eyes, there were significant differences at week 2, week 4, and termination of the follow-up period in the groups treated with cevimeline, 20 mg three times daily, and cevimeline, 30 mg three times daily. (filled triangle) Placebo; (filled circle) 20 mg three times daily; (filled square) 30 mg three times daily. †Statistically significant, $P < .05$.

TABLE 6. Efficacy*

	Placebo	Cevimeline, 20 mg tid	Cevimeline, 30 mg tid
Useful, n (%)	0 (0.0)	5 (23.8) (0.23)	2 (11.8) (.124)
(*P* value)			
Not useful, n (%)	19 (100)	16 (76.2)	15 (88.2)

P value: compared with placebo.

*"Useful" indicates ratings of "remarkably useful" and "useful"; "not useful" indicates ratings of "slightly useful," "neither useful nor useless," and "not useful."

TABLE 7. Safety*

	Placebo	Cevimeline, 20 mg tid	Cevimeline, 30 mg tid
Safe, n (%)	13 (68.4)	15 (71.4)	8 (47.1)
Unsafe, n (%)	6 (31.6)	6 (28.6)	9 (52.9)

*"Safe" indicates rating of "quite safe"; "unsafe" indicates ratings of "almost safe," "safety questionable," and "unsafe."

TABLE 8. Adverse Events

	Placebo, n (%)	Cevimeline, 20 mg tid, n (%)	Cevimeline, 30 mg tid, n (%)
Gastrointestinal system			
Diarrhea		1 (4.8)	5 (27.8)
Nausea		4 (19.0)	1 (5.6)
Abdominal pain	1 (5)	2 (9.5)	5 (27.8)
Dyspepsia	1 (5)		1 (5.6)
Vomiting		2 (9.5)	
Thirst		2 (9.5)	
Stomatitis	1 (5)		
Rough tongue	1 (5)		
Flatulence			1 (5.6)
Central and peripheral nervous system			
Headache	1 (5)	1 (4.8)	2 (11.1)
Dizziness		1 (4.8)	
Body as a whole			
Hot flashes	2 (10)		
Malaise			1 (5.6)
Swelling of eyelid		1 (4.8)	
Eyes			
Eye sensation			1 (5.6)
Eye pain	1 (5)		
Increased sweating		2 (9.5)	2 (11.1)
Heart rate		1 (4.8)	
Tinnitus		2 (9.5)	
Acute parotitis		1 (4.8)	

been obtained because the increase of tear volume was undetectable by current tear measurement methods, including the Schirmer test with eyedrop anesthesia, the cotton threat method, and the tear clearance test. The minute details of improvement of rose bengal score on cevimeline 30 mg three times daily are shown in Figure 8 to determine whether some areas improved to a greater extent than the others. This tendency of improvement is observed on the nasal and temporal bulbar conjunctiva and central cornea. In this study, the results indicate an overall poor improvement when calculating an average, but the figures indicate the areas that showed greater improvement. Possible changes in secretion by accessory lacrimal glands may be another reason. Assessment of accessory gland function is generally difficult, because of their small size, small amount of secretion, and difficulty in identifying their position and distribution. In patients with SS, secretory dysfunction attributable to the dystrophy of meibomian glands (which are some of the accessory glands) has been reported.[30] Like the main lacrimal gland, the accessory glands are regulated by the nervous system[31–33] and endocrine system, including androgens.[34,35] In the present study, the improvement of BUT and corneoconjunctival epithelial damage, despite no detectable increase in tear volume, may have been because of the improvement of stimulated secretion by the conjunctival goblet cells,[32,33] but further investigation is necessary to allow for the evaluation of this possibility.

Recently, we reported that defective cellular trafficking of lacrimal gland aquaporin-5 in SS might contribute to decreased lacrimation and dry eye in these patients.[36] Also, further investigation might be necessary on how cevimeline improves dry eye in SS and whether cevimeline increases cellular water transportation in the acinar and ductal cells of SS lacrimal glands.

The results show that among patients who participated, there was a trend toward improvement in their symptoms with cevimeline, 20 mg three times daily. With these

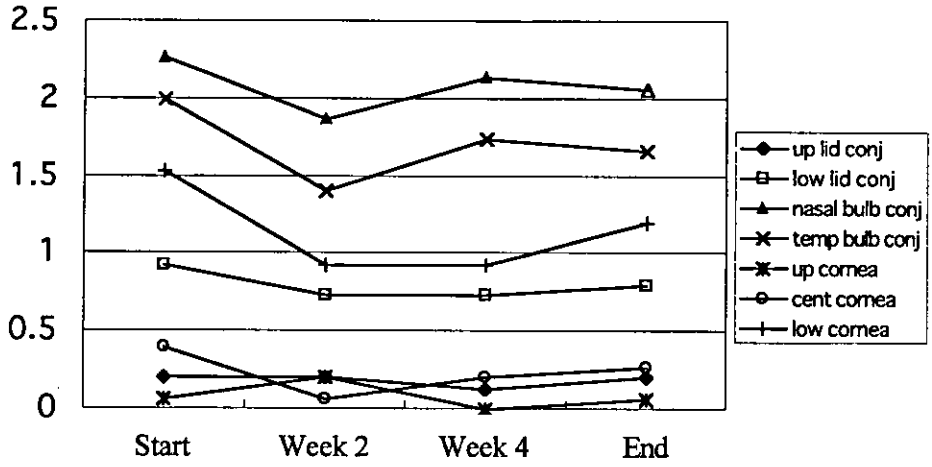

FIGURE 8. Rose bengal staining. Fine detail of rose bengal staining of cevimeline 30 mg three times daily.

encouraging results, further studies are needed to define more clearly the optimum dosage of cevimeline for the treatment of dry eye in patients with SS.

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