Treatment of Chronic Nonhealing Neurotrophic Corneal Epithelial Defects With Thymosin Beta 4

Neurotrophic keratopathy is a degenerative disease of the corneal epithelium and stroma that results from impaired corneal innervation. Reduced corneal sensitivity is responsible for producing recurring or chronic epithelial defects that may lead to subsequent ulceration and/or perforation. It is most frequently associated with topical medications, long-standing diabetes mellitus, herpes zoster ophthalmicus (HZO), herpes simplex keratitis, neurologic disease, or localized trauma.

Conventional treatments include prophylactic topical antibiotic drops or ointment, frequent nonpreserved ocular lubricants, patching, and bandage contact lenses. In recalcitrant cases, oral doxycycline, autologous serum, and the surgical application of an amniotic membrane, tarsorrhaphy, or a conjunctival flap are used alone or in combination. Successful modulation healing in these patients is erratic at best and vexing for both the patient and ophthalmologist.

The potent wound healing and anti-inflammatory effects of thymosin beta 4, a naturally occurring, 43-amino acid, G-actin-sequestering molecule, has been demonstrated in numerous animal and cellular models of corneal injury. We sought to evaluate thymosin beta 4 in a human disorder that did not have an infectious component or one in which stem cell dysfunction or conjunctival disruption was extensive. A preliminary unpublished evaluation of thymosin beta 4 in diabetic corneal defects was encouraging. Here we describe the treatment results of 4 patients with chronic neurotrophic corneal epithelial defects who were treated under a Food and Drug Administration investigational new drug compassionate use protocol (approved by the Wayne State University Human Investigation Committee) with a sterile, single-dose, nonpreserved formulation of thymosin beta 4 eye drops supplied by RegeneRx Biopharmaceuticals, Inc (Rockville, Maryland).

Report of Cases. Case 1. An 81-year-old white man with a 12-year history of diabetes subsequently developed HZO in the right eye with decreased corneal sensation. A persistent right corneal epithelial defect of 11 weeks’ duration developed and was unresponsive to treatment that included nonpreserved lubricants, Vigamox (Alcon, Fort Worth, Texas), and a bandage contact lens. His epithelial defect measured $2.0 \times 1.0$ mm at the start of treatment with 2 drops 4 times daily of thymosin beta 4 for 4 weeks. Treatment with Vigamox 4 times daily and preservative-free tears were continued. The defect was healed on day 25 and remained so during the 30-day follow-up period (Table, Figure 1, and Figure 2).

Case 2. A 47-year-old white woman with a 23-year history of diabetes and associated peripheral neuropathy and retinopathy developed a persistent right corneal epithelial defect of 6 weeks’ duration that was unresponsive to treatment including nonpreserved lubricants, Vigamox, and a bandage contact lens. Corneal sensation was decreased. Her epithelial defect measured $3.5 \times 3.0$ mm prior to treatment with 2 drops 4 times daily of thymosin beta 4 for 4 weeks. Treatment with Vigamox twice daily and erythromycin ophthalmic ointment were continued. On cessation of thymosin beta 4 treatment the defect measured $1.0 \times 0.25$ mm. At

### Table. Patient Demographics and Treatment Response Using Thymosin Beta 4

<table>
<thead>
<tr>
<th>Case No./Sex/Age, y</th>
<th>Eye</th>
<th>Sensation</th>
<th>Diagnosis</th>
<th>Defect Prior to Treatment</th>
<th>Approximate Size of Defect, mm</th>
<th>Day 0</th>
<th>Days 25-28</th>
<th>Days 63-70</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/81</td>
<td>Right</td>
<td>Decreased</td>
<td>HZ ophthalmicus; diabetes; dry eye</td>
<td>11 wk</td>
<td>$2.0 \times 1.0$</td>
<td>$0.0 \times 0.0$, Healed</td>
<td>$0.0 \times 0.0$, Healed</td>
<td></td>
</tr>
<tr>
<td>2/F/47</td>
<td>Right</td>
<td>Decreased</td>
<td>Diabetes, 23 y; erosion</td>
<td>6 wk</td>
<td>$3.5 \times 3.0$</td>
<td>$1.0 \times 0.5$</td>
<td>Silverlike defect, $0.2 \times 1.5$</td>
<td></td>
</tr>
<tr>
<td>3/M/84</td>
<td>Left</td>
<td>Absent</td>
<td>HZ ophthalmicus</td>
<td>14 mo</td>
<td>$1.0 \times 0.5$</td>
<td>$1.1 \times 0.5$</td>
<td>Healing line</td>
<td></td>
</tr>
<tr>
<td>4/M/57</td>
<td>Left</td>
<td>Absent</td>
<td>HZ ophthalmicus; diabetes, 10 y; dry eye</td>
<td>12 wk</td>
<td>$4.5 \times 2.8$</td>
<td>$0.3 \times 0.8$</td>
<td>$0.0 \times 0.0$, Healed</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: HZ, herpes zoster.
the termination of follow-up (week 8), a sliverlike defect remained that measured 1.5 × 0.2 mm (Table and Figure 2).

Case 3. An 84-year-old white man with a history of HZO in the left eye developed a persistent left corneal epithelial defect of 14 months’ duration that was unresponsive to treatment including nonpreserved lubricants, erythromycin ophthalmic ointment, and a bandage contact lens. Corneal sensation was absent. His epithelial defect measured 1.0 × 0.5 mm prior to treatment with 2 drops 4 times daily of thymosin beta 4 for 4 weeks. Use of erythromycin ophthalmic ointment was continued. On cessation of thymosin beta 4 treatment, the defect measured 1.1 × 0.3 mm. At the termination of follow-up (day 70), a fine healing line was present (Table and Figure 2).

Case 4. A 57-year-old white man with a 10-year history of diabetes subsequently developed HZO in the right eye with an anesthetic cornea. He developed a persistent left corneal epithelial defect of 12 weeks’ duration that was unresponsive to treatment including nonpreserved lubricants, topical antibiotics, oral doxycycline, and a bandage contact lens. His epithelial defect measured 4.5 × 2.8 mm at the start of treatment with 2 drops 4 times daily of thymosin beta 4 for 4 weeks. Treatment with erythromycin ophthalmic ointment and oral doxycycline were continued. One drop of prednisolone acetate, 1%, with benzalkonium chloride, 0.01%, preservative was continued to maintain control of a smoldering uveitis. On cessation of thymosin beta 4 treatment, the defect measured 0.8 × 0.3 mm. The defect was healed on day 42 and remained that way through the remainder of the follow-up period (day 56) (Table and Figure 2).

Comment. Our study evaluated the clinical benefit of adding thymosin beta 4 eye drops to the existing treatment regimens of patients with long-standing neurotrophic defects due to HZO, diabetes, or both. The 1-month follow-up period allowed us to monitor both healing and subsequent surface breakdown after cessation of treatment. Our choice of dose and length of treatment were based on laboratory studies that demonstrated corneal epithelial cell migration and wound healing when

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Figure 1. Slitlamp photographs of the cornea of case 1. A, Prominent epithelial defect at onset of treatment is shown. B, Healed epithelial defect on day 31 is shown. Black arrows indicate areas of surface irregularity with fluorescein pooling; white arrows, the edges of the epithelial defect; F, flash reflex from slitlamp camera.

Figure 2. Changes in the size of the corneal epithelial defect following treatment with thymosin beta 4 (area = longest × shortest dimension). The horizontal line indicates the end of the treatment period.
Thymosin beta 4 was applied topically to healthy rats and mice in scrape and alkali injury models. Our limited study was not designed to determine the optimal dose or length of treatment.

All patients in this study showed clinically significant reduction in the size of their epithelial defects. Case 1 demonstrated complete healing within the 28-day treatment period (Figure 1). Some fluctuation in the size of the defects was seen in most patients; however, regression to the pretreatment defect size did not occur. Case 3 healed completely by the end of the follow-up period (day 36). These findings are particularly noteworthy given that the defects showed no inclination toward healing in the 6 to 12 weeks prior to initiating treatment. No evidence of stromal thinning or vascular ingrowth of the corneal stroma on the iris surface or retina was observed in any of the patients (2 of 3 patients with diabetes had been treated previously for proliferative diabetic retinopathy).

No patient reported discomfort associated with the drops. Improved ocular comfort and decreased conjunctival injection was correlated with healing.

In conclusion, this study suggests a promising role for thymosin beta 4 drops in the treatment of patients with recalcitrant, nonhealing, neurotrophic corneal epithelial defects. This pilot study is to be continued; if the initial results are supported, then a controlled clinical trial will be warranted to explore the full effects and proper dosing of thymosin beta 4 in the modulation of corneal wound healing in patients with neurotrophic keratopathy.

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Cellular Composition of the Ridge in Retinopathy of Prematurity

In retinopathy of prematurity (ROP), elevated levels of oxygen arrest the normal posterior-to-anterior growth of intraretinal blood vessels; in some patients, the demarcation zone between vascular and avascular retina thickens into an ophthalmoscopically visible ridge that designates stage 2 ROP. The ridge consists of a larger anterior collection of spindle-shaped cells in the nerve fiber layer (the “vanguard”) and a variably present smaller posterior vascularized reguard. Although the ridge is an important aspect of ROP pathogenesis, its cellular composition has not been well characterized.

Report of Cases. Four eyes were obtained at autopsy from 2 children clinically diagnosed with stage 2 ROP at the Stanford University Medical Center.

Case 1. A male baby was born at 24 weeks’ gestational age and treated with oxygen for respiratory distress from birth. Stage 2 ROP was diagnosed 8 weeks after birth. The child died at age 12 weeks of multiple organ failure.

Case 2. A male baby was born at 27 weeks with hydrops fetalis and an 11p13-15.1 deletion. The patient received supplemental oxygen and died 6 weeks after birth.

Immunohistochemical examination was performed on formalin-fixed paraffin-embedded sections using antisera to identify mature and immature vascular endothelial cells (CD31; Dako, Glostrup, Denmark, or CD34; Becton, Dickinson, and Co, Franklin Lakes, New Jersey), astrocytes (glial fibrillary acidic protein [GFAP]; Dako), astrocyte precursor cells (PAX2; Zymed Laboratories, San Francisco, California), myeloid lineage cells (microglia and macrophages; CD68; Dako), pericytes (desmin; Dako), neurons (neuron-specific enolase; Dako), or proliferating cells (Ki67; Dako).}

Ridge Vanguard. A thickened ridge of spindle cells (the vanguard) in the nerve fiber layer just anterior to the most distal blood vessels was verified in eosin-stained sections in the temporal and nasal aspects of all 4 eyes; a smaller vascular proliferation in the reguard was evident in some cases (Figure 1A). CD34 and CD31 labeled intraretinal blood vessels (Figure 1B and C). No CD34 or CD31 immunoreactivity was detected in ridge spindle cells (Figure 1B and C). Scattered CD68+ cells (presumed resident retinal microglia) were present in the inner retina posterior to the ridge, often adjacent to blood vessels, but were found only rarely within the ridge (Figure 1D and E). Occasional lightly GFAP-immunoreactive cells were detected in the ridges (Figure 1F).