

The Effect of Nerve Growth Factor on Corneal Sensitivity After Laser In Situ Keratomileusis

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Objective: To determine if topically administered nerve growth factor (NGF) plays a role in accelerating the recovery of corneal sensitivity after laser in situ keratomileusis (LASIK).

Methods: A prospective double-masked study comparing the effect of topical NGF with balanced salt solution on corneal sensitivity after LASIK in rabbits. Preoperative and postoperative corneal sensitivities were assessed using an esthesiometer (Cochet-Bonnet esthesiometer).

Results: Eyes that were treated with topical NGF demonstrated an earlier and faster recovery of corneal sensitivity after LASIK ($P=.007$). A statistically significant

difference in corneal sensitivity was found between the topical NGF and control group postoperatively at 2 ($P=.01$), 3 ($P=.03$), and 4 ($P=.03$) weeks.

Conclusion: Topically administered NGF may play a significant role in accelerating corneal reinnervation after LASIK.

Clinical Relevance: Our results showed that topical NGF had beneficial effects in the early recovery of corneal sensitivity after LASIK. Nerve growth factor can be a new therapeutic approach for dry eye syndrome after LASIK.

Arch Ophthalmol. 2004;122:1338-1341

LASER IN SITU KERATOMILEUSIS (LASIK), which is widely used for correcting refractive errors, combines lamellar corneal surgery with excimer laser photoablation. The automated microkeratome is used to create either a superior or a nasal hinged keratectomy that inevitably truncates a portion of the corneal nerve supply. The nerves of the stromal bed are subsequently exposed to excimer laser photoablative decomposition. Damaging the corneal nerve supply during LASIK contributes to a reduction in corneal sensitivity.¹ The extent and duration of sensory loss depend on the location and number of nerves damaged by the surgery. Experimental and clinical studies^{2,3} have demonstrated that impairment of corneal sensitivity leads to decreased vitality and metabolism of the corneal epithelium, frequently associated with epithelial erosion and delay or absence of spontaneous wound-healing capability. Consequently, a rapid recovery of corneal reinnervation is important for restoring the normal physiological features, tear secretion, and healing properties of the cornea.

Nerve growth factor (NGF) is a polypeptide discovered in the early 1950s by Levi-Montalcini.⁴ It is essential for regulating the growth and survival of developing peripheral and central nervous system neurons. It also serves as a survival and differentiating factor for neural crest sensory and sympathetic neurons.⁴ Nerve growth factor induces neurite sprouting by neuronal cells and restores the function of injured neurons.^{4,5} Various tissues and cell types produce and release NGF. Specific high- and low-affinity NGF receptors have been identified on cell membranes, including the ocular surface.^{6,7} Bonini et al⁸ have demonstrated that topical NGF eyedrops improved corneal sensitivity and promoted corneal epithelial healing in patients with moderate and severe neurotrophic keratitis. Although performed in an uncontrolled and non-randomized series of patients, therapy with topical NGF demonstrated promise for the restoration of ocular surface integrity and visual function in those with neurotrophic corneal disease.

While strong evidence suggests that improved corneal sensitivity could be attributed to NGF, which induces sensory

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neuron sprouting into areas of denervated cornea,⁸⁻¹⁰ no study, to our knowledge, has investigated the role of NGF in promoting corneal nerve regeneration after LASIK.

This study investigates whether topically administered NGF plays a role in accelerating the recovery of corneal sensitivity after LASIK in a rabbit model.

METHODS

ANIMALS

Sixteen corneas of 16 New Zealand white rabbits (approximate weight, 3.5-4.5 kg) underwent LASIK. All animals were treated according to established institutional guidelines regarding animal experimentation and the Association for Research in Vision and Ophthalmology Regulations for the Use of Animals in Research.

LASIK TECHNIQUE

Intramuscular ketamine hydrochloride (Ketaject), 30 mg/kg of body weight, and intramuscular xylazine hydrochloride (Xylaject), 5 mg/kg of body weight, were used to induce anesthesia. A Barraquer-style speculum was placed between the eyelids, and the eye was rinsed with balanced salt solution (BSS). A paraxial linear mark with gentian violet pencil was applied to the corneal surface. After placement of the suction ring, the intraocular pressure was verified to be higher than 65 mm Hg, using a Barraquer tonometer. A nasal-based 160-mm-thick and 8.5-mm-wide hinged corneal flap was created, using an automated microkeratome (SKBM microkeratome; Alcon-Summit Technologies, Cork, Ireland). Subsequently, the microkeratome and the suction ring were removed from the eye and the corneal flap was lifted and retracted against the peripheral cornea.

Excimer laser photoablation was performed on the stromal bed, using an excimer laser (Summit Apex Plus excimer laser; Summit Technologies, Cork). A single-zone approach (a laser zone diameter of 6.0 mm) was used in all eyes undergoing LASIK. A myopic correction of -3.0 diopters was performed in all eyes for an approximate ablation depth of 36 μm. After the photoablation, the corneal flap was carefully repositioned. A temporary tarsorrhaphy was then performed using a 6-0 black silk suture to keep the eyelids closed for the first week. Antibiotic (0.3% ofloxacin [0.3% Ocuflax; Allergan Inc, Irvine, Calif]) and corticosteroid (0.1% fluorometholone [0.1% FML; Allergan Inc]) eyedrops were instilled 4 times a day for the first 7 days.

The treatment agent consisted of murine NGF (200 mg in 1 mL of BSS) purified from the submaxillary gland, as previously described.¹¹ The rabbits received 10 μL of NGF or BSS, using a micropipette. The first topical application was applied directly to the exposed stromal bed immediately after photoablation and then onto the corneal surface at a frequency of 4 times a day for 3 days. The investigators (M.-J.J.) were masked to the use of NGF solution and BSS.

CORNEAL SENSITIVITY

A prospective study comparing post-LASIK corneal sensitivities of rabbits treated with topical NGF vs BSS control was performed. A preoperative basic ocular examination was performed using a portable slitlamp. The corneal sensitivity was assessed with an esthesiometer (Cochet-Bonnet esthesiometer; Luneau Ophthalmologie, Chartres, France).¹² Under direct visual control, the nylon filament of the esthesiometer touched the center of the cornea smoothly and perpendicularly. The diameter of the nylon filament was 0.12 mm, and its

Corneal Sensitivity Values After LASIK

Time, wk	Corneal Sensitivity, Mean ± SD, cm*		P Value
	NGF-Treated Group	Control Group	
Preoperatively	4.06 ± 0.12	4.06 ± 0.12	NA
Postoperatively			
2	1.59 ± 0.42	0.92 ± 0.48	.01†
3	2.52 ± 0.66	1.73 ± 0.64	.03†
4	2.50 ± 0.78	1.58 ± 0.72	.03†
5	2.13 ± 0.70	1.50 ± 0.79	.18
6	2.48 ± 0.78	1.92 ± 0.90	.27
7	2.41 ± 1.12	1.75 ± 1.02	.42
8	2.03 ± 1.22	1.50 ± 1.25	.56

Abbreviations: LASIK, laser in situ keratomileusis; NA, data not applicable; NGF, nerve growth factor.

*Data are given as filament length, as measured by an esthesiometer.

†The difference was statistically significant.

length could be varied from 0 to 60 mm. The pressure applied to the cornea, thus, ranged from 0.4 to 15.9 g/mm². Contact was detected by the slightest bend of the nylon; sensitivity was taken as the length of the filament (in centimeters) that gave a 50% positive corneal reflex (blinking reflex) response from a minimum of 6 stimulus applications. Corneal sensitivities were checked twice a week, and the mean of 2 measured sensitivities was used as the corneal sensitivity of the week.

STATISTICAL ANALYSIS

A *t* test was used to analyze the difference in corneal sensitivity between treatment with topical NGF and treatment with BSS at given points. Estimates of the average effect of treatments on the corneal sensitivity over time were calculated using a linear mixed model.¹³ Variability between samples at different points was treated as a random effect, and variability due to treatment as a fixed effect. The analysis was performed using the MIXED procedure in SAS statistical software.¹⁴

RESULTS

The mean ± SD preoperative measured corneal sensitivity was 4.06 ± 0.12 cm. The corneal sensitivities of eyes treated with NGF and eyes in the BSS control group were significantly decreased at 2 weeks from preoperative levels and gradually increased after 2 weeks. The mean corneal sensitivities of the NGF-treated group and the control group at every point are shown in the **Table** and depicted in **Figure 1**. Eyes treated with topical NGF demonstrated an earlier recovery in corneal sensitivity after LASIK (*P* = .007) (Figure 1 and **Figure 2**). A statistically significant difference was found between eyes treated with topical NGF and those treated with topical BSS at the post-LASIK points of 2, 3, and 4 weeks (Table).

COMMENT

The cornea is innervated by sensory nerve fibers originating from the ophthalmic division of the trigeminal nerve (cranial nerve V), whose cell bodies lie in the trigeminal ganglion, and by sympathetic nerves, whose cell bodies lie in the superior cervical ganglion.¹⁵ The nerve

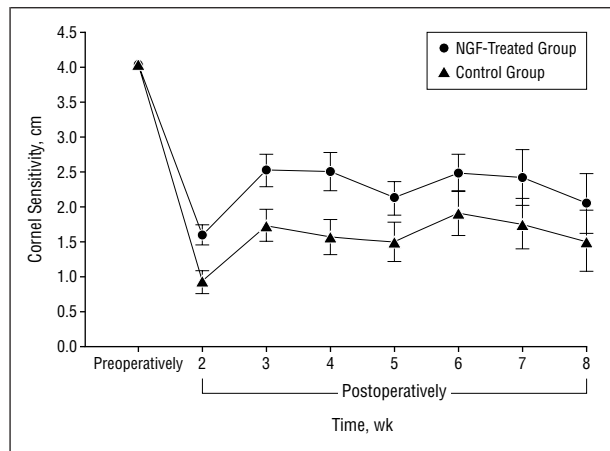


Figure 1. Effects of nerve growth factor (NGF) on corneal sensitivity (which is given as filament length, as measured by an esthesiometer) recovery. Topical administration of NGF accelerated corneal regeneration ($P=.007$) when compared with the control group treated with balanced salt solution. Data are given as mean \pm SEM.

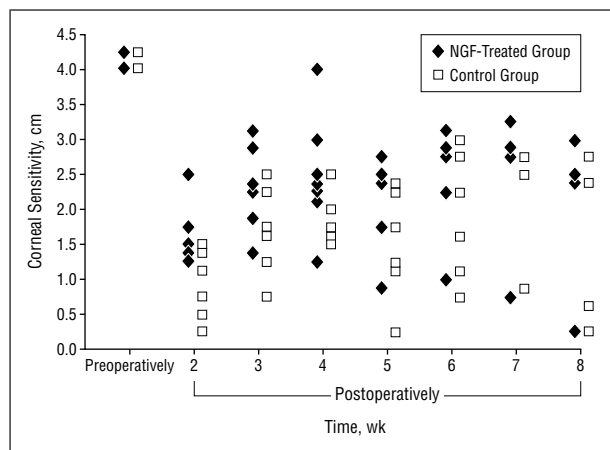


Figure 2. Effect of nerve growth factor (NGF) on corneal sensitivity (which is given as filament length, as measured by an esthesiometer) following laser in situ keratomileusis. A single point may represent multiple values.

fibers penetrate the limbus and form thick nerve bundles in the anterior third of the stroma. As these nerves course to the center of the cornea, they branch horizontally and vertically to form the basal epithelial/subepithelial nerve plexus between the basal epithelial cells and the Bowman layer.¹⁶ Normal corneal sensitivity is essential for proper maintenance of a healthy ocular surface. Experimental and clinical studies have demonstrated that corneal nerve damage alters the metabolism and vitality of the epithelium, impairs epithelial healing, and is responsible for trophic ulceration.

During LASIK, a corneal flap is created using a microkeratome. During this procedure, the superficial stromal nerves are severed in the lamellar flap margin, and the nerves in the stromal bed under the flap are subsequently exposed to laser photoablation. Corneal sensitivity decreases after LASIK because of surgical amputation and excimer laser ablation of the nerve fibers innervating the central corneal surface.¹⁷⁻¹⁹

Corneal hypoesthesia may consequently trigger a cascade of events that degrade the corneal integrity by re-

ducing the protective blinking reflex, delaying epithelial wound healing, and decreasing aqueous tear layer production.^{16,20,21} Moreover, the loss of corneal sensitivity may disrupt the release of nerve-derived trophic factors that are required to maintain the integrity of the corneal epithelium.²² Ultimately, the ocular surface is compromised by changes resulting in corneal punctate epitheliopathy and corneal surface irregularity that manifest clinically as ocular surface irritative symptoms and fluctuating vision.²³

Toda et al²⁴ reported that patients undergoing LASIK developed dry eye with compromised tear function for at least 1 month after surgery. Use of artificial tears in the early postoperative period may help ameliorate irritative symptoms and ocular surface damage. Wilson²⁵ reported that staining of the epithelium with rose bengal solution in patients without preexisting dry eye after LASIK is likely a result of neurotrophic epitheliopathy, noting that there is no difference in mean tear production between patients who have significant punctate epithelial erosions and development of rose bengal staining on the flap and those who do not. The signs and symptoms of LASIK-induced neurotrophic epitheliopathy tend to resolve spontaneously approximately 6 months after surgery. This correlates well with the point when reinnervation of LASIK flaps is complete. Linna et al²⁶ have demonstrated in the rabbit cornea that, at 2.5 and 5 months, more regenerating nerve sheathes were observed to emerge from the cut stromal nerve trunks. They seemed to send anastomosing fibers among the neighboring stromal nerves. By this time, the epithelial, basal epithelial/subepithelial, and anterior stromal innervation had gained an almost normal nerve density and architecture.

In this study, an esthesiometer was used to measure corneal sensitivity. This instrument requires the subject's cooperation not to move during the measurement, so some skills were needed to obtain reliable data from rabbits, which cannot be trained to cooperate. The tester (M.-J.J. or K.R.Y.) gently held the body of the rabbit and was cautious not to touch the cilia or eyelids with the instrument. A false-positive reaction was monitored intermittently by simply approaching the instrument without touching the corneal surface. Because corneal sensitivity has a topographical variation, data were taken at the apex of the cornea under direct visual control.

Our results suggest that topically applied NGF accelerates the recovery of corneal sensitivity. We hypothesized that this earlier recovery may lead to an accelerated return of tear function and improvement in the ocular surface. Moreover, the early recovery of corneal sensation may ameliorate the symptoms of dry eyes and associated fluctuating vision after LASIK by restoring tear secretion from the lacrimal gland and the normal blink reflex.

There have been reports^{8,9} that topically applied exogenous NGF restored corneal integrity and increased the best-corrected visual acuity progressively during treatment and follow-up in patients with corneal neurotrophic ulcers. The mechanism of action of NGF on the ocular surface is not well understood. Nerve growth factor treatment may restore a deficit of synthesis or a re-

lease of endogenous NGF. This hypothesis is supported by evidence from an animal study by Brewster et al.²⁷ In this study, diabetic rats show reduced expression of target-derived NGF and reduced expression of neuronal genes that are responsive to NGF. The latter is corrected by administration of exogenous NGF. Thus, insufficient neurotrophic support might contribute to the pathogenesis of diabetic neuropathy, and any successful treatment might include exogenous neurotrophins or other strategies to correct their deficiency of action.²⁷ Neurotrophic factors are proteins that promote the survival of specific neuronal populations. Many have other physiological effects on neurons, such as inducing morphological differentiation, enhancing nerve regeneration, stimulating neurotransmitter expression, and otherwise altering the physiological characteristics of neurons. These properties suggest that neurotrophic factors are highly promising as potential therapeutic agents for neurological disease.²⁸ Another indirect mechanism could also be involved, such as increasing neuropeptide synthesis that promotes epithelial healing or invoking immune cells through the release of cytokines.²⁹

The results of this study suggest that topical NGF can induce an earlier recovery in corneal sensitivity after LASIK. Topical NGF application may be effective for the early recovery of post-LASIK hypoesthesia. Earlier recovery should contribute to a reduction of the symptoms of sensory nerve denervation after LASIK. A better understanding of the mechanism of action of NGF on the ocular surface may allow the broadening of its indications to other external eye diseases. Further studies to assess the potential of topically administered NGF to avoid the post-LASIK nerve denervation-induced symptoms are warranted.

Submitted for publication January 10, 2003; final revision received January 29, 2004; accepted March 4, 2004.

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