Nerve Regeneration in the Cornea

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Introduction

Sensory Innervation to the Cornea from the Trigeminal Ganglion is important for the Perception of Stimuli, Maintenance of Hydration and Avoidance of Injury.

Corneal Nerve Dysfunction is the pathophysiological basis of several Ocular Diseases that cause considerable morbidity (e.g., Neurotrophic Keratitis and Dry Eye Disease).

Adapted from Blarski 2006 and Bonini et al, Eye; 2003

Ophthalmic Surgical Procedures like Corneal Transplantation, Radial Keratotomy, Photorefractive Keratectomy (PRK) and Laser-Assisted In Situ Keratomileusis (LASIK), cause Corneal Nerve Disruption that can lead to Neurotrophic Epitheliopathy.

Clinical studies have shown that corneal nerves regenerate over several years after surgical transection; however, the nerve density never returns to presurgery values.

Subbasal nerve density decreased by 82% in 5 days after LASIK. A gradual increase in density was observed at 2 weeks after surgery; however, even 2 years after LASIK, nerve density was only 64% of preoperative values (Moiinanen et al; BJO; 2008)

Subbasal nerve density is not restored to normal even 40 years after penetrating keratoplasty (Niederer et al IOVS; 2007)

Median subbasal nerve density in clear grafts is also significantly lower than that in normal corneas (Patel et al; TAOS; 2007)

Adapted from SE Wilson et al; Ophthalmology; 2001

Slit-lamp photographs of corneas with Epitheliopathy and Rose Bengal staining after Laser In Situ Keratomileusis (LASIK)

Adapted from SE Wilson et al; Ophthalmology; 2001
• Despite the clinical need to promote corneal nerve regeneration in neurotrophic corneas, few specific therapeutic interventions are available.

• One reason for insufficient progress in this area is the limited availability of methodologies to investigate the effect of interventions on corneal nerve regeneration.

The recent introduction of Neurofluorescent thy1-YFP mice has made sequential in vivo investigations of corneal nerves feasible (Yu and Rosenblatt, IOVS, 2007)

Objective - 1

To determine the effect of lamellar transection surgery on the nerve fiber density (NFD) and pattern of nerve regeneration in the cornea of thy1-YFP transgenic mice.

Methods

Wide-field Stereo fluorescence microscopy:
Used to obtain serial images of nerves in live thy1-YFP mice.

Nerve Fiber Density NFD (mm/mm²):
Calculated from maximum intensity projection images as the total length of fibers within the area of the contour in which nerves were traced.

Whole-mount Confocal Microscopy:
To analyze the arrangement of nerves and types of regenerating fibers.

In vivo maximum intensity projection image of fluorescent nerves in the normal thy1-YFP mouse cornea

• Stromal network formed by thick nerve trunks
• Second network formed by thinner subbasal hairpin-like nerves

Namavari et al; IOVS, 2011
Nerves in Normal thy1-YFP murine corneas

Subbasal hairpin nerve pattern, but not the stromal trunk pattern, changed over these serial observations.

Namavari et al; IOVS, 2011

Dissection of Hinged Lamellar Corneal Flap

A: After marking the central cornea with a 2-mm trephine, an initial cut is made with a diamond blade and a stromal pocket is created using a 15-degree 5.0-mm standard angle knife.

B: The stromal pocket is extended by lamellar dissection using a 1.0-mm paracentesis knife.

C: The stromal pocket is opened with scissors circumferentially, except at the 3 hinges to leave the flap attached to the stromal bed.

D: Cornea with the hinged lamellar flap. Arrows point to the hinges.

Chaudhary et al; Cornea; 2012

Cornea immediately after the lamellar dissection surgery

The same cornea 2 wks after surgery.

No sign of scar formation, inflammation, or neovascularization are present.

H and E staining of a sagittal corneal section immediately after lamellar surgery.

Namavari et al; IOVS, 2011

In vivo serial imaging of regenerating nerves after lamellar corneal surgery

NFD at 2 and 4 Weeks was reduced as compared to baseline levels.

Namavari et al; IOVS, 2011
**Objective - 2**

To determine and characterize the effect of topical application of Benzalkonium Chloride (BAK) on corneal nerves in vivo and in vitro.
Effect of BAK on corneal nerves in the thy1-YFP mouse

- In vivo MIP Nerve Imaging
- Fluorescein Staining

Sequential in vivo imaging of cornea showing BAK-induced Neurotoxicity

A1-D1: Reversible Neurotoxicity (Axonopathy and Recovery)

A2-D2: Irreversible Neurotoxicity (Degeneration and Regeneration)

Stromal nerves tracings with Neuroulcida for calculating NFD

Bar diagrams showing changes in stromal NFD, Aqueous Tear production.

Western blot analyses of corneal lysate for IL6, CD3, TUBB3 and GAP43.

Compartmental Culture of dissociated trigeminal ganglion neurons

Significant reduction in Neurite extension after BAK addition (B2, C2)
Although these studies described phenomena-based processes associated with corneal reinnervation, the associated molecular events remain largely unknown.

The robust regenerative response after nerve injury is thought to correspond with the coordinated expression of a number of neurotrophins (NTs) and regeneration associated genes (RAGs) that aid rapid regeneration.

Expression of NTs and RAGs have been reported in the cornea, however their expression in the setting of nerve regeneration is not known.

Objective - 3

To evaluate the in vivo expression of Neurotrophins (NTs) and Nerve Regeneration - Associated Genes (RAGs) after lamellar surgery in thy1-YFP mice.

NTs represent a family of neurotrophic growth factors comprising nerve growth factor (Ngf), brain-derived neurotrophic factor (Bdnf), neurotrophin 3 (Ntf3), and neurotrophin 4/5 (ntf5)

Two widely known RAGs are the growth-associated protein-43 (Gap43) and small proline–rich repeat protein 1A (Sprr1a)

Gene Expression During Corneal Nerve Regeneration

• Among NTs, Bdnf is most significantly expressed.
• Among RAGs, Sprr1a is most significantly expressed.
Summary

1. Transection of axons in the cornea causes regenerative sprouting from stromal trunks which peaks up to 6 weeks.

2. Regenerated axons do not completely recapitulate the normal arrangement of corneal nerves.

3. Topical BAK application causes Reversible Neurotoxicity (axonopathy and recovery) and Irreversible Neurotoxicity (degeneration and regeneration)

4. NTs and RAGs are expressed in the cornea after corneal nerve transection, particularly Bdnf and Sprr1a, which are robustly expressed.

5. YFP+ cells (inflammatory cells) in the stroma serve as a source for Bdnf in the cornea and epithelium as a source of Sprr1a.

Conclusion

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