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Diabetic Cornea: Clinical and Pathogenetic Features of Neurotrophic Corneal Disorders in Diabetes

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Abstract

Diabetic neuropathy is associated with neurotrophic ulcerations of the skin and cornea. Decreased corneal sensitivity and impaired innervation lead to weakened epithelial wound healing predisposing patients to ocular complications such as corneal infections, stromal opacification, and surface irregularity. This review presents recent findings on impaired corneal innervation in diabetic individuals, and the findings suggest that corneal neuropathy might be an early indicator of diabetic neuropathy. Additionally, the recent findings for neuroprotective and regenerative therapy for diabetic keratopathy are presented.

Introduction

Diabetes mellitus is a major disease worldwide, and the incidence of diabetes has risen markedly in the past several decades. The complications associated with diabetes are the leading cause of blindness in the working age adults. Diabetic neuropathies characterized by a progressive loss of nerve fibers are common complications affecting about 50% of patients with diabetes [1].

It was recently demonstrated that retinal neuronal components were associated with the pathogenesis of diabetic retinopathy, and the neuronal degeneration in the retina may be dependent on the mitochondrial- and caspase dependent cell-death pathway. It is known that neuronal abnormalities directly affect visual function in diabetic retinopathy, and those neuronal changes are probably also the reason for diabetic keratopathy, as cornea is one of the most highly innervated tissues [2]. However, there are limited numbers of studies available that focused on evaluation of corneal innervation changes in diabetic patients. Early diagnosis of neuropathy is very important for evaluation of risks and therapeutic management. Thus in this review we aim to summarize the most recent findings on impaired corneal innervation in diabetic patients and findings on neuroprotective and regenerative therapy approach for diabetic keratopathy.

Corneal Innervation

The density of corneal epithelial nerves is 300–600 times higher than that of the skin with approximately 7000 nociceptors/mm2. The sensory nerve fibers in the peripheral cornea have a myelinated shell and can be seen by slit lamp bio microscopy. The central cornea is acutely sensitive especially along the horizontal meridian and less sensitive along the vertical meridian [3].

The ophthalmic nerve and occasionally the maxillary branch of the trigeminal nerve innervate the cornea, and the superior cervical ganglion supplies sympathetic innervation to the limbus and peripheral cornea. Light and electron microscopic studies and more recently confocal microscopic studies have provided detailed information on the distribution of the nerves in the human cornea.

There are about 70 to 80 large diameter myelinated nerves that enter the cornea at the posterior to mid-stromal level, and they run radially and anteriorly toward the center of the cornea. The anterior stromal layers are innervated by multiple branches of these nerves that have no perineurium and myelin. They penetrate the cornea approximately 1 mm from the limbus, pass through Bowman's membrane [4], and turn in a clockwise direction forming the sub basal nerve plexus that

lies between Bowman's layer and the epithelium forming the sub basal nerve vortex. Its geographic center is located between 2.18 and 2.92 mm from the corneal apex. In some cases, the sub basal nerves do not form a prominent spiral but end on opposite sides of an imaginary interface.

The functioning of the corneal nerve is assessed by corneal sensitivity tests. There are three main groups of receptors in the cornea: mechanical or mechano-nociceptors, chemical or polymodal nociceptors, and thermal or cold receptors.

Mechanisms of Impaired Corneal Innervation and Neurotrophic Role of Corneal Nerves

Recent studies have provided strong evidence that glycation plays an important role in the pathogenesis of retinal diabetic neuropathy with the triggering of different mechanisms that result in neuronal dysfunction. Obrosova and Julius reported that oxidative stress and poly (ADP-ribose) polymerase activation were fundamental mechanisms that play a role in the pathogenesis of diabetic neuropathy. Byun et al. confirmed that poly (ADP-ribose) polymerase inhibition prevented the loss of epithelial innervation and promoted epithelial wound healing. They concluded that poly (ADP-ribose) polymerase activation played a role in the pathogenesis of diabetic neuropathy [5].

The results of a recent study have shown that one of the functions of corneal nerve fibers is in maintaining a healthy cornea and promoting wound healing after eye injuries. The results of in vitro studies suggested that there is a trophic support between corneal epithelial cells and neurons [6]. Accordingly, corneal epithelial cells release soluble factors such as NGF and GDNF that stimulate neurite survival and extension. Lambiase et al. reported that stromal keratocytes also produce neurotrophins, for example, neurotrophins 3 and 4; however their trophic influences on corneal nerve fibers still remain undetermined. BDNF is found in the corneal epithelial cells and stromal keratocytes,

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and it is believed to be also contained in corneal sensory neurons.

Nerve-derived trophic factors regulate the biochemistry of the corneal epithelium and control the normal and renewal processes of maintaining the corneal epithelial cells. Thus, patients with impaired corneal innervation, for example, after herpetic keratitis, diabetes, prolonged contact lens wear, advanced age, and refractive surgery, are at increased risk of corneal damage because of diminished trophic support. Ferrari et al. reported that nerve-secreted neuropeptides influence corneal cell proliferation in vitro, and the rate of corneal epithelial cell mitosis is altered in denervated corneas of rats. Ciliary neurotrophic factor (CNTF) has been detected in corneal endothelial cells [7]. Zhou et al. discovered that the mRNA of CNTF was more significantly down regulated in diabetic mice than in normal mice. A subconjunctival injection of CNTF significantly reduced the size of the corneal epithelial defect of $67.89 \pm 12.27\%$ to $30.10\% \pm 10.13\%$ after

48 hours. These results suggest that impaired corneal epithelial wound healing in diabetic mice can be caused by reduced levels of CNTF, and CNTF supplementation can promote corneal epithelial wound healing by activating corneal epithelial stem/progenitor cells.

A study of the effects of neuropathy showed that the diabetes-induced denervation of the cornea reduces the viability of the corneal epithelial cells and their ability to recover from injury. Guo et al. reported that the trigeminal ganglion neurons and the innervation of the cornea were impaired in diabetic mice. A detailed listing of the neurotrophic factors present in the cornea is shown in (Table 1).

Corneal Sensitivity in Diabetes

Patients with diabetes have decreased corneal sensitivity and thus are very vulnerable to trauma. In the study by Nielsen it was demonstrated that corneal sensitivity in 83% of diabetic patients was

Table 1: Neurotrophic factors in the cornea.

Growth factor	Healthy cornea	Injured cornea	Topical application
Nerve growth factor (NGF)	(i) Found in corneal epithelium and stromal keratocytes	Upregulated during reinnervation after nerve surgical transection, and in dry eye syndrome, in inflamed conjunctiva of patients with vernal keratoconjunctivitis	(i) Augments corneal wound healing and provides recovery of corneal sensitivity and photophobia
	(ii) Critical for corneal nerve survival and maintenance, axonal branching, elongation, neuronal sprouting, and regeneration following nerve damage		(ii) Has potent antiviral properties (restrict herpes simplex virus-1)
Brain-derived neurotrophic factor (BDNF)	(i) Found in corneal epithelium and stromal keratocytes, originate from corneal sensory neurons	Expressed after experimental flap surgery in putative corneal stromal and/or inflammatory cells in a positive association with neurite extension	
	(ii) Exact role related to corneal nerves is unclear		
Glial cell-derived neurotrophic factor (GDNF)	Expressed in human corneal stromal keratocytes and may operate similarly to or synergistically with NGF by triggering gene transcription governing epithelial cell migration and wound healing	Possibly plays an important role in corneal regeneration and wound healing	Produces complete epithelial healing in a patient with a progressive neurotrophic ulcer
Neurotrophins 3, 4/5 (NT-3, NT-4/5)	(i) NT-3 transcribed in epithelial cells and stromal keratocytes	Minimal changes in NT-3 gene expression following surgical transection of corneal nerves	
	(ii) NT-4 is present in corneal epithelium and is a neurotrophic factor that may be involved in the regulation of stromal keratocytes by epithelial cells		
Ciliary neurotrophic factor (CNTF)	Promotes corneal epithelial wound healing by activating corneal epithelial stem/progenitor cells	(i) Upregulated in corneal epithelium after injury in mice	
		(ii) Down regulated in corneal epithelium of diabetic mice	
Vascular endothelial growth factor (VEGF)	Minimally present	(i) Upregulated in the injured cornea	VEGF supplementation promotes trigeminal nerve repair, and abrogation of VEGF signaling reduces corneal nerve growth
		(ii) Required for efficient corneal nerve regeneration	
Hepatocyte growth factor (HGF)	Expressed in stromal keratocytes, stimulates corneal epithelial proliferation	Upregulated after corneal epithelial wounding and probably contributes to the epithelial wound healing process	
Keratocyte growth factor (KGF)	(i) Expressed in stromal keratocytes, fibroblasts	Upregulated in corneal epithelial wounding	
	(ii) Stimulates corneal epithelial proliferation, acts specifically on cells of epithelial origin as a paracrine mediator		
Transforming growth factor-α (TGF-α), interleukin-1β (IL-1β), and platelet-derived growth factor-B (PDGF-B)	(i) Exclusively expressed in the corneal stroma		
	(ii) TGF-α and IL-1β can upregulate the transcription of neurotrophins, such as NGF in 3T3 mouse fibroblasts		

reduced below 60 mm against 38% of the controls alongside with reduced perception of vibrations (vibratory perception of the left index finger and great toe by biothesiometer). A decrease in corneal sensitivity may cause a delay in epithelial wound healing and be the cause of recurrent erosions. This is because the corneal nerves release epitheliotropic substances that promote the maintenance of the integrity of corneal surface [8]. Alterations of the corneal nerves decrease the corneal sensitivity resulting in corneal hypoesthesia that disrupts the epithelial architecture and function. These changes would further delay the reepithelization of the cornea.

Confocal microscopy has shown promise as a noninvasive method for quantifying the damage and repair of corneal sensory nerves that can serve as markers for diabetic neuropathy. Thus, Rosenberg et al. using confocal microscopy found decreased corneal sensitivity together with a decreased number of long nerve fiber bundles in the sub basal nerve plexus. In addition, patients with diabetes had fewer nerve fiber bundles than healthy control subjects possibly due to the presence of polyneuropathy. In all patients with diabetes with neuropathy, the sub basal nerve densities were significantly reduced. Additionally, the authors found that even if most patients with diabetes had nerve fiber bundles with a normal morphology, patients under dialysis with mild to moderate neuropathy had abnormally tortuous nerve fiber bundles in the sub basal nerve plexus [9]. This confirmed the presence of an impairment of corneal sensitivity, and the duration of diabetes was significantly and directly correlated with the degree of polyneuropathy.

In the sub basal nerve plexus, a decrease in the nerve density, number of branches, single nerve fiber length, and increased tortuosity have been found to be significantly correlated with established electromyography and nerve conduction parameters and with the results of the skin biopsy.

Several studies have confirmed that the cornea innervations were altered in animal models of diabetes. Davidson et al. reported a 50% loss of corneal nerve fibers after 12 weeks of high fat diet in low-dose streptozotocin-induced diabetic rats. Yin et al. described corneal changes in streptozotocininduced type 1 diabetic rats. A 50% decrease in tear secretion was found after eight weeks of diabetes induction in SD rats [10], corneal sensitivity was decreased, and the corneal nerves had fewer branches and were thinner and shorter by 75%.

Ueno et al. found a decreased density of the corneal sub basal nerve plexus and corneal epithelial branches in leptin receptor mutant mice which are an accepted animal model of type 2 diabetes. The corneal sub basal nerves were more tortuous in these mutant mice than in normal mice. Wang et al. reported delayed corneal wound healing in the Akita diabetic mice, a model of chronic complications of type 1 DM. In a longitudinal study of corneal nerve density in a rat model of type 1 diabetes, Chen et al. found that density of nerves was initially increased in the subbasal plexus after 8 and 16 weeks of diabetes. However, the density remained unchanged in the stromal layer. An increase could be explained by increased nerve tortuosity or collateral sprouting as reported in patients with impaired glucose tolerance.

It is difficult to translate the results from the streptozotocin-induced animals to type 1 human diabetes and that from the db/db mouse to type 2 human diabetes due to recessive homozygous mutation in the leptin receptor (fa/fa) [11].

Diabetic neurotrophic keratopathy, a manifestation of diabetic polyneuropathy, also plays a significant role in limiting epithelial wound healing. Neurotrophic keratopathy according to Mackie has three stages: Stage 1, positive Bengal staining of the inferior palpebral conjunctival surface, punctate keratopathy, and a decrease in tear breakup time; Stage 2, epithelial breakdown with epithelial deficits surrounded by loose epithelium that becomes hazy, edematous, and poorly adherent to Bowman's layer; and Stage 3, corneal ulceration that can lead to melting and perforation of the cornea.

Therapeutical Management

A diabetic corneal ulcer is a challenging clinical condition. The development of persistent corneal epithelial defects is often associated with severe peripheral neuropathy [12]. The success in the management of neurotrophic keratopathy is generally based on achieving epithelial healing and preventing a progression of the corneal damage. Pathogenetic-orientated pharmacological treatment for neurotrophic keratopathy is still not available. Preservative-free artificial tears, topical antibiotics, bandage contact lenses; amniotic membrane transplantation, tarsorrhaphy, and a conjunctival flap are useful methods that have been used to treat refractory neurotrophic corneal ulcers. Therefore, the establishment of a pathogenetic treatment that can lead to neuroprotection and neuroregeneration is extremely important.

Different nerve-secreted factors, such as NGF and BDNF, are important factors necessary for epithelial regeneration. The application of exogenous NGF was able to reverse the damage of peripheral nerves and completely heal corneal epithelial defects in patients with neurotrophic keratitis. According to Ueno, corneal stem/progenitor cells are closely associated with corneal wound healing and insulin-like growth factor-I administration may be a promising agent that can be used to prevent persistent corneal ulcers in patients with type 2 diabetes [13].

There are other reports that miRNAs, including miR21 and miR-29b, can be used as therapeutic agents to stimulate peripheral nerve regeneration. Wang et al. reported that miR-182 can protect trigeminal neurons from peripheral nerve damage in an experimental mouse model of diabetes. They suggested that this was accomplished by its ability to enhance neurite outgrowth in isolated trigeminal sensory neurons [14]. This overcame the detrimental effects of hyperglycemia by stimulating corneal nerve regeneration by decreasing the expression of one of its target genes, NOX4.

Conclusion

Impaired corneal innervation in diabetes is an important early indicator of diabetic neuropathy. The decrease in corneal sensitivity with a longer duration of diabetes is correlated with the severity of neuropathy. Further investigations on mechanisms of corneal nerve damage and establishment of pathogenetic-orientated therapy for diabetic neuropathy should be the main focus of future research.

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