Histol Histopathol (2010) 25: 771-780

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

Neurotrophic keratopathy; its pathophysiology and treatment

Yuka Okada[1], Peter S. Reinach[2], Ai Kitano[1], Kumi Shirai[1], Winston W.-Y. Kao[3] and Shizuya Saika[1]

[1]Department of Ophthalmology, Wakayama Medical University, Wakayama, Japan, [2]School of Optometry, State University of New York, New York, NY, USA and [3]Department of Ophthalmology, University of Cincinnati School of Medicine, Cincinnati, OH, USA.

Summay. Neurotropic keratopathy is one of the major refractory corneal disorders, which was first recognized in 1824. This category of diseases is caused by damage to the trigeminal nerve and the consequent loss of corneal sensation. It leads to various types of corneal disorder, including superficial keratopathy, persistent epithelial defects and corneal ulcers. In the present article, we review the pathobiology and prevention/ treatment of neurotrophic corneal diseases.

The goals of treatment of neurotrophic keratopathy are to prevent the progression of corneal damage to promote epithelial healing in case that the original damage in the trigeminal nerve or its braches is difficult. The therapy should be prompt and based on the clinical stage of the disease. Although at present, there are no accurate treatment methods for neurotrophic keratopathy, several effective treatments have been reported.

Signals derived from TRP channels are involved in homeostasis of tissues by controlling cell behaviors, i. e., gene expression for inflammation, cell migration, or cell survival/cell death. Targeting TRP channel in the cornea is expected to contribute to the development of a new treatment method for neurotrophic keratopathy. Further study and clinical trial are need to establish this new treatment strategy.

Key words: Neurotrophic keratopaty, Pathology, Treatment

Offprint requests to: Yuka Okada, M.D., Ph.D., Department of Ophthalmology, Wakayama Medical University, 811-1 Kimiidera, Wakayama, 641-0012, Japan. e-mail: yokada@wakayama-med.ac.jp

Introduction

Neurotropic keratopathy is one of the refractory corneal disorders, which was first recognized in 1824 (Magendie, 1824), results from damage to the trigeminal nerve and the consequent loss of corneal sensation. It leads to various types of corneal disorder, including superficial keratopathy, persistent epithelial defects and corneal ulcers (Mishima, 1957; Lim, 1976; Beuerman et al., 1980; Klyce et al., 1985). Corneal nerves are critical to the detection of noxious stimuli at the surface of the cornea and for protection against corneal injury (Acosta et al., 2001; Belmonte et al., 2004). Several reports have shown the biological mechanisms underlying the phenomenon that a loss of corneal sensory innervation leads to a decrease in the vitality, metabolism, and mitosis of epithelial cells, and consequently to epithelial breakdown (Sigelman and Friedenwald, 1954). In the present article, we review the pathobiology and prevention/treatment of neurotrophic corneal diseases.

Anatomy and histology of the cornea

The cornea is not an isolated tissue, forming, together with the sclera, the outer shell of the eyeball (Fig. 1). This tissue lacks the vascular supply and the tear fluid (anteriorly) and aqueous humor (posteriorly) are the main routes of the nutrients and O_2 for the tissue. Although both the cornea and sclera are composed of dense connective tissue, the physiological roles of these two parts of the eye shell differ from each other. The cornea is the transparent "window" of the eye that allows light to penetrate. These functions require the cornea to be transparent and possess a smooth surface (Maurice, 1984; Gipson, 1994; Nishida, 1997).

Histological analysis reveals the layers of the

corneal tissue: epithelium, Bowman's membrane, stroma, Descemet's membrane, and endothelium. The corneal stroma is a layer of avsculartхick connective tissue of collagen lamellae. Proteglycan components occupy the space among the collagen fibers and modulate their diameter that is critical to the corneal transparency. Mesenchymal cells, i.e., keratocytes or corneal fibroblasts, locates among the matrix lamellae. The epithelium serves as an important barrier that protects the integrity of the corneal stroma. The persistence of corneal epithelial defects often results in stromal melting, the infiltration of inflammatory cells, and corneal opacity, leading to vision loss.

The cornea is suffused by the tear film anteriorly and by the aqueous humor posteriorly. This characteristic is important for understanding both the regulatory mechanisms that underlie maintenance of normal corneal integrity and the pathobiology of various corneal diseases. Alterations in the components of tear fluid or the aqueous humor may result in pathological changes in the cornea.

Sensory innervation in the cornea

The sensory innervation of the eye originates at the trigeminal ganglion. Most sensory axons directed to the eye run with the first division of the ganglion, the ophthalmic nerve that enters the superior orbital fissure, branching into nesociliary, frontal, and lacrimal nerves. Trigeminal sensory nerve fibers end the connective tissue, epithelia, and blood vessels of the orbit, uvea, ciliary body, extraocular muscles, choroid, scleral spur, lids, sclera, cornea, and conjunctiva. The cornea is the most sensitive tissue in the body, being 300 to 400 times more sensitive than the skin. It is innervated by dense nerve endings, with numerous nerve fibers forming a network structure at various levels of the tissue (Tervo and Palkama, 1978; Marfurt et al., 1989, 2001; Muller et al., 1996, 1997, 2003) (Fig. 2).

The majority of axons of corneal sensory neurons (about 70%) are polymodal nociceptors. Sensory nerves are mainly detected in the anterior two thirds of the thickness of the stroma. The posterior one third of this layer of the stroma as well as Descemet's membrane do not contain sensory nerve fibers. They are activated by near-noxious mechanical energy, heat, and chemical irritants, and by a large variety of endogenous chemical mediators released by damaged corneal tissue and resident inflammatory cells, or leakage from limbal vessels (Belmonte and Giraldez, 1981; Belmonte et al., 1991; Gallar et al., 1993). About 15-20% of the peripheral axons innervating the cornea, all thinly myelinated, respond only to mechanical forces in the order of magnitude close to that required to damage corneal epithelial cells.

Another category of corneal nerve fibres, which represents 10-15% of the total population, is the group of cold-sensitive thermal receptors. These are Ad and C fibers, which are transiently silenced on warming (Brock et al., 1998; Tanelian and Beuerman, 1984; Jaffe, 1938; Pannabecker, 1944).

Neurotransmitters, i.e., substance P (SP), calcitonin gene-related peptide (CGRP), and acetylcholine, are secreted by sensory nerve fibers in the corneal stroma, as revealed by employing immunohistochemistry or biochemical techniques (Tervo et al., 1981, 1982; Stone and McGlinn, 1988; Uusitalo et al., 1989; Beckers et al., 1992). In certain epithelial tissues, activation of transient receptor potential (TRP) vanilloid subtype 1 (TRPV1) by noxious stimuli induces pro-inflammatory cytokine release, which helps to mitigate the challenge. While the corneal epithelium elicits such responses to a variety of challenges, it remains unknown whether TRPV1 mediates pro-inflammatory cytokine secretion. TRPV1 has been suggested to be involved in neurogenic inflammation or in the process of certain allergic

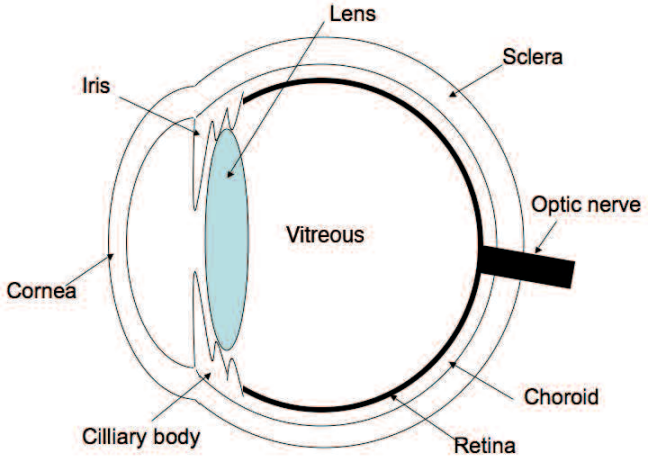

Fig. 1. Structure of a human eye. Light reaches the retina through a transparent vitreous gel after refraction by the cornea and lens. The iris modulates the volume of light that comes into the eye. The choroid, which contains abundant blood vessels and melanin cell is located between retina and sclera.

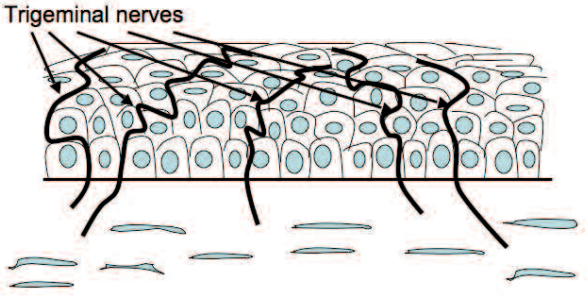

Fig. 2. Trigeminal sensory nerve fibers end in cornea. It is innervated by dense nerve endings, with numerous nerve fibers forming a network structure.

inflammation, and its co-existence in nerves containing SP or CGRP has been confirmed (Murata and Murata, 2006).

Healing process of corneal epithelial wounds

In the clinical setting, various corneal epithelial disorders are encountered, some of them exhibit a delay in the healing process. In the treatment of corneal epithelial disorders, to clarify the cause of delayed epithelial wound healing, the states of both the corneal epithelium and its environment should be accurately evaluated. Diagnosis and treatment markedly differ between whether the corneal epithelial disorder is limited to the corneal epithelium or involves the entire ocular surface including the conjunctival epithelium. Stem cells, the progenitor cells of the corneal epithelium, locates to the limbus which is the birder region between cornea and conjunctiva. Therefore, in addition, the degree of stem cell damage in the corneal limbus markedly affects corneal epithelial repair. When the corneal limbus is normal, even in the presence of corneal epithelial defects, no cell proliferation occurs around the defects, cell elongation and migration occur first, and the defect is repaired by a cell layer and subsequently regenerated and repaired by cell proliferation.

Thoft proposed the concept of the "ocular surface" and the XYZ theory for the understanding of corneal epithelial wound healing (Thft and Friend, 1983). Sun et al. clarified the importance of stem cells in the corneal limbus as a source of corneal epithelial cells (Sun and Green, 1977; Cotsarelis et al., 1989).

When the corneal limbus is normal, and only corneal epithelial defects are present, the wound healing process can be classified into 3 phases: the elongation/migration (first phase), proliferation (second phase), and differentiation (third phase) of epithelial cells.

When corneal epithelial defects develop, adhesion glycoproteins , i. e., fibronectin appears and provide a scaffold for cell migration (Fujikawa et al., 1981; Suda et al., 1981, 1982; Murakami et al., 1992). The remaining epithelial cells are attached to the fibronectin matrix as a temporal basement membrane, and migrate, initiating wound healing as the first step. Once the defect is covered with a layer of epithelial cells, epithelial cells begin to proliferate, increasing the epithelial thickness (the second phase). Then the epithelial cells begin to differentiate, forming a stratified, ordered structure in association with upregulation of molecules related to maturation of stratified epithelium in the third phase, as is observed in the normal corneal epithelium, and wound healing is completed (Tervo et al., 1992; Nishida, 1993; Dua et al., 1994).

Humoral factors involved in wound of corneal epithelium

Blood vessels are absent in cornea, and epithelial cells are in contact with tear fluid containing various physiologically active substances such as growth factors and cytokines, and their function is regulated by humoral factors from the limbal capillary loop and conjunctiva. There are also receptors on corneal epithelial cells for these humoral factors. Such humoral factors interact for both maintenance of the integrity of the normal cornea as wellas for the control and regulation of epithelial cell response to wounding.

The factors involved in the control/regulation of epithelial cell migration include humor factors and matrix components; epidermal growth factor (EGF), keratinocyte growth factor (KGF), transforming growth factor (HGF), transforming growth factor (TGFb), interleukin, fibronectin (Nishida et al., 1983, 1984, 1990), and hyaluronic acid (Miyauchi et al., 1990; Nishida et al., 1991). EGF is present at an adequate concentration in lacrimal fluid for corneal epithelial cell proliferation, is considered to be supplied by the lacrimal gland (Wilson et al., 1991; Watanabe et al., 1993), and to promote the proliferation of both corneal epithelial and parenchymal cells, i. e., keratocyes. KGF is secreted by corneal parenchymal cells and acts on epithelial cells in a paracrine fashion (Sotozono et al., 1994), promoting corneal epithelial repair (Wilson et al., 1993). HGF as well as KGF is produced by corneal parenchymal cells and acts on epithelial cells, promoting corneal epithelial repair (Wilson et al., 1994). A cytokine/growth factor activates intracellular signaling cascades upon its binding to its cell surface receptor. The ligand/receptor signaling cascades often converge via mitogen-activated kinases (MAPK) that include three subfamilies, i.e., p42/44 ERK (external regulated kinase, MAP kinase), c-Jun N-terminal kinase (JNK), and p38 MAP kinase (Javelaude and Mauviel, 2005). These signaling pathways mediate different biological responses. For example, pP42/44 MAP kinase is involved in cell proliferation regulation whereas JNK and p38 are activated upon cellular stresses and modulate cell survival/cell death or control expression of stress-response genes.

TGFß, one of the most important growth factor involved in connective tissue healing and remodeling, has also a critical role in the epithelial healing. TGFß family membersincludes three isoforms, i. e., TGFß1 to ß3, TGFß activates various signaling cascades which regulate gene expressions in various setting. Such cascades include Smad, MAPK, p38MAPK and JNK. Smad signal is characteristic to TGFß superfamily, while the later three signals can ben activated by various growth factor and cytokines. During the healing of corneal epithelial defects, endogenous TGFß activates p38MAPK for cell migration and the suppression of cell proliferation and up-regulates Smad7 for the inhibition of Smad2 and Smad3 signaling, resulting in rapid initial resurfacing of the epithelium (Saika et al., 2004, 2008; Saika, 2004, 2006).

Many interleukins (ILs) are involved in inflammation and immune reactions in the cornea. These interleukins do not directly affect cell proliferation but

regulate growth factor expression and recruit inflammatory cells to local areas. Among them, IL-1, -6, -8 are considered to be involved in corneal wound healing (Nishida et al., 1992; Boisjoly et al., 1993; Cubitt et al., 1993).

Several studies have focused on the role of sensory neuromediators in the corneal epithelium. These studies have shown the depletion of substance P (SP) and acetylcholine (Ach) after sensory nerve injury (Mishima, 1957). SP, a member of the tachykinin family, is an 11-amino-acid peptide. The physiological functions of SP including regulation of miosis and neurogenic inflammation. In addition to functioning as a neurotransmitter (Elsas et al., 1994), SP also acts as a neurotrophic factor. Indeed, SP is present in the nerve fibers of corneal epithelium (Miller et al., 1981; Tervo et al., 1981, 1982; Shimizu, 1982; Stone et al., 1982; Stone and Kuwayama, 1985).

Neuroparalytic keratopathy

Clinical aspects

The cornea is provided with the richest innervation of all body tissues (40 times more than the tooth pulp and 400 times more than skin). Neurotrophic keratitis is a rare degenerateive corneal disease caused by an impairment of trigeminal corneal innervation caused by different ocular and systemic diseases, leading to a decrease or absence of corneal sensation. In most cases, the clinical diagnosis of neurotrophic keratopathy is easily made on the basis of history, but this condition is one of the most difficult and challenging corneal diseases to manage. The pathology of neurotrophic keratopathy is basically delayed corneal epithelial wound healing.

As examined by using a slit lamp microscopy, Neurotrophic keratopathy is clinically characterized by various corneal epithelial disorders such as conjunctival hyperemia, stippled haze due to corneal edema, bulla formation, and superficial punctate keratopathy (SPK), and epithelial defects are dry and cloudy (Jaffe, 1938; Pannabecker, 1944). Clinical findings are classified by the severity of the damage of the corneal tissue; they include SPK, recurrent corneal erosion, persistent corneal epithelial defects, which are defects in full-thickness corneal defects without re-coverage, or corneal ulcer with parenchymal melting (Paton, 1926; Cavanagh et al., 1979; Cavanagh and Colley, 1989).

The most frequent cause of decreased corneal sensitivity is herpetic keratitis due to herpes simplex or zoster infection (Cavanagh et al., 1976; Wormack and Liesegang, 1983; Liesegang, 1985; Harding et al., 1987; Cobo, 1988; Liesegang, 1988), and a decrease in corneal sensitivity is a major clinical finding for the differential diagnosis. Constantly decreased corneal sensitivity is also observed after corneal transplantation or cataract surgery involving nerve transection (Rao et al., 1985; Jone et al., 1988; Campos et al., 1992; Tugal et al., 1993; Ishikawa et al., 1994), the necrosis of corneal scleral tissue including nerve fibers due to corneal burn or alkali injury, and toxic keratopathy due to the frequent use of topical anesthetics or drugs for glaucoma (Van Buskrik, 1979; Chang et al., 1984; Rosenwasser et al., 1990; Weissman and Asbell, 1990). These are considered disorders at the periphery of the trigeminal nerve. In trigeminal palsy associated with surgery for brain and auditory tumors (Onofrio, 1975; Rose, 1982; Adams and Cullen, 1987; Sterkers et al., 1994) or congenital familial dysautonomia (Goldberg et al., 1968), corneal sensitivity is decreased at the level of the trigeminal nucleus or ganglion. In patients with diabetes mellitus, decreased corneal sensitivity is often observed, suggesting the involvement of sensory nerves in the pathology of diabetic keratopathy (Schwarts, 1974; Schultz et al., 1983; Saini and Khandalavla, 1995). (Table.1)

Clinical staging of neurotrophic keratopaty

As discussed above the neurotrophic ulcer is classified by its degree of the damage in the corneal tissue. Stage 1 is characterized by rose bengal staining of the inferior palpebral conjunctival surface, a decrease in breakup time, and a punctate keratopaty. If these changes become chronic, superficial vascularization, epithelial hyperplasia and irregularity and stromal scarring.

Table 1. Causes of reduced corneal sensitivity.

Genetic diseases
Corneal distrophies
Familiar corneal hypoaesthesia
Goldenar-Gorlin syndrom
Multiple endocrine neoplasia IIb
Hereditary sensory and autonomic neuropathy types III, IV or V
Other systemic diseases
Diabetes
Leprosy
Neurosarcoidosis
Orbital tumours and inflammations
Brainstem diseases (cerebrovascular disease, multiple sclerosis)
Fifth nerve palsy due to trigeminal leasions in posterior fossa (aneuysm, acoustic neuroma, meningioma)
Eye Surgery
Catarect surgery
Refractive surgery
Retinal surgery
Cycloablation
Infections
Viral infections (herpes simplex and zoster)
Large bacterial ulcers
Late Acanthamoeba ulcers
Drugs
Topical b-blockers
Topical non-steroidal anti-inflammatory drugs
Topical anaesthetics
Other conditions
Chemical and thermal burns
Contact lens wear

Stage 2 is characterized by a present epithelial defect with an oval or circular shape that is often localized in superior half cornea. Usually, around the epithelial defect, there is an area poorly adherent opaque and oedematous epithelium that can spontaneously detach leading to an enlargement of the defect. The edges of the defect become smooth and rolled as the defect ages without appreciable epithelial growth (Cavanagh et al., 1976). Stromal swelling and folds in the Descemet membrane my also be observed with an inflammatory reaction in the anterior chamber and rarely, sterile hypopion may be observed.

Stage 3 is characterized by stromal involvement with a corneal ulcer that may progress to perforation and/or stromal melting. Secondary infection or topical treatment with corticosreroids increases the risk for perforation.

Pathobiology of neuronal regulation of integrity of corneal epithelium

It is generally accepted that corneal sensory nerves play a key role in maintaining the anatomical integrity and function of the cornea and particularly of the epithelium. On the other hand, it has been shown that impairment of the corneal sensory nerves affects corneal epithelial function and vitality by decreasing the metabolism and mitosis of epithelial cells and increasing the epithelium's permeability (Sigelman and Friedenwald, 1954; Mishima, 1957), leading the development of neurotrophic corneal diseases. The mechanisms of the phenomenon is to be explored, but explanations include that a decrease in cell proliferation due to a decrease in acetylcholine in corneal epithelial cells, thinning of the cell layer, an increase in permeability due to impaired intercellular tight junctions and dilated intercellular spaces, and delayed wound healing due to a decrease in adhesion between corneal epithelial cells and the basement membrane (Cavanagh and Colley, 1989; Alper, 1976; Beuerman and Schimmelpfenning, 1980; Schimmelpfenning and Beuerman, 1982; Barker et al., 1993; Araki et al., 1994).

A similar background of impaired tissue healing is observed in diabetic foot ulcder. Diabetic patients are consequently more prone to develop foot ulcers, resulting from a multitude of factors, including neuropathy, vascular disease and foof deformities (Jude and Boulton, 1997; Jeffcoate and Harding, 2003). In the skin of diabetic patients, including those having ulcers, and of genetically diabetic mice, diminishing staining was seen for such nerve fibres (Kennedy et al., 1996; Gibran et al., 2002), as well as for neuropeptides, including SP (Levy et al., 1989, 1992).

Treatment of neurotrophic keratopathy

The goals of treatment of neurotrophic keratopathy are to prevent the progression of corneal damage to promote epithelial healing in case that the original damage in the trigeminal nerve or its braches is difficult. The therapy should be prompt and based on the clinical stage of the disease (Lambiase et al., 1999). Although at present, there are no accurate treatment methods for neurotrophic keratopathy, several effective treatments have been reported.

Treatment methods at present

Classical treatment

Stage 1 requires the discontinuation of all topical medications and the evaluation of side effects of systemic therapies such as neuroleptic, antipsychotic and antihistamine drugs. Administration of topical preservative-free artificial tears may help in improving the corneal surface (Bonini et al., 2000). The therapy at this stage aims to improve epithelial quality and transparency and to avoid epithelial breakdown. Some authors have suggested the use of scleral or therapeutic contact lenses, but these lenses may increase the risk forsecondary infections and their use may by complicated by development of a sterile hypopyon (Kent et al., 1990; Pfister, 1992).

When an epithelial defect develops (stage 2), the aims of treatment are to avoid the development of corneal ulcer to promote healing of epithelial defect and to prevent the recurrence of the epithelial breakdown. Various approaches are suggested; the goal is to cover the area of epithelial deficit to promote healing. The simplest and most used procedure is a lateral tarsorrhaphy (Alper, 1976). If healing occurs, the tarsorraphy opening may be enlarged after a few weeks, but opening the tarsorraphy prematurely may results in a recurrence of corneal epithelial breakdown. Alternatively, it is possible to cover the epithelial defect by means of an amniotic membrane transplantation (Lee and Tseng, 1997) or to use a palpebral spring or botulium A toxin injection of eyelid elevator (Kirkness et al., 1988; McNeill and Oh, 1991). Tropical steroids have been suggested because prostaglandins inhibit the epithelial growth and the use of steroids could reduce the activity of these inflammatory mediators (Canvanagh et al., 1979; Canvanagh and Colley, 1989). However, steroid by inhibiting stromal healing, may increase the risk of corneal stromal melting and perforation, thus their use should be considered with caution. Topical nonsteroid anti-inflammatory drug treatment does not induce improvement of the healing process (Hersh et al., 1990).

When a corneal ulcer develops (stage 3), the therapy is aimed at promoting corneal healing and preventing corneal melting and perforation. In the case of stromal melting, topical collagenase inhibitors ß such as N-acetylcysteine, tetracycline or medroxyprogesterone may be administered (Davis and Dohlman, 2001). Alternative treatments include use of a conjunctival flap, tectonic lamellar keratoplasty, use of tissue adhesive (Alper, 1976), and amniotic membrane transplantation (Kirkness

et al., 1988). Large defects require lamellar or penetrating keratoplasty (Alper, 1976; Gersh et al., 1990). The success rates of these corneal transplants are low because of poor wound healing and the persistent risk for epithelial defects due to corneal hypoesthesia.

SP and insulin-like growth factor (IGF-1) in the treatment of neurotrophic keratopathy

SP has evolved considerably since it was first discovered in 1931. SP, a member of the tachykinin family, is an 11-amino-acid peptide. The physiological functions of SP include regulation of miosis and neurogenic inflammation. SP is, however, easily degraded and inactivated by neuropeptidases, i. e., carboxypeptidases and endopeptidases (Guyon et al., 1979; Matsas et al., 1983, 1984; Lebien and McCormack, 1989). In addition to functioning as a neurotransmitter (Elsas et al., 1994), SP also acts as a neurotrophic factor. SP is present in the nerve fibers of corneal epithelium (Miller et al., 1981; Tervo et al., 1981, 1982; Shimizu, 1982; Stone et al., 1982, 1985).

SP exerts its biological effects through binding to G protein-coupled receptors, among which the neurokin type 1 (NK-1) receptor shows the highest affinity for SP (Regoli et al., 1994).

Human IGF-1 contains 70 amino acids, woth a molecular mass of 7649 daltons, and it comprises four domains designated A, B, C and D. It is also known as aomatomedin C and possesses insulin-like activity. Although the A and B domains of the three proteins share many strucyural similarities, the C domains of the IGFs show no homology to the C peptide of proinsulin, which is not retained in mature insulin. In addition, IGFs possess the D domain at their carboxyl termini, whereas proinsulin has no corresponding sequence.

Nishida et al. observed that the effect of SP on corneal epithelial migratin (Nishida et al., 1992; Nakamura et al., 2000). Whereas SP alone had no effect, EGF by itself significantly increased the oath length of epithelial migration. Neither IGF-1, basic fibroblast growth factor (bFGF) nor TGFß alone affected epithelial migration. However, EGF and IGF-1 each acted synergistically with SP to promote corneal migration. This combined effect of SP and IGF-1 depends on the concentration of each factor (Nishida et al., 1996). The synergestic action of SP with IGF-1 was found to be mediated by the NK-1 receptor, but not by NK-2 or NK-3 (Nakamura et al., 1997). In in vitro, SP, as well as cholecystokinin gene-related peptide (CGRP), and Ach, induces epithelial proliferation (Cavanagh and Colley, 1989; Mikulec and Tanelian et al., 1996; Reid et al., 1993).

Following injury to soft tissue, SP and other neuropeptides are released by cutaneous neurons and modulate the function of immunocompetent and inflammatory cells, as well as epithelial and endothelial cells (Bockers et al., 1989; Hagen et al., 1990; Ziche et al., 1990; Dunnick et al., 1996). The elicidation of SP-mediating mechanisms is crucial to firmly establishing the involvement and interaction of the peripheral nervous system ans the immune system in cutaneous repair. SP participates in the complex network of mediators involved in cutaneous inflammation and wound healing (Delgado et al., 2005).

The carboxyl-terminal for amino acids of SP (Phe-Gly-Leu-Met-amide, or FGLM-amide) are sufficient for this effect (Nakamura et al., 1997). The minimum nucleotide sequence of SP required for the synergistic effects of SP and IGF-1 has been identified as FGLM-NH_2 (Yamada et al., 2006).

SP (or FGLM) and IGF-I instillation is a potential treatment method for neurotrophic keratopathy.

Concept of new strategies based on the roles of TRP channel

Vanilloid receptor subtype 1 (VR1) as a capsaicin receptor is a non-selective cation channel with high Ca^{2+} permeability and 6 transmembrane regions that were cloned in 1997, and its activation induces neuronal excitation by depolarization, changing nociceptive stimuli to electrical signals (Fig. 3). VR1 belongs to the transient receptor potential (TRP) family and, therefore, is called transient receptor potential vanilloid subtype 1 (TRPV1). The TRP superfamily is classified into 7 subfamilies: TRPC, TRPV, TRPM, TRPN, TRPP, TRPA, and TRPML (Montell, 2005). TRPV1 is specifically expressed on sensory nerves as well as in corneal epithelium (Murata and Masuko, 2006; Zhang et al., 2007) and plays a central role in nociception activated by multiple nociceptive stimuli such as capsaicin, acid, and heat (>43°C). TRPV1 expression at the gene and protein levels has been evaluated.

The corneal sensory neurons have been well documented that their somata are mainly located in the

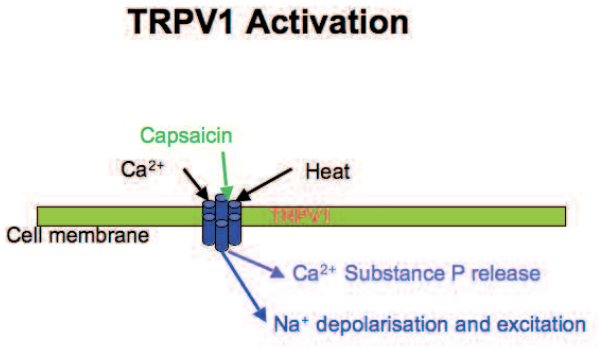

Fig. 3. TRPV1 Activation. The TRPV1 elicits responses to a variety of diverse noxious stimuli that include capsaicin, hypertonic challenges, a decline in pH (<6.0) or by moderate heat (≥ 43°C), all of which lead to nocioceptions., TRPV1 activation induces in addition to Ca^{2+} influx also substance P release , Na^+ influx and membrane depolarization leading to neuronal excitation.

dorsal part of the ophthalmic region of the ipsilateral trigeminal ganglion (Marfurt and Del Toro, 1987; Takemura et al., 1991). SP and CGRP coexist in single of TG (Lee et al., 1985; Skofitsch and Jacobowitz, 1985; Ma, 2002) and possibly in the corneal fibers (Beckers et al., 1993). TRPV1-containing neurons co-express CGRP in TG (Guo et al., 1999; Ichikawa and Sugimoto, 2001), and capsaicin evokes CGRP release via a vanilloid-receptor-mediated exocytotic mechanism from rat buccal mucosa of trigeminal field of innervation (Flores et al., 2001). Capsaicin has been reported to promote the in vitro repair of corneal epithelial cell defects (Zhang et al., 2007). In addition, our study using TRPV1 knockout mice confirmed a delay in the repair of corneal epithelial defects but infrequent corneal inflammation after alkali injury, corneal opacity, or new blood vessel invasion (Okada et al, unpublished data). We also confirmed the reproducibility of this phenomenon by the administration of an antagonist for TRPV1. The clarification and control of the action mechanism of the TRP channel in the cornea are expected to contribute to the development of a new treatment method for neurotrophic keratopathy. Further study and clinical trial are need to establish this new treatment strategy.

References

Acosta M.C., Tan M.E., Belmonte C. and Gallar J. (2001). Sensations evoked by selective mechanical, chemical, and thermal stimulation of the conjunctiva and cornea. Invest. Ophthalmol. Vis. Sci. 42, 2063-2067.

Adams G.G and Cullen J.F. (1987). Neuroparalytic keratitis and the effect of cervical sympathectomy following operative procedures for trigeminal neuralgia. Scott. Med. J. 32, 86-88.

Alper M.G. (1976). The anethetic eye: An investigation of changes in the anterior ocular segment of the monkey caused by interrupting the trigeminal nerve at various levels along its course. Trans. Am. Ophthalmol. Soc. 73, 323-365.

Araki K., Ohashi Y., Kinoshita S., Hayashi K., Kuwayama Y. and Yano Y. (1994). Epithelial wound healing in the genervated cornea. Curr. Eye. Res. 13, 203-211.

Beckers H.J., Klooster J., Vrensen G.F. and Lamers W.P. (1992). Ultrastructural isentification of trigeminal nerve endings in the rat cornea and iris. Invest. Ophthalmol. Vis. Sci. 33, 1979-1986.

Beckers H.J., Klooster J., Vrensen G.F. and Lamers W.P. (1993). Substance P in rat corneal and iridal nerves: anultrastructural immunohistochemical study. Ophthalmic Res. 25, 192-200.

Belmonte C. and Giraldez F. (1981). Responses of cat corneal sensory receptors to mechanical and thermal stimulation. J. Physiol. 321, 355-368.

Belmonte C., Gallar J., Pozo M.A. and Rebollo I. (1991). Excitation by irritant chemical substances of sensory afferent units in the cat's cornea. J. Physiol. 437, 709-725.

Belmonte C., Acosta M.C. and Gallar J. (2004). Neural basis of sensation in intact and injured corneas. Exp. Eye. Res. 78, 513-325.

Beuerman R.W. and Schimmelpfenning B. (1980). Sensory denervation of the rabbit cornea affects epithelial properities. Exp. Neurol. 69, 196-201.

Bockers M., Benes P. and Bork K. (1989). Persistent skin ulcers, mutilations and acro-osteolysis in herediary sensory and autonomic neuropathy with phospholipids excretion. J. Am. Acad. Dermatol. 21, 736-739.

Boisjoly H.M., Laplante C., Bernatchez S.F., Salesse C., Giasson M. and Joly M.C. (1993). Effects of EGF, IL-1 and their combination on in vitro corneal epithelial wound closure and cell chemotaxis. Exp. Eye Res. 57, 293-300.

Bonini S., Lambiase A., Rama P., Caprioglio G. and Aloe L. (2000). Topical treatment with nerve growth factor for neurotrophic keratitis. Ophthalmology 107, 1347-1352.

Brock J.A., McLachlan E.M. and Belmonte C. (1998). Tetrodotoxin-resistant impulses in single nociceptor nerve terminal in guinea-pig cornea. J. Physiol. 512, 211-217.

Campos M., Hertzog L., Garbus J.J. and Mc Donnell P.J. (1992). Corneal sensitivity after photorefractive keratectomy. Am. J. Ophthalmol. 114, 51-54.

Cavanagh H.D. and Colley A.M. (1989). The molecular bsis of neurotrophic keratitis. Acta. Ophthalmol. 67 (Suppl 192), 115-134.

Cavanagh H.D., Pihlaja D., Thoft R.A. and Dohlman C.H. (1976). The pathogenesis and treatment of persistent epithelial defects. Trans. Am. Acad. Ophthalmol. Otolaryngol. 81, 754-769.

Cavanagh H.D., Colley A. and Pihlaja D.J. (1979). Persistent corneal epithelial defects. Int. Ophthalmol. Clin. 19, 197-206.

Cavanagh H.D., Pihlaja D., Thoft R.A. and Dohlman C.H. (1976). The pathogenesis and treatment of persistent epithelial defects. Trans. Am. Acad. Ophthalmol. Otol. 81, 754-769.

Chang F.W., Reinhart S. and Fraser N.M. (1984). Effect of 30% sodium sulfacetamide on corneal sensitivity. Am. J. Ophtom. Physiol. Opt. 61, 318-320.

Cobo L.M. (1988). Corneal complications of herpes zoster ophthalmicus. Prevention and treatment. Cornea 7, 50-56.

Cotsarelis G., Cheng S.Z., Dong G., Sun T.T. and Lavker R.M. (1989). Existence of slow-cycling limbal epithelial basal cells that can be preferentially stimulated to proliferate: implications on epithelial stem cells. Cell 57, 201-209.

Cubitt C.L., Tang Q., Monteiro C.A., Lausch R.N. and Oakes J.E. (1993). IL-8 gene expression in cultures of human corneal epithelial cells and keratocytes. Invest. Ophthalmol. Vis. Sci. 34, 3199-3206.

Davis E.A. and Dohlman C.H.(2001). Neurotrophic keratitis. Int. Ophthalmol. Clin. 41, 1-11.

Delgado A.V., McManus A.T. and Chambers J.P. (2005). Exogenous administration of substance P enhances wound healing in a novel skin-injury model. Exp. Biol. Med. 230, 271-280.

Dua H.S., Gomes J.A. and Singh A. (1994). Corneal epithelial wound healing. Br. J. Ophthalmol. 78, 401-408.

Dunnick C.A., Gobran N.S. and Heimbach D.M. (1996). Substance P has a role in neurogenic mediation of human burn wound healing. J. Burm. Care. Rehabil.17, 390-396.

Elsas T., Edvinsson L., Sundler F. and Uddman R. (1994). Neuronal pathways to the rat conjunctiva revealed by retrograde tracing and immunocytochemistry. Exp. Eye Res. 58, 117-126.

Flores C.M., Leong A.S., Dussor G.O., Harding-Rose C., Hargreaves K.M. and Kilo S. (2001). Capsaicin-evoked CGRP release from ratbuccal mucosa: development of a model system for studying trigeminal mechanisms of neurogenic inflammation. Eur. J. Neurosci. 14, 1113-1120.

Fujikawa L.S., Foster C.S., Harrist T.J., Lanigan J.M. and Colvin R.B. (1981). Fibronectin in healing rabbit corneal wounds. Lab. Invest. 45, 120-129.

Gallar J., Pozo M.A., Tuckett R.P. and Belmonte C. (1993). Response of sensory units with unmyelinated fibers to mechanical, thermal and chemical stimulation of the cat's cornea. J. Physiol. 468, 609-622.

Gibran N.S., Jang Y.C., Isik F.F., Greenhalgh D.G., Muffley L.A., Underwood R.A., Usui M.L., Larsen J., Smith D.G., Bunnett N., Ansel J.C. and Olerud J.E. (2002). Diminish neuroeptide levels contribute to impaired cutaneous healing response associated with diabetes mellitus. J. Surg. Res.108, 122-128.

Gipson I.K. (1994). Anatomy of the conjunctiva, cornea, and limbus. In: The cornea: Scientific foundations and clinical practice. Smolin G. and Thoft R.A. (eds). Little Brown and Company. pp 3-24.

Goldberg M.F., Payne J.W. and Brunt P.W. (1968). Ophthalmologic studies of familial dysautonomia. The Riley-Day syndrome. Arch. Ophthalmol. 80, 732-743.

Guo A., Vulchanova L., Wang J., Li X. and Elde R. (1999). Immunocytochemical localization of the vanilloid receptor 1 (VR1): relationship to neuropeptides, the P2X3 purinoceptor and IB4 binding sites. Eur. J. Neurosci. 11, 946-958.

Guyon A., Roques B.P., Guyon F., Fougault A., Perdrisot R., Swerts J.P. and Schwartz J.C. (1979). Enkephalin degradation in mouse brain studied by a new H.P.L.C. method: further evidence for the involvement of carboxydipeptidase. Life. Sci. 25, 1605-1612.

Hagen N.A., Stevens J.C. and Michet C.J. (1990). Trigeminal sensory neuropathy associated with connective tissue disease. Neurology 40, 891-896.

Harding S.P., Lipron J.R. and Wells J.C.D. (1987). Natural history of herpes zoster ophthalmicus: Predictors of postherpetic neuralgia and ocular involvement. Br. J. Ophthalmol. 71, 353-358l.

Hersh P.S., Rice B.A., Baer J.C., Wells P.A., Lynch S.E., McGuigan L.J.B. and Foster C.S. (1990). Topical nonsteroidal agents and corneal wound healing. Arch. Ophthalmol. 108, 577-583.

Ichikawa H. and Sugimato T. (2001). VR-immunoreactive primary sensory neurons in the rat trigeminal ganglion. Brain. Res. 890, 184-188.

Ishikawa T., Park S.B., Cox C, del Cerro M. and Aquavella J.V. (1994). Corneal sentation following excimer laser photorefractive keratectomy in humans. J. Refract. Corneal. Surg. 10, 417-422.

Jaffe M. (1938). Neuroparalytic keratitis. Arch. Ophthalmol. 20, 688-689.

Javelaud D. and Mauviel A. (2005). Crosstalk mechanisms between the mitogen-activated protein kinase pathways and Smad signaling downstream of TGF-ß: implications for carcinogenesis. Oncogene 24, 5742-5750.

Jeffcoate W.J. and Harding K.G. (2003). Diabetic foot ulcers. Lancet 361, 1545-1551.

Jude E.B. and Boulton A.J.M. (1997). End-stage complications of diabetic neuropathy. Diabet. Rev. 7, 395-410.

Kennedy W.R., Wendelschafer-Crabb G. and Johnson T. (1996). Quantitation of epidermal nerves in diabetic neuropathy. Neurology 47, 1042-1048.

Kent H.D., Cohen E.J., Laibson P.R. and Arentsen J.J. (1990). Microbial keratitis and corneal ulceration associated with therapeutic soft contact lens. CLAO J. 16, 49-52.

Kirkness C.M., Adams G.G.W., Dilly P.N. and Lee J.P. (1988). Botulinum toxin A-induced protective ptosis in corneal disease. Ophthalmology 95, 473-480.

Klyce S.D., Beuerman R.W. and Crosson C.E. (1985). Alteration of corneal epithelial ion transport by sympathectomy. Invest. Ophthalmol. Vis. Sci. 26, 434-442.

Lambiase A., Rama P., Aloe L. and Bonini S. (1999). Management of neurotrophic keratopathy. Curr. Opin. Ophthalmol. 10, 270-276.

Lavielle S., Chassaing G., Ploux O., Loeuillet D, Besseyre J., Julien S., Marquet A., Convert O., Beaujouan J.C. and Torrens Y. (1988). Analysis of tachykinin binding site interactions using constrained analogues of tachykinins. Biochem. Pharmacol. 37, 41-49.

Lebien T.W. and McCormack R.T. (1989). The common acute lymphoblastic leukemia antigen (CD10)- emancipation from a functional enigma. Blood. 73, 625-635.

Lee S.H. and Tseng S.C. (1997). Amniotic membrane transplantation for persistent epithelial defects with ulceration. Am. J. Ophthalmol. 123, 303-312.

Lee Y., Kawai Y., Shiosaka S., Takami K., Kiyama H., Hillyard C.J., Girgis S., MacIntyre I., Emson P.C. and Tohyama M. (1985). Coexistence of calcitonin gene-related peptide and substance P-like peptide in single cells of the trigeminal ganglion of the rat: immunohistochemical analusis. Brain Res. 330, 194-196.

Levy D.M., Terenghi G., Gu X.H., Abraham R.R., Springal D.R. and Polak J.M. (1992) Immunohistochemical measurements of nerves and neuropeptides in diabetic skin: relationship to tests of neurological function. Diabetologia 35, 889-897.

Lim C.H. (1976). Innervation of the cornea of monkeys and the effects of denervation. Br. J. Physiol. Opt. 31, 38-42.

Liesegang T.J. (1985). Corneal complications from herpes zoster ophthalmicus. Ophthalmology 92, 316-324.

Liesegang T.J. (1988). Ocular herpes simplex infection: Pathogenesis and current therapy. Mayo Clin. Proc 63, 1092-1105.

Ma Q.P. (2002). Expression of capsacin receptor (VR1) by myelinated primary afferent neurons in rats. Neurosci. Lett, 319, 87-90.

Magendie P.A. (1824). De l'influence de la cinquieme paire de nerfs sur la nutrition et les functions de l'oeil. J. Physiol. 4, 176-177.

Marfurt C.F. and Del Toro D.R. (1987). Cornral sensory pathway in the rat: a hprseradish peroxidase tracing study. J. Comp. Neurol. 261, 450-459.

Marfurt C.F., Kingsley R.E. and Fchtenkamp S.E. (1989). Sebsory and sympathetic innervation of the mammalian cornea. A retrograde tracing study. Invest. Ophthalmol. Vis. Sci. 30, 461-472.

Marfurt C.F., Murphy C.J. and Florczak L. (2001). Morphology and neurochemistry of canine corneal innervation. Invest. Ophthalmol. Vis. Sci. 42, 2242-2251.

Maurice D.M. (1984). The cornea and sclera. In: The eye. Davson H. (ed). Academic. Press. pp 1-158.

Matsas R., Fulcher I.S., Kenny A.J. and Turner A.J. (1983). Substance P and [Leu] enkephalin are hydrolyzed by an enzyme in pig caudate synaptic membranes that is identical with the endopeptidase od kidney microvilli. Proc. Natl. Acad. Sci. 80, 3111-3115.

Matsas R., Kenny A.J. and Turner A.J. (1984). The metabolism of neuropeptides. The hydrolysis of peptides, including enkephalins, tachykinins and their amaligues, by endopeptidase-24.11. Biochem. J. 223; 433-440.

McNeill J.I. and Oh Y.H. (1991). An improved papebral spring for the management of paralytic lagophthalmos. Ophthalmology 98, 715-719.

Mikulec A.A. and Tanelian D.L. (1996). CGRP increases the rate of corneal re-epithelization in an in vtro whole mount preparation. J. Ocul. Phamacol. Ter. 12, 417-423.

Miller A., Costa M., Furness J.B. and Chubb I.W. (1981). Substance P immunoreactive sensory nerves supply the rat iris and cornea. Neurosci. Lett. 23, 243-249.

Mishima S. (1957). The effects of the denervation and the stimulation of

the sympathetic and trigeminal nerve on the mitotic rate of the corneal epithelium in rabbit. Jpn. J. Ophthalmol. 1, 65-73.

Miyauchi S., Sugiyama T. Machida A., Sekiguchi T., Miyazaki K., Tokuyasu K. and Nakazawa K. (1990). The effect of sodium hyaluronate on the migration of rabbit corneal epithelium. An *in vitro* study. J. Ocular Pharmacol. 6, 91-99.

Montell C. (2005). TRP superfamily of cation channels. Sci. STKE. re3

Muller L.J., Pels L. and Vrensen G.F. (1996). Ultrastructural organization of human corneal nerves. Invest. Ophthalmol. Vis. Sci. 37, 476-488.

Muller L.J., Vrensen G.F., Pels L., Cardozo B.N. and Willekens B. (1997). Architecture of human corneal nerves. Invest. Ophthalmol. Vis. Sci. 38, 985-994.

Muller L.J., Marfurt C.F., Kruse F. and Tervo T.M. (2003). Corneal nerves: Structure, contents and function. Exp. Eye. Res. 76, 52542.

Murakami J., Nishida T. and Otori T. (1992). Coordinated apperance of ß1 integrins and fibronectin during corneal wound healing. J. Lab. Clin. Med. 120, 86-93.

Murata M. and Masuko S. (2006). Peripheral and central distribution of TRPV1, substance P and CGRP of rat corneal neurons. Brain. Res. 1085, 87-94.

Nakamura M., Ofuji K., Chikama T. and Nishida T. (1997). The NK1 receptor and its participation in the synergistic enhancement of corneal epithelial migration by substance P and insulin-like growth factor-1. Br. J. Pharmacol. 120, 547-552.

Nakamura M., Chikama T.I. and Nishida T. (2000). Characterization of insulin-like growth factor-1 receptors in rabbit corneal epithelial cells. Exp. Eye. Res. 70, 199-204.

Nakanishi S. (1991). Manmalian tachykinin receptors. Annu. Rev. Neurosci. 14, 123-136.

Nishida T., Nakagawa S., Awata T., Ohashi Y. Watanabe K. and Manabe R. (1983). Fibronectin promotes epithelial migration of cultured rabbit cornea in situ. J. Cell Biol. 97, 1653-1657.

Nishida T. (1993). Extracellular matrix and growth factors in corneal wound healing. Curr. Opin. Ophthalmol. 4, 4-13.

Nishida T. (1997). Cornea. In: Cornea: Fundamentals of cornea and external disease. Krachmer J.H., Mannis M.J. and Holland E.J. (eds). Mosby-Year Book. Inc. pp 3-27.

Nishida T., Nakamura M., Mishima H. and Otori T. (1990). Differential modes of action of fibronectin and epidermal growth factor on rabbit corneal epithelial migration. J. Cell. Physiol. 145, 549-554.

Nishida T., Nakamura M., Mishima H. and Otori T. (1991). Hyaluronan stimulates corneal epithelial migration. Exp. Eye. Res. 53, 753-758.

Nishida T., Nakamura M., Mishima H. and Orori H. (1992). Interleukin 6 promotes epithelial migration by a fibronectin-dependent mechanism. J. Cell. Physiol. 153, 1-5.

Nishida T., Nakamura M., Ofuji K., Reid T.W., Mannis M.J. and Murphy C.J. (1996). Synergistic effects of substance P with insulin-like growth factor-1 on epithelial migration of the cornea. J. Cell. Physiol. 169, 159-166.

Onofrio B.M. (1975). Radiofrequency percutaneous Gass erian ganglion lesions. Result in 140 patients with trigeminal pain. 42, 132-139.

Pannabecker C.L. (1944). Keratitis neuroparalica. Arch. Ophthalmol. 32, 456-463.

Paton L. (1926). The trigeminal nerve and its ocular lesions. Br. J. Ophthalmol. 10, 305-342.

Pfister R.R. (1992). Clinical measures to promote corneal epithelial healing. Acta. Ophthalmol. 70, 73-83.

Rao G.N., John T., Ishida N. and Aquavella J.V. (1985). Recovery of corneal sensitivity in grafts following penetrating keratoplasty. Ophthalmology 93, 1408-1411.

Regoli D., Boudon A. and Fauchere J.L. (1994). Receptors and antagonists for substance P and related peptides. Pharmacol. Rev. 46, 551-599.

Reid T.W., Murphy C.J., Iwahashi C.K., Foster B.A. and Mannis M.J. (1993). Stimulation of epithelial cell growth by the neuropeptide substance P. J. Cell. Biochem. 52, 476-485.

Rose W. (1982). Surgical treatment of trigeminal neuralgia. Lancet 1, 295-302.

Rosenwasser G.O., Holland S., Pflugfelder S.C., Lugo M., Heidemann D.G., Cullbertson W.W. and Kattan H. (1990). Topical anesthetic abuse. Ophthalmology 97, 967-972.

Saika S. (2004). TGF-beta signal transduction in corneal wound healing as a therapeutic target. Cornea 23 (8 Suppl), S25-30.

Saika S. (2006). TGFbeta pathobiology in the eye. Lab. Invest. 86, 106-115.

Saika S., Okada Y., Miyamoto T., Yamanaka O., Ohnishi Y., Ooshima A., Liu C.Y., Weng D. and Kao W.W. (2008). Role of p38 MAP kinase in regulation of cell migration and proliferation in healing corneal epithelium. Invest Ophthalmol Vis Sci. 45, 100-109.

Saika S., Yamanaka O., Sumioka T., Miyamoto T., Miyazaki M., Okada Y., Kitano A., Shirai K., Tanaka S. and Ikeda K. (2008). Fibrotic disorders in eye: Targets of gene therapy. Prog. Retin. Eye Res. 23, 283-305.

Saini J.S. and Khandalavla B. (1995). Corneal epithelial fragility in diabetes mellitus. Can. J. Ophthalmol. 30, 142-146.

Schimmelpfenning B. and Beuerman R. (1982). A technique for controlled sensory denervation of rabbit cornea. Graefes. Arch. Clin. Exp. Ophthalmol. 218, 287-293.

Schultz R.O., Peters M.A., Sobocinski K., Nassif K., Schultsz K.J. (1983). Diabetic keratopathy as a manifestation of peripheral neuropathy. Am. J. Ophthalmol. 96, 368-371.

Schwarts D.E. (1974). Corneal sensitivity in diabetics. Arch. Ophthalmol. 91, 174-178.

Shimizu Y. (1982). Localization of neuropeptides in the cornea and uvea of the rat: an immunohistochemical study. Cell. Mol. Biol. 28, 103-110.

Sigelman S. and Friedenwald J.S. (1954). Mitotic and wound-healing activities of the corneal epithelium. Arch. Ophthalmol. 53, 46-57.

Sterkers J.M., Morrison G.A., Sterker O. and El-Dine M.M. (1994). Preservation of facial, cochlear, and, other nerve functions in acoustic neuroma treatment. Otolaryngol. Head Neck Surg. 110, 146-155.

Skofitsch G. and Jacobowitz D.M. (1985). Calcitonin gene-related peptide coexists with substance P in capsaicin sensitive neurons and sensory ganglia of the rat. Peptides 6, 747-754.

Stone R.A. and Kuwayama Y. (1985). Substance P-like immunoreactive nerves in the human eye. Arch. Ophthalmol. 103, 1207-1211.

Stone R.A., Latles A.M. and Brecha N.C. (1982). Substance P-like immunoreactive nerves in the anterior segment of rabbit, cat and monkey eye. Neuroscience 7, 2459-2468.

Stone R.A. and McGlinn A.M. (1988). Calcitonin gene-related peptide immunoreactive nerves in human and rhesus monkey eyes. Invest. Ophthalmol. Vis Sci. 29, 305-310.

Suda T., Nishida T., Ohashi Y., Nakagawa S. and Manabe R. (1981/1982). Fibronictin appers at the site of corneal stromal wound in rabbits. Curr. Eye. Res. 1, 553-556.

Sun T.T. and Green H. (1977). Cultured epithelial cells of cornea,

conjunctiva and skin: absence of marked intrinsic divergence of their differentiated states. Nature 269, 489-493.

Takemura M., Sugimato T. and Shigenaga, (1991). Difference in central projection of primary afferents innervating facial and intraoral structures in the rat. Exp. Neurol. 111, 324-331.

Tanelian D.L. and Beuerman R.W. (1984). Responses of rabbit corneal nociceptors to mechanical and thermal stimulation. Exp. Neurol. 84, 165-178.

Tervo T. and Palkama A. (1978). Innervation of the rabbit cornea. A histochemical and of ocular surface. Ann. Med. 24, 19-27.

Tervo K., Tervo T., Eranko L. and Eranko O. (1981). Substance P immunoreactive nerves in the rodent cornea. Neurosci. Lett. 25, 95-97.

Tervo K., Tervo T., Eranko L., Vannas A., Cuello A.C. and Eranko O. (1982). Supstance P-immunoreactive nerves in the human cornea and iris. Invest. Ophthalmol. Vis. Sci. 23, 671-674.

Tervo T., van Setten G.B., Päällysaho T., Tarkkanen A. and Tervo K. (1992). Wound healing of the ocular surface. Ann. Med. 24, 19-27.

Thft R.A. and Friend J. (1983). The X, Y, Z hypothesis of corneal epithelial maintenance (letter). Invest. Ophthalmol. Vis. Sci. 24, 1442-1443.

Tugal T.I., Akarcay K., Kozer B.L, Sansoy N. and Urgancioglu M. (1993). Corneal sensitivity after penetrating keratiplasty. Eur. J. Ophthalmol. 3, 66-70.

Uusitalo H., Krootila K. and Palkama A. (1989). Calcitonin gene-related peptide (CGRP) immnunoreactive sensory nerves in the human and guinea pig uvea and cornea. Exp Eye Res. 48, 467-475.

Van Buskirk E.M. (1979). Corneal anesthesia after timolol maleate therapy. Am. J. Ophthalmol. 88, 739-743.

Weissman S.S. and Asbell P.A. (1990). Effects of topical timolol (0.5%) and betaxolol (0.5%) on corneal sensitivity. Br. J. Ophthalmol. 74: 409-412.

Watanabe H., Lloyd S.A. and Kennedy R.H. (1993). Distribution of epidermal growth factor in rat ocular and periocular tissues. Graefe. Arch. Clin. Exp. Ophthalmol. 231, 228-232.

Wilson S.E., He Y.G. and Lloyd S.A. (1993). Hepatocyte growth factor, keratinocyte growth factor, their receptors, fibroblast growth factor recceotor-2, and the cells of the cornea. Invest. Ophthalmol. Vis. Sci. 34, 2544-2561.

Wilson S.E., He Y.G., Weng J., Zieske J.D. and Jester J.V., Schultz G.S. (1994). Effect of epidermal growth factor, hepatocyte growth factor, and keratinocyte growth factor, on proliferation, motility and differentiation of human corneal epithelial cells. Exp. Eye. Res. 59, 665-678.

Wilson S.E., Lloyd S.A. and Kennedy R.H. (1991). Epidermal growth factor messenger RNA production in human lacrimal gland. Cornea 10, 519-524.

Womack L.W and Liesegang T.J. (1983). Complications of herpes zoster ophthalmicus. Arch. Ophthalmol. 101, 42-45.

Yamada N., Yanai R., Kawamoto K., Nagano T., Nakamura M., Inui M. and Nishida T. (2006). Promotion of corneal epithelial wound healing by a tetrapeptide (SSSR) derived from IGF-1. Invest. Ophthalmol. Vis. Sci. 47, 3286-3292.

Zhang F., Yang H., Wang Z., Mergler S., Liu H., Kawakita T., Tachado S.D., Pan Z., Capó-Aponte J.E., Pleyer U., Koziel H., Kao W.W. and Reinach P.S. (2007). Transient receptor potential vanilloid 1 activation induces inflammatory cytokine release in corneal epithelium through MAPK signaling. J. Cell. Physiol. 213, 730-739.

Ziche M., Morbidelli L., Pacini M., Geppetti P., Alessandri G. and Maggi C.A. (1990). Substance P stimulates neovascularization in vivo and proliferation of cultured endothelial cells. Microvasc. Res. 40, 264-278.

Accepted November 20, 2009