

## CLINICAL APPLICATION OF NERVE GROWTH FACTOR ON HUMAN CORNEAL ULCER

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### INTRODUCTION

Corneal transparency is essential for the maintenance of visual function and is contingent on the flawless integrity of all its components: the epithelium, stroma and endothelium (1). Disruption of the epithelial anatomical barrier activates healing and remodeling processes, which can predispose the tissue to stromal ulceration and/or cause stromal opacity, ultimately leading to irreversible visual deficit (2). Epithelial/stromal integrity is compromised by any insult to the ocular surface: infection, trauma, chemical burns, contact lens wear, topical drug abuse, and post-operative damage (3). Indeed, the key role of corneal sensory innervation in corneal trophism and recovery after injury is well known (4). Despite numerous studies completed in recent years which have indicated that cytokines, growth factors and neuropeptides may influence the epithelial healing process in vitro, a suitable therapeutic approach to modulate the healing process has not yet been defined (5, 6).

It was recently shown that nerve growth factor (NGF) plays a crucial role in corneal trophism and in the healing process after corneal injury.

### EXPERIMENTAL DATA

Nerve growth factor (NGF) is an endogenously produced molecule essential for the survival and growth of sympathetic and sensory neurons and for differentiation of neurons in the central nervous system (7). NGF induces neurite sprouting by neural cells and restores the function of injured neurons (7-9). In the past few years several studies have focused on the role of NGF in the pathophysiology of the posterior segment of the eye (retina and optic nerve). Recently our group has highlighted the important function of NGF in the anterior segment of the eye (cornea and conjunctiva). We reported, for the first time in 1995, the potential activity of NGF in the pathophysiology of the ocular surface demonstrating that NGF plasma levels were

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increased in patients affected by allergic conjunctivitis and that NGF levels were correlated with the number of mast cells infiltrating the conjunctiva (10). A number of studies have demonstrated the involvement of NGF in the pathophysiology of the ocular surface, showing that NGF is stored and produced by human and rat cornea (11, 12), and that NGF receptors are expressed by human and rat corneal cells (11, 13). The co-expression of both NGF and its receptor on the same corneal cell suggests the presence of an autocrine and/or paracrine circuit that supports the survival and/or function of these cells, according to the well-known NGF trophic function on neuronal and immune cells (14-16). In line with this hypothesis animals with a targeted mutation for the NGF receptor develop corneal opacity (17, 18).

In addition to a trophic function, NGF also modulates the healing processes of the cornea. In fact, experimental corneal epithelial injuries induce an increase of NGF production and concentration in the cornea, and inhibition of endogenous NGF by Ab-NGF significantly delayed corneal epithelial healing (11). Conversely exogenous NGF treatment in live rats with lesioned corneas significantly accelerated epithelial healing confirming the direct involvement of NGF in corneal recovery after injury (11). In line with these observations, *in vitro* NGF induced rabbit corneal epithelium to proliferate and differentiate (19).

Another interesting function of NGF in stimulating corneal healing is related to its demonstrated activity in promoting sensory nerve differentiation and function (7-9). The cornea is a virtually avascular tissue, but it has very dense innervation (40 times more than the tooth pulp and 400 times more than skin). Thus, any inflammatory reaction and subsequent healing are controlled by this neuronal innervation (20). Experimentally, corneal nerve damage induces severe alterations in the metabolism and vitality of the epithelium, and clinically, surgical damage (as may occur during trigeminal nerve manipulation or penetrating keratoplasty) or chemical damage (such as that caused by abuse of local anesthetics) of corneal innervation impairs epithelial healing and induces trophic ulcers (21). Corneal nerves are dependent on NGF as demonstrated in an animal model of TrkA knockout mice (22), and an impairment of the sensory nerves induces a decrease of NGF concentration in the tissues, leading to skin ulcers as demonstrated in patients affected by diabetes mellitus, leprosy and nerve trauma (23, 24). Indeed, the activity of NGF in supporting the survival, function and regeneration of sensory nerves has been well demonstrated by *in vitro* and *in vivo* studies (25, 26).

#### CLINICAL DATA

Based on the previous experimental evidence we have recently performed some clinical trials to evaluate the efficacy and safety of NGF topical treatment in patients affected by corneal diseases unresponsive to any standard treatment.

In 1998 a pilot study was carried out on 12 patients (14 eyes) affected by neurotrophic keratitis (27). Neurotrophic keratitis is a degenerative corneal condition that results from a variety of ocular and systemic diseases such as fifth nerve palsy,

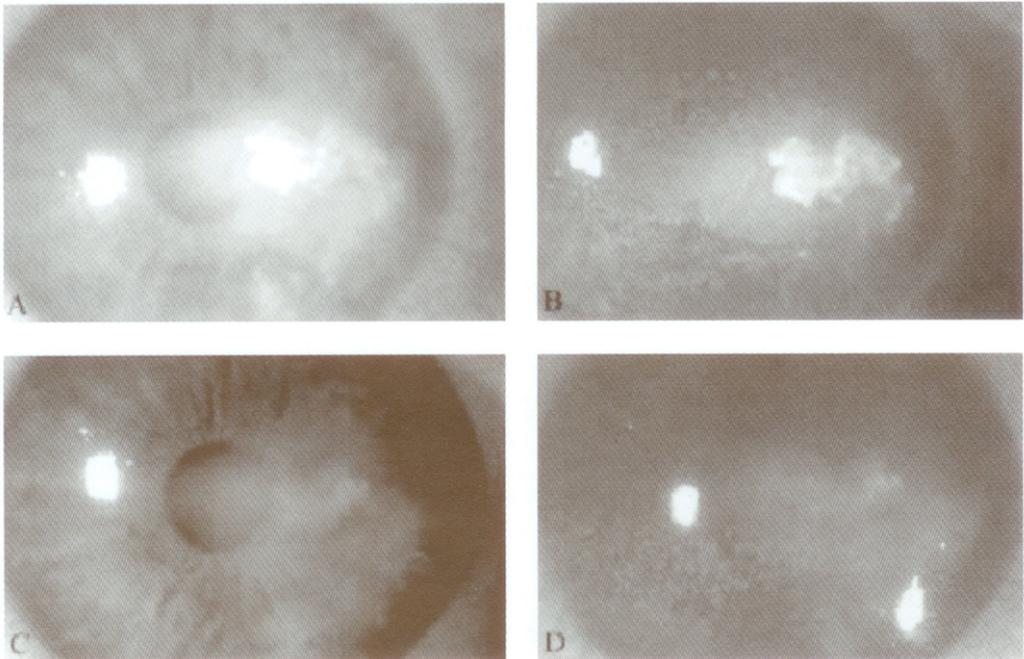


Fig. 1. - Patient with stage 2 neurotrophic keratitis (persistent epithelial defect) before (A) and after treatment with NGF (C). Fluorescein staining evidences the size of the corneal epithelial defect (B) and the complete epithelial healing after NGF treatment (D).

viral infections, chemical burns, corneal surgery, abuse of topical anesthetic, diabetes mellitus and multiple sclerosis (21). In all of these diseases, a lesion of the trigeminal nerve occurs, resulting in impairment of corneal sensitivity and consequent degenerative changes to the corneal epithelium. The clinical stages of neurotrophic keratitis range from punctate corneal keratopathy with tear film abnormalities (stage 1), to persistent epithelial defects (PED) without (stage 2) or with corneal ulcers (stage 3), stromal edema or melting and perforation (21). Although such ulcers are uncommon, their effects are potentially devastating and may lead to blindness. All the eyes included in the study showed neurotrophic corneal ulcers before NGF treatment, and a complete recovery after NGF therapy. Corneal healing began 2 to 14 days after the initiation of treatment with nerve growth factor, and all patients had complete healing of their corneal ulcers after 10 days to 6 weeks of treatment. Corneal sensitivity improved in 13 eyes, and returned to normal in 2 of the 13 eyes. Corneal integrity and sensitivity were maintained during the follow-up period (range, 3 to 15 months) and visual acuity progressively increased during treatment and follow-up in all patients. No systemic or local side effects were observed during treatment.

This preliminary observation was confirmed and extended with an open study on 45 eyes affected by stage 2 and stage 3 neurotrophic keratitis (28). Patients were treated with topical NGF and follow-up period was  $15.8 \pm 11.5$  months. In this series

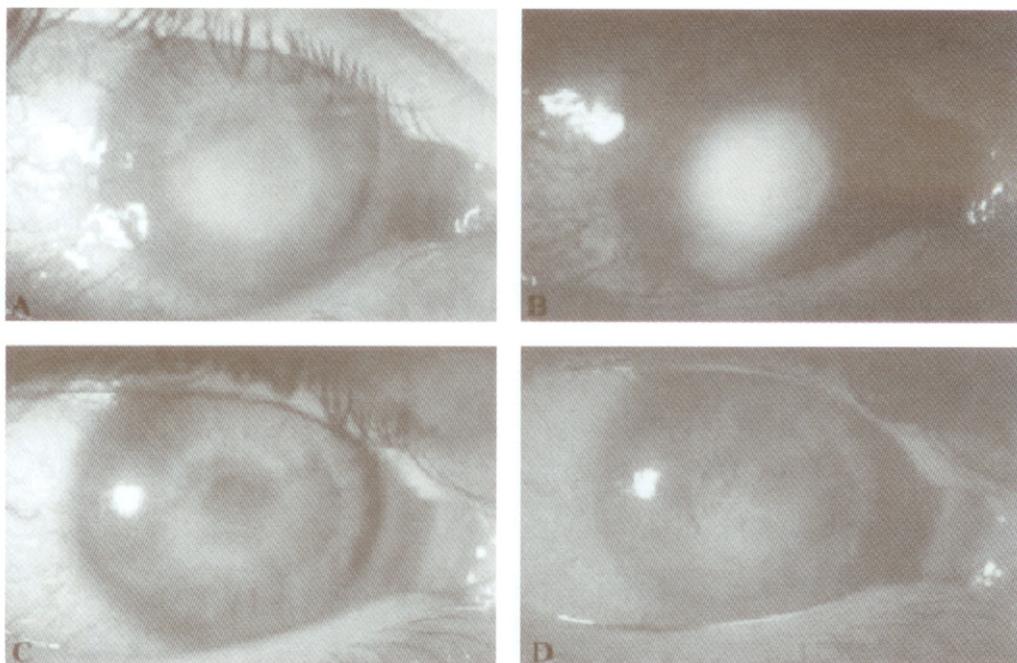


Fig. 2. - Patient with stage 3 neurotrophic keratitis (corneal ulcer) before (A) and after treatment with NGF (C). Fluorescein staining evidences the size of the corneal lesion (B) and the complete healing after NGF treatment (D).

of patients, topical administration of NGF was found to be an effective therapy for both moderate and severe clinical stages of neurotrophic keratitis. Its rapid onset of action allowed the cornea to heal in approximately one month, thus reducing the risk of ocular infections. The ability of topically applied NGF to restore the integrity of the corneal epithelium prevented further corneal melting and perforation and preserved corneal integrity after the treatment was discontinued. In fact, except in the three patients with trigeminal nerve resection, no recurrence of the disease was observed during the follow-up period. These findings, together with the observed improvement of corneal sensitivity in these patients, suggest that recovery of sensory innervation plays a key role in maintaining the trophism and function of the corneal epithelium. This study confirmed that no major ocular or systemic side effects were observed during NGF treatment or in the follow-up period. The most common ocular side effects were conjunctival hyperemia, photophobia and ocular pain during the first few days of treatment. These side effects may be easily explained by the improvement of corneal sensitivity after NGF treatment in the partially or completely anesthetized cornea. Increased tearing was another frequent symptom reported by our patients during treatment. Neurotrophic keratitis may be associated with decreased aqueous tear production (29, 30). Although we found an increase of tear production in some patients, extended studies are required to evaluate the relationship between NGF and tear production.

In a more recent study we evaluated the efficacy of NGF treatment in 4 patients with severe corneal melting as a consequence of immune-related corneal peripheral ulcers (31). Immune corneal ulcers are rare ocular surface diseases with multiple etiologies (32). Immunosuppressive drugs and systemic or topical steroids may occasionally control the inflammatory process, but in more severe cases, the ulcer may progress to corneal melting and perforation. No suitable therapy is currently available for these patients (32). All corneal ulcers treated with NGF healed within 8 weeks, and no relapse of the disease was observed in any patient during follow-up (3-12 months). The only adverse effect observed during NGF treatment was local pain and photophobia, which preceded the healing process and disappeared soon after the healing was completed. Several reports showed the involvement of NGF in the immune process. In fact, inflammatory responses within and outside the nervous system are associated with a transient increase of NGF (33). Moreover, an increase of immune cells expressing NGF receptors has been reported in ocular inflammatory diseases, such as scarring pemphigoid and vernal keratoconjunctivitis (13). The functional significance of these changes is not known. However, it is possible that NGF is involved in treating ocular inflammation, reducing cell damage, and promoting corneal healing. The hypothesis of the anti-inflammatory role of NGF has already been reported. It has been shown that NGF exerts an anti-inflammatory action on experimentally induced inflammation, is 10 times more active than dexamethasone, and a 1000 times more active than nonsteroid anti-inflammatory drugs in animals (34, 35).

It is possible to hypothesize that the efficacy of NGF treatment in this disease is related to the direct effect of NGF in promoting corneal healing as well as to the modulation of the functional activity of inflammatory cells on the ocular surface.

## CONCLUSION

Cumulatively, experimental evidence demonstrates that NGF plays a crucial role in corneal trophism and healing processes. Clinical studies show the efficacy and safety of NGF topical treatment for neurotrophic and autoimmune corneal ulcers.

Though the mechanism of action of NGF on the ocular surface remains not clearly defined, the available data clearly indicate that: 1) NGF treatment may restore a deficit of synthesis or release of endogenous NGF; 2) a direct mechanism may involve sensory innervation and the proliferation and differentiation of epithelial cells; 3) an indirect mechanism could also be involved, such as increasing those neuropeptides that promote epithelial healing or invoking immune cells through the release of cytokines (36). 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.

Until now, the topical use of murine NGF in humans has been experimental and is, therefore, only to be used in cases in which conventional therapy has failed.

## SUMMARY

Nerve growth factor (NGF) is a neurotrophic and immunomodulatory factor contributing of the control of cutaneous morphogenesis, wound healing and inflammatory responses. Following the evidence that topical administration of NGF leads to healing of human corneal ulcers, we investigate the therapeutic action of NGF on immune and/or autoimmune cutaneous ulcers. We found that 1-10 µg of highly purified murine NGF dissolved in 50 µl of physiological solution and topically applied to skin ulcer leads, after 4-12 weeks of daily treatment (depending on the size and depth of the lesion) to complete healing of the ulcer. Thus, NGF was able to promote complete repair in human skin and corneal ulcers which were poorly or non-responsive to conventional topical and systemic treatments. No side effects were observed and a follow up after 4 months showed no signs of relapse.

These findings indicate that failure of cutaneous tissues to produce sufficient amounts of NGF might represent a prominent mechanism implicated in the clinical manifestation of ocular ulcers.

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