

Clinical applications of NGF in ocular diseases

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ABSTRACT

Nerve Growth Factor (NGF) and its receptors TrkA and p75 are expressed in physiological states in the anterior and posterior segments of the human eye, where they exert several tissue-specific functions. The roles played by NGF in the homeostasis of the eye and in vision are, therefore, crucial and have been widely investigated both in vitro and in vivo, with growing evidence of a NGF-pathway alteration in several ocular diseases. In this review we describe the functions of NGF in health and disease states of the eye, and discuss the potential therapeutic effectiveness of NGF in preliminary clinical reports performed in severe ocular diseases unresponsive to any standard treatment. In fact, pharmacodynamic studies showing that NGF administered topically on the ocular surface affects not only the ocular surface but is also able to reach the retina, optic nerve and brain, recently opened new perspectives for the treatment of challenging ocular surface diseases, optic nerve diseases, and degenerative diseases of the retina currently lacking an effective therapy.

Key words

NGF • Eye • Glaucoma • Keratitis

The eye

The eye is composed of ocular annex and bulb, the former being composed of eyelids and lacrimal gland, the latter being composed of three layers – sclera, uvea and retina – divided into an anterior and a posterior segment (Fig. 1). The anterior ocular segment is composed by tear film, conjunctiva, cornea, anterior chamber (filled with the aqueous humor), iris, posterior chamber (filled with the aqueous humor), lens and ciliary bodies. The posterior ocular segment is composed by vitreous body, retina, optic nerve head and choroid. The visual pathway continues from the optic nerves, crossing in the optic chiasm, into the lateral geniculate bodies and reaches the occipital visual cortex through the optic tracts (Snell and Lemp, 1998).

NGF in the anterior ocular segment

Nerve growth factor (NGF) and its receptor trkA are expressed in the anterior ocular segment in physiological states and NGF is normally released in the aqueous humor (Lambiase et al., 2000). NGF has also been quantified in human tears, indicating that NGF is basally released by the lacrimal gland, suggesting a role in the maintenance of the tear film homeostasis (Lee et al., 2005). In line with this hypothesis, NGF and its receptors, trkA and p75, are proven to be expressed by the rat lacrimal gland tissue (Ghinelli et al., 2003; Muzi et al., 2010). *In vitro* studies have shown that NGF is produced, stored, released and utilized by conjunctival cells (both epithelial cells, goblet cells, immune cells and fibroblasts). NGF, directly or via induction of

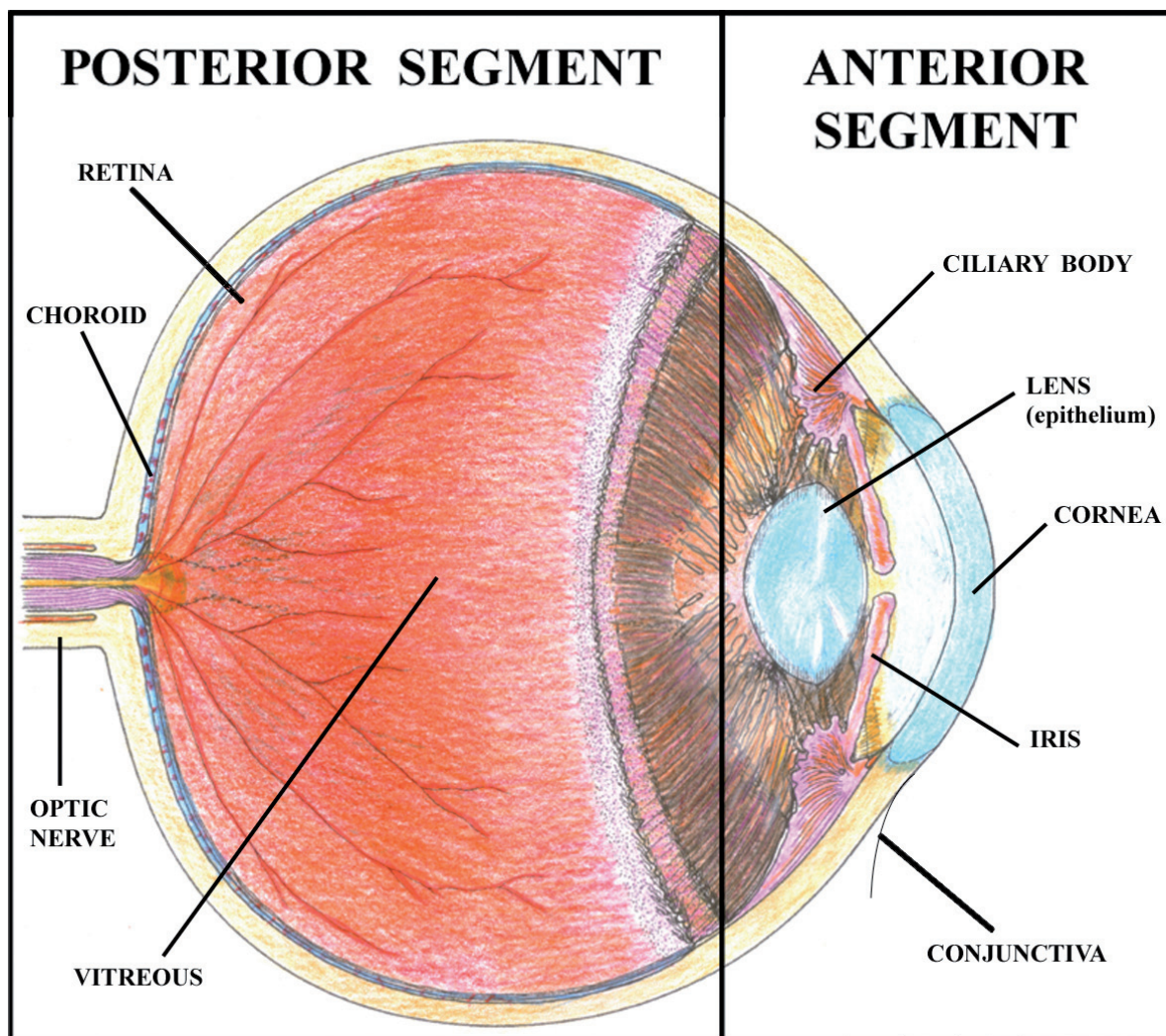


Fig. 1. - This drawing depicts the section of a human eye and the sites of action of NGF.

other cytokines/growth factors, seems to modulate, *in vitro* and *in vivo*, some of the principal functions of conjunctival epithelial and goblet cells, as well as immune cells and fibroblasts (Lambiase et al., 2004; Micera et al., 2006 Lambiase et al., 2009). In humans and in several experimental models, not only conjunctival but also corneal cells (epithelial, stromal and endothelial cells), show the ability to produce and release NGF, and to express its receptors *trkA* and *p75* (Lambiase et al., 1998, 2000). In line with this data, compelling evidence from *trkA*- or *p75*-null mice indicate that NGF also plays a role in the trophobiology of the cornea (Bonini et al., 2002; Kawamoto et al., 2004). Specifically, corneal innervation appears to be NGF dependent, with a

decrease of corneal nerve density and sensitivity observed in *trkA*-null mice (de Castro et al., 1998). Moreover, NGF has been shown to induce *in vitro* corneal epithelial cell proliferation and differentiation and it is involved in maintaining limbal epithelial cell stemness (Kruse et al., 1993; Qi et al., 2007). The expression of both NGF receptors by corneal endothelium also strongly supports the hypothesis of a NGF biological activity, although NGF function on these cells is still largely unclear (Lambiase et al., 2000, 2002; Sornelli et al., 2010). NGF is also expressed by iris and ciliary body, that overexpress and release NGF following ocular injury (Lambiase et al., 2002). Experimental study demonstrated that rat lens epithelium also expresses and synthe-

sizes NGF and TrkA. Indeed, in a culture model of cataractogenesis, NGF administration delays, while neutralizing NGF-antibody increases cataract development (Ghinelli et al., 2003).

Not only the role of NGF in normal states has been widely investigated, but NGF is also known to play a major role in disease states of the ocular surface. Specifically, the immune cells infiltrating cornea and conjunctiva during chronic inflammatory ocular conditions, such as allergic and autoimmune diseases, have been shown to be widely influenced by alterations of NGF levels (Bonini et al., 1999; Aloe et al., 2008). During these inflammatory diseases of the ocular surface, NGF and trkA are overexpressed in the conjunctiva, as well as in serum. A significant correlation was also observed between NGF concentrations and increased numbers of mast cells in the allergic conjunctiva (Lambiase et al., 1995; Bonini et al., 1996). It is still unclear what cells are responsible for the increase of NGF and how NGF participates in allergic inflammation (Aloe et al., 1999). Activated mast cells and eosinophils may be one source of NGF, as demonstrated *in vitro* and *in vivo*. It is well known that NGF affects all cells that play a pivotal role in allergic inflammation, such as Th2 lymphocytes, mast cells and eosinophils. Specifically, NGF induces mast cell differentiation, degranulation (including cytokines/growth factor release) and apoptosis/survival, as well as eosinophil activation, apoptosis and cytokine/growth factor release (Lambiase et al., 1998; Solomon et al., 1998; Bonini et al., 2003).

In summary, considering that NGF potentially affects all the components of the anterior ocular segment, it is believed that it might play an important role in a wide range of ocular surface diseases, including dry eye, neurotrophic keratitis, herpetic keratitis, limbal stem cell deficiency, and in corneal transplant outcomes (Lambiase et al., 1998, Bonini et al., 2000; Coassin et al., 2005; Lee et al., 2005; Gong et al., 2007; Lambiase et al., 2008, 2009). In line with this hypothesis: 1) NGF eye drop administration in a dog experimental model of dry eye, increases tear production, conjunctival goblet cell density and corneal features (Coassin et al., 2005); 2) increased tear concentration of NGF has been reported also in patients affected by dry eye (Lee et al., 2005); 3) NGF stimulates conjunctival goblet cells glycoconjugate secretion, without affecting cell proliferation (Lambiase et al., 2009).

NGF in the posterior ocular segment

NGF and both NGF receptors (NGFR) are widely expressed in the central visual pathway (lateral geniculate nucleus and visual cortex) as well as in the optic nerve and retina. Recently, it has also been shown that NGF and NGFR are expressed by the intraocular tissues of the posterior segment, including vitreous, choroid, and retina (Micera et al., 2004; Lambiase et al., 2010). In these tissues, NGF has been shown to modulate the development and differentiation of retina and optic nerve, and to promote the survival and recovery of retinal ganglion cells (RGCs) (Ayer-Lelievre et al., 1983; Carmignoto et al., 1991; Maffei et al., 1992).

In the retina, NGF is produced and utilized in a local paracrine/autocrine fashion by RGCs, bipolar neurons and glial cells. A retrograde/anterograde transport along the axons of the RGCs (optic nerve) and geniculate nucleus has been also reported (Ayer-Lelievre et al., 1983; Yip and Johnson, 1983; Carmignoto et al., 1991; Maffei et al., 1992; Micera et al., 2004; Lambiase et al., 2010). NGF has also been proven to affect retinal plasticity, with massive cell death observed during retinal development associated with a consistent p75 expression. Conversely, several experimental studies demonstrated that NGF/TrkA pathway promotes RGCs survival. In fact, intraocular injection of NGF is known to promote recovery of damaged RGCs after ischemic injury, optic nerve transection, and ocular hypertension, in contrast to detrimental effects of the administration of neutralizing anti-NGF antibodies (Carmignoto et al., 1989; Chakrabarti et al., 1990; Siliprandi et al., 1993; Haamedi et al., 2001; Harada et al., 2006; Coassin et al., 2008; Lambiase et al., 2010). Recently, it has also been demonstrated that intravitreal injection of exogenous NGF protects retinal cells degeneration and apoptosis in experimental retinal detachment (Sun et al., 2007). In line with these data, in an animal models of diabetes, both RGCs and Müller cells underwent apoptosis by overexpression of p75, while intraocular administration of NGF triggered recovery of damaged retina and even exogenous NGF treatment appeared to prevent cell loss and tissue damage (Sun et al., 2007; Coassin et al., 2008). In addition to the original discovery of NGF as a survival and neurite growth-promoting factor, in the retina it appears to exert multiple effects on dif-

ferent cell types, including proliferation, transmitter synthesis, cytoskeletal changes, synaptic transmission, reorganization, and plasticity. More recently, experimental data on the effects of NGF in animal models of glaucoma, a neurodegenerative disease involving retina and optic nerve, an increase of intraocular NGF during the progression of retinal degeneration was demonstrated (Lenzi et al., 2005). Exogenous NGF administration in these animals rescued RGCs, while its neutralizing antibodies exacerbated RGCs loss. The crucial role of TrkA/p75 ratio in determining the fate of RGC is further supported by a recent study showing that TrkA activation, but not p75, is able to rescue RGCs degeneration (Bai et al., 2010; Colafrancesco et al., 2011).

Challenging ocular disease before and after the advent of NGF

At the end of 1990s, the concept of NGF involvement in the pathophysiology of the ocular surface was introduced, with rapidly growing evidence showing a role of this neurotrophin in the modulation of immune reaction, trophic support and healing of both cornea and conjunctiva (Lambiase et al., 1998; Bonini et al., 2002). As a consequence, the efficacy of topical treatment with NGF eye drops was rapidly evaluated in animal models and in patients affected by challenging ocular surface diseases including neurotrophic keratitis and autoimmune corneal ulcers, showing that the therapeutic effectiveness was associated with a satisfying safety profile (Lambiase et al., 1998, 2000, 2007). Following these first studies, the involvement of NGF in limbal stem cell differentiation and corneal transplant rejection has been addressed, suggesting a potential use of NGF eye drops in patients affected by limbal stem cell deficiency and in the postoperative management of corneal transplant surgery (Kawamoto et al., 2004; Gong et al., 2007; Lambiase et al., 2009). More recently, pharmacodynamic studies highlighted that NGF administered on the ocular surface reaches the ocular posterior segment and brain area, opening new perspectives for the treatment of challenging optic nerve diseases, and degenerative diseases of the retina currently lacking an effective therapy, such as glaucoma and retinitis pigmentosa (Lambiase et al., 2005).

Neurotrophic keratitis

Neurotrophic keratitis is a rare degenerative corneal condition that results from a variety of ocular and systemic diseases such as fifth nerve palsy, viral infections, chemical burns, corneal surgery, abuse of topical anesthetic, diabetes mellitus, and multiple sclerosis. 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. Persistent epithelial defects and corneal ulcers such as those occurring in neurotrophic keratitis are known to often be unresponsive to different therapeutic approaches including tarsorrhaphy or patching, unpreserved artificial tears, or soft contact lens bandage. Other surgical measures, such as amniotic membrane transplantation, are under evaluation, however, to date neurotrophic keratitis remains a disease orphan of a specific treatment (Bonini et al., 2003).

Topical administration of murine-derived NGF eye drops at a concentration of 200 micrograms/ml was found to be an effective therapy in humans for both moderate and severe clinical stages of neurotrophic keratitis. In different case series, its rapid onset of action has been proven to induce cornea healing in approximately 1 month (Fig. 2). The ability of topically applied NGF to restore the integrity of the corneal epithelium is able to preserve corneal integrity even long after the treatment is discontinued (Lambiase et al., 1998, 2008).

Dry eye

The tear film plays a crucial role in maintaining a healthy conjunctival and corneal epithelium. Dry eye, or keratoconjunctivitis sicca, is a chronic ocular surface condition characterized by an impairment of tear film quantity and/or quality resulting in chronic symptoms of discomfort, visual disturbance, and tear film instability, with potential severe damage to the ocular surface leading to corneal blindness for corneal opacities and/or keratinisation. According to the 2007 Dry Eye Workshop consensus, it is amongst the most prevalent ocular diseases, with 3.23 million women and 1.68 million men, for a total of 4.91 million Americans 50 years and older affected by moderate-to-severe dry eye and tens

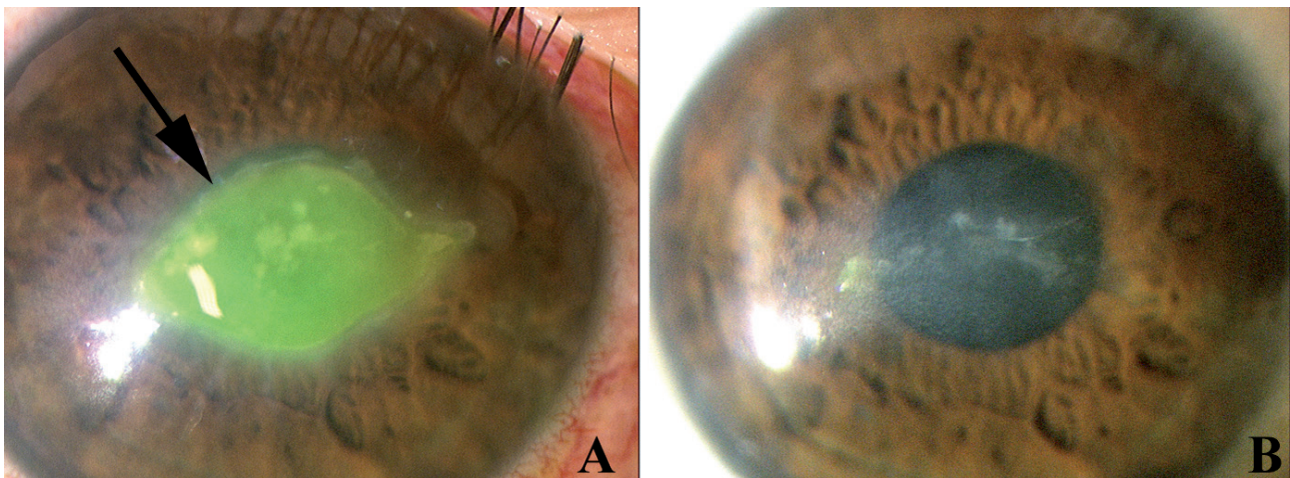


Fig. 2. - Neurotrophic corneal ulcer (arrow) before (A) and after NGF eye drop treatment (B).

of millions more with less severe presentation. Nevertheless, currently no specific therapy exist and medications are often used chronically only to treat long-term complications or to reduce symptoms, resulting in enormous costs for patients and health care institutions.

Dry eye can be divided in two types: aqueous-deficient – including Sjögren's and non-Sjögren's – and evaporative. In the former there is a reduction in tear production, in the latter there is a reduction in tear film stability due to alterations in tear components such as ocular surface mucins. NGF and its receptors may play a role in both aqueous-deficient and evaporative dry eye. In fact, increasing evidence is showing a role for NGF in the regulation of tear production and tear film homeostasis. NGF and its receptor p75 have been localized in the human lacrimal gland tissue, and NGF has been quantified in human tears, indicating that it may be basally released by the lacrimal gland (Lambiase et al., 1998, Ghinelli et al., 2003; Lee et al., 2005; Lambiase et al., 2009). It is therefore possible that NGF plays a pivotal role and may be used as a novel therapeutic option in dry eye disease. In line with this hypothesis: (i) NGF eye drop administration in a dog experimental model of dry eye, increases tear production and conjunctival goblet cell density (Coassin et al., 2005); (ii) increased tear concentration of NGF has been reported also in patients affected by dry eye (Lee et al., 2005). Moreover, NGF has recently been shown to regulate conjunctival goblet cell secretion in rats (Lambiase et al., 2009). The main secretory product

of conjunctival goblet cells is the large gel-forming mucin MUC5AC. Ocular surface mucins play a critical role in the protection of corneal and conjunctival epithelia and a decrease in goblet cell numbers and MUC5AC expression is one of the features of dry eye disease. Another feature of dry eye disease is an increase in tear osmolarity, which results in damage and apoptosis of corneal epithelial cells. Recently, Chang et al. (2008) have demonstrated an up-regulation of NGF in corneal epithelial cells under hyperosmolar stress. The authors suggest that NGF may contribute, at least in part, to reduce the apoptosis of corneal epithelial cells and that, therefore, enhanced NGF expression may be beneficial in recovering corneal damage due to chronic hyperosmolar stress. These results open new therapeutic possibilities for NGF eye drops in dry eye disease.

Herpetic keratitis

Herpes simplex virus (HSV) keratitis, a primary cause of blindness worldwide, results from chronic episodes of viral reactivation leading to permanent corneal scarring, vision impairment and blindness. During primary infection, latency is established in neurons when HSV reaches sensitive ganglia by retrograde axonal transport. Reactivation of latent HSV seems to be related to the loss of trophic support provided by the peripheral target, leading to herpetic keratitis recurrence and, ultimately, to corneal scarring. Corneal opacity is a difficult challenge for

the ophthalmologist, since corneal grafts are often unsuccessful due to the reactivation of latent HSV and rejection reactions (Kaye et al., 2006).

Prophylactic treatment with oral acyclovir significantly improves the prognosis, however, novel and long-acting effective treatments for herpetic keratitis are still under investigation. NGF has been proposed as one of these future options to treat and inhibit recurrences of herpetic keratitis. In fact, *in vivo* and *in vitro* studies have shown that NGF also exerts antiviral activity. *In vitro*, NGF has a cytoprotective effect, as demonstrated on HSV-infected cell cultures. Indeed, NGF treatment preserves HSV latency in rat sympathetic neuronal cell cultures, while neutralizing anti-NGF antibodies induce virus reactivation. This antiviral action of NGF has been confirmed by *in vivo* studies showing that NGF treatment decreases neuronal damage induced in young rat cervical ganglia in an animal model of HSV encephalitis (Ungheri et al., 1993). Moreover, systemic treatment with neutralizing anti-NGF antibodies induces reactivation of HSV keratitis in a rabbit model of HSV infection. Data from our group also demonstrates that anti-NGF antibodies induce a more severe keratitis, while topical treatment with NGF induces a significant amelioration of clinical and laboratory parameters of HSV keratitis in rabbits. In line with these results, a report on a patient with HSV keratitis unresponsive to acyclovir treatment, showed that NGF eye drops was able to induce a complete corneal healing (Mauro et al., 2007).

Glaucoma

Glaucoma, one of the leading causes of blindness worldwide, is a chronic and progressive optic neuropathy characterized by degeneration of the RGC and loss of axons of the optic nerve with a progressive and consequent deficit of the peripheral and central visual field (Lambiase et al., 2010). The main risk factor developing this disease is an elevated intraocular pressure (IOP). However, in spite of several successful treatment options to reduce IOP, up to 20% glaucoma patients show progression of visual field defects with RGC and optic nerve degeneration despite successful management of ocular hypertension. An approach that would vastly improve the treatment of this challenging dis-

ease would, therefore, involve neuroprotection with exogenous neurotrophic factors. Pharmacodynamic studies have shown NGF eye drops' ability to target the optic nerve and brain, thus opening up avenues of research in pursuit of a novel drug for the treatment of glaucoma (Lambiase et al., 2007).

Recently, it has been demonstrated that murine NGF administered topically to the eye rescued RGCs from apoptosis in a rat model of glaucoma. The beneficial effect of NGF eye drop on RGC survival was demonstrated to be due to inhibition of apoptosis, as shown by the reduction in TUNEL RGC immunostaining and the greater retinal Bcl-2/Bax ratio (Colafrancesco et al., 2011). It is known that RGCs express NGF receptor (TrkA) and that NGF binding to TrkA up-regulates Bcl-2 protein, which protects cells from apoptosis by preventing caspase activation. The crucial role of TrkA/p75 ratio expression in determining the fate of RGC is supported by the evidence that in animal models of glaucoma an unbalance of TrkA/p75 ratio is associated with progression of retinal damage. In addition, a recent study on an animal model of glaucoma has shown that TrkA, but not p75, activation is able to rescue RGCs degeneration (Lambiase et al., 2010). It has also been shown in a small case series in humans with advanced glaucoma that NGF eye drop treatment induced long lasting improvements in visual field, optic nerve function as measured by electrofunctional parameters, contrast sensitivity, and visual acuity (Lambiase et al., 2009) (Fig. 3).

To summarize our findings

Once regarded solely as a target-derived survival factor for the nervous system, NGF is now known to exert different roles in modulating the homeostasis of the ocular surface, as well as the retina and optic nerve. NGF affects resident ocular surface cells (corneal and conjunctival epithelium, fibroblasts and endothelium), immune cells, sensory nerves, tear production, but also retinal ganglion cells, photoreceptors and optic nerve. As a consequence, NGF is involved in several ocular diseases from the anterior to the posterior ocular segment with different etiopathology. Most of the experimental evidence show an effectiveness of NGF treatment in degenerative diseases of the retina, such as

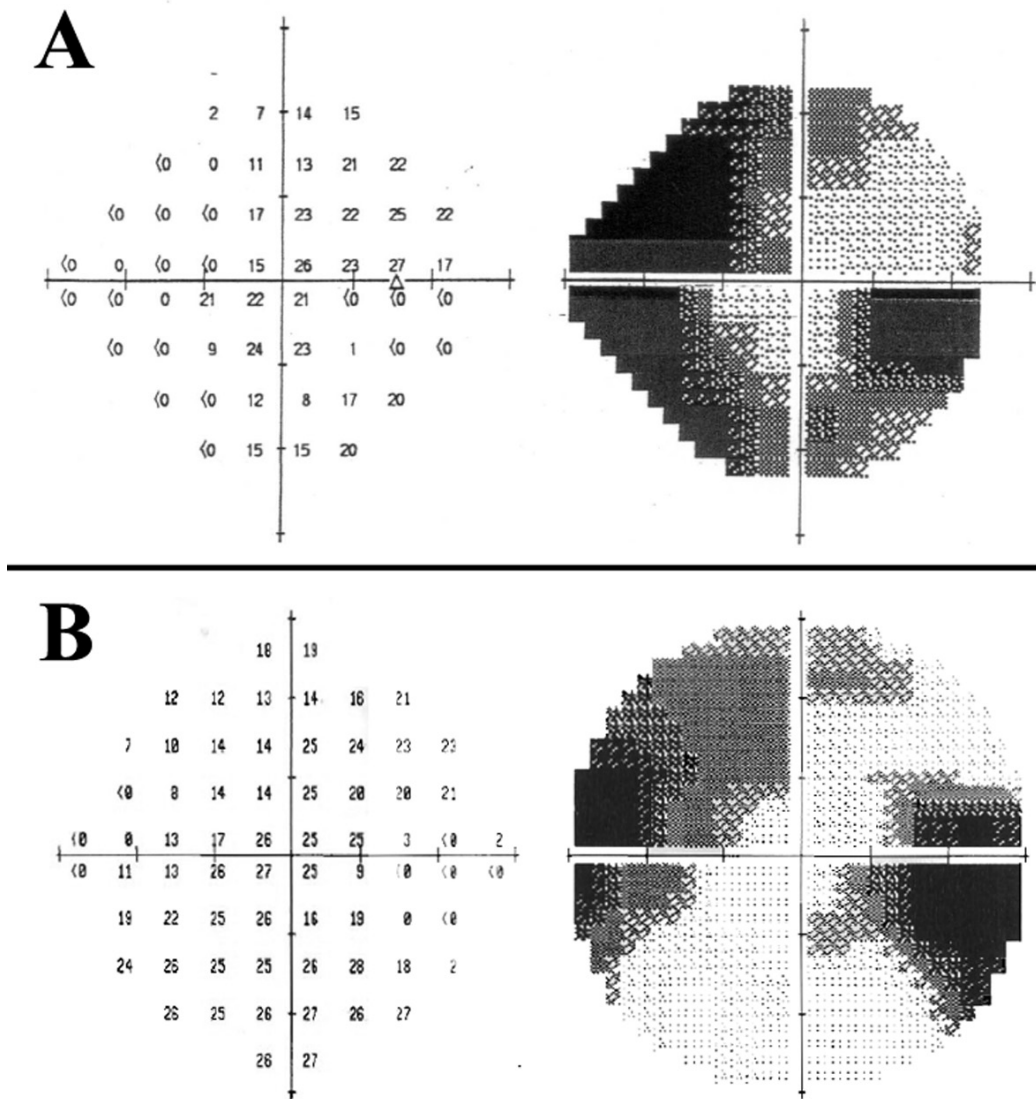


Fig. 3. - Computerized visual field analysis showing decreased sensitivity in a patient with advanced glaucoma (darker areas, A) and the improvement observed in the same patient after topical NGF eye drop treatment for six months (B).

glaucoma and retinitis pigmentosa, as well as of the cornea, such as neurotrophic keratitis and herpetic keratitis. Moreover, increasing evidence also suggest a role of NGF also in promoting corneal healing in a wide spectrum of diseases including infective keratoconjunctivitis such as herpes virus infection. Preliminary clinical reports, performed in severe ocular diseases unresponsive to any standard treatment, confirm the therapeutic effectiveness of NGF eye drop treatment in patients affected by challenging ocular diseases such as neurotrophic and herpetic keratitis, and glaucoma.

Acknowledgements

This work was partially supported by a grant from Fondazione Roma.

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