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Nerve Growth Factor as an Ocular Therapy: Applications, Challenges, and Future Directions

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Abstract

Nerve growth factor (NGF), the prototypical neurotrophin first discovered in the 1950s, has recently garnered increased interest as a therapeutic agent promoting neuronal health and regeneration. After gaining orphan drug status within the last decade, NGF-related research and drug development has accelerated. The purpose of this article is to review the preclinical and clinical evidence of NGF in various applications, including central and peripheral nervous system, skin, and ophthalmic disorders. We focus on the ophthalmic applications including not only the FDA-approved indication of neurotrophic keratitis but also retinal disease and glaucoma. NGF represents a promising therapy whose therapeutic profile is evolving. The challenges related to this therapy are reviewed, along with possible solutions and future directions.

Keywords

Nerve growth factor; NGF; neurotrophic keratitis; neurotrophic keratopathy; neurotrophin

INTRODUCTION

Nerve growth factor (NGF) was first identified as a protein secreted by mouse sarcomas with the ability to stimulate growth in sympathetic and sensory neurons.^{1,2} Since then, NGF has become prototypical of a class of proteins – neurotrophins – characterized by their involvement in neuronal protection and growth, including brain-derived nerve growth factor, neurotrophin-3, and neurotrophin-4/5.

The proenzyme of NGF, pro-NGF, is secreted and latently present in tissue until activation by cleavage. Pro-NGF consists of a complex of three subunits: alpha (α), beta (β), and gamma (γ). The active form of NGF is a dimer of β -NGF (molecular weight 26-kDa) consisting of two non-covalently bonded β subunits. Upon cleavage of pro-NGF, dimerized β -NGF is free to bind cell-surface receptors.^{3,4} Neurotrophic effects are primarily mediated by tropomyosin kinase receptor A (TrkA^{NGFR}),^{5,6} with secondary effects mediated by the p75 neurotrophin receptor (p75^{NTR}).⁷ TrkA is a high-affinity receptor whose activation

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acts via a mitogen activated protein kinase pathway, a phosphoinositide 3-kinase pathway, and a phospholipase 3 pathway to produce downstream effects.^{8, 1} In addition, the activated NGF/TrkA^{NGFR} complex is internalized in an endocytic vesicle and undergoes retrograde transport from the synaptic terminal membrane to the neuronal cell body to effect transcriptional responses,^{9–11} which may have effects distinct from the cell surface signaling pathways.¹² Equally as versatile as its molecular pathways are the various cell signaling mechanisms of NGF, including endocrine, paracrine, and autocrine pathways.¹³ Moreover, the signaling mechanisms and pathways of NGF interact with various neuronal and non-neuronal cells, including fibroblasts, glial cells, epithelial cells, immune cells, and neuroendocrine cells.^{13–16}

Considering its diversity of targets and effects, NGF has been investigated for use in a variety of clinical applications, including peripheral sensory neuropathies, skin ulcers, neurotrophic keratopathy, and central nervous system disorders such as Alzheimer's and Parkinson's disease.¹⁷ In this article, we review the clinical applications and challenges of using NGF as a therapy, with an emphasis on ocular indications.

NON-OPHTHALMIC APPLICATIONS

Central Nervous System

NGF has been extensively studied in the treatment of degenerative diseases of the central nervous system such as Alzheimer's Disease and Parkinson's Disease. Part of the pathophysiology of these neurodegenerative conditions is thought to be related to diminished production of neurotrophic factors, especially among the cholinergic neurons of the basal forebrain.^{18,19} Oral and parenteral routes of NGF administration have had limited success, due to poor blood-brain barrier penetration and enzymatic degradation.^{20,21} Therefore, NGF has been delivered to the CNS primarily through intracerebroventricular administration. Intracerebroventricular NGF was found to promote survival and regeneration of central cholinergic neurons after axonal transection in rodents.^{22–25} However, clinical studies of NGF have been limited by poor efficacy and frequent adverse events.²⁶

Due to the challenges associated with intracerebroventricular administration, non-invasive approaches have also been investigated, including intranasal^{27–29} and topical ocular administration.^{30,31} Gene- and cell-based therapies, protein conjugates, and sustained release cerebral implants have also been investigated for CNS NGF distribution, with varying degrees of success.^{32,33}

Peripheral Nervous System

In addition to CNS pathologies, NGF has been investigated as a treatment in peripheral nervous system (PNS) disease. While the blood-brain barrier may hinder NGF delivery to the CNS, access to the PNS is less protected and affords better drug penetration to target neurons. Early studies on rats with streptozotocin-induced diabetes and diabetic peripheral neuropathy showed that subcutaneous injection of rhNGF prevented the reduction in substance P, calcitonin gene-related peptide, and temperature sensitivity typically seen in this model.^{34,35} While phase I studies of systemic and subcutaneous rhNGF administration

found a substantial incidence of injection site hyperalgesia and myalgias,³⁶ subcutaneous rhNGF injections were found to significantly improve subjective and objective sensory measures in a phase II trial of diabetic polyneuropathy.³⁷ However, similar improvements in subjective and objective sensory measures were not replicated in phase III trials of diabetic polyneuropathy,³⁸ nor in phase II trials of HIV-associated peripheral neuropathy.³⁹ Explanations for the discrepancy between these trials include potential confounding by unblinding as a result of prominent local hyperalgesia in the rhNGF injection, differences in rhNGF formulation, and a lower clinical dose than used in preclinical animal models.⁴⁰

Skin

In addition to promoting cutaneous neuronal regeneration through regulation of complex neuroendocrine pathways, NGF and other neurotrophins exert effects on keratinocytes, fibroblasts, vascular endothelial cells, and immune cells to modulate cutaneous tissue remodeling and wound healing.⁴¹ Epithelial cells and keratinocytes adjacent to skin wound edges express higher levels of NGF. In a diabetic mouse model⁴² as well as various other mouse models of skin wounds,⁴³ topical NGF application has been found to accelerate cutaneous wound healing. Clinically, topical mNGF has been applied to pressure ulcers,⁴⁴ diabetic foot ulcers,⁴⁵ and vasculitic ulcers in patients with rheumatoid arthritis⁴⁶ in non-controlled studies. In a randomized controlled trial, topical mNGF demonstrated efficacy in the treatment of pressure ulcers.⁴⁷ Despite this early success, larger and more rigorous trials establishing the utility of topical NGF have not yet been performed.

OPHTHALMIC APPLICATIONS

Ocular Surface Disease

Presently, NGF as an ocular therapy has been furthest advanced in the treatment of ocular surface disease. NGF is thought to regulate ocular surface homeostasis in multiple ways, including epithelial health, limbal stem cell proliferation, immune modulation, and tear production.⁴⁸ TrkA^{NGFR} and p75^{NTR} are constitutively expressed in the basal epithelial cells and the stroma of the conjunctiva, as well as the epithelial and endothelial cells of the cornea.^{49–51} In patients with ocular surface diseases, such as vernal keratoconjunctivitis and ocular cicatricial pemphigoid, higher levels of TrkA^{NGFR} are found in the conjunctival stroma, along with inflammatory stromal infiltration,⁴⁹ suggesting a potential role in ocular surface disease. Moreover, increased levels of NGF are seen in the tear film of patients with various ocular surface diseases, including contact lens-related dry eye, Sjögren's syndrome, ocular cicatricial pemphigoid, and post-refractive dry eye.^{52–55}

Given the purported influence of NGF on the ocular surface, NGF has been investigated as a therapy in various ocular surface disease applications. Rabbits with iatrogenic corneal epithelial defects were found to have increased corneal NGF expression, and treatment with topical NGF decreased epithelial defect closure times.⁵⁰ Dogs with dry eyes, induced by lacrimal gland excision, experienced significant improvement in clinical dry eye parameters – including degree of punctate keratopathy and Schirmer test – and increased conjunctival goblet cell density, when compared to controls.⁵⁶ In a rabbit model, topical NGF along with docosahexaenoic acid was found to enhance the regrowth of epithelial and subbasal nerve

bundles while also promoting wound healing after photorefractive keratectomy.⁵⁷ Another rabbit study demonstrated significantly improved corneal sensitivity in those rabbits treated with topical NGF after LASIK when compared to vehicle.⁵⁸

Based on the preponderance of promising preclinical testing, pilot clinical studies were performed in patients with neurotrophic keratopathy (NK) in the late 1990s and early 2000s.^{59,60} These studies were not controlled, but the safety of topical NGF was demonstrated. Decades passed before another clinical trial was performed, in part due to the relative rarity of NK as a clinical entity. In 2013, a phase I trial established the tolerability of rhNGF in escalating doses up to 180 ug/mL.⁶¹ In 2018, a phase I and a subsequent double-masked phase II clinical trial (NGF0212/REPARO phase I/II) reaffirmed the safety of topical rhNGF, demonstrated significantly decreased epithelial defect healing time, and reduced recurrence rate versus control in patients with moderate to severe NK.^{62,63} Despite improvements in the ocular surface, significant improvements in corneal sensitivity were not seen in this study. Nonetheless, following the overall success of these clinical trials, topical NGF has received approval from the European Medicines Agency and the United States Food and Drug Administration for the treatment of moderate to severe NK. For this use, the medication is approved as a six times per day eye drop, to be used for eight weeks.

A recently published phase II trial was the first to investigate the use of rh-NGF eye drops in dry eye syndrome, and significant improvements were seen in symptoms, ocular surface staining, and tear production.⁶⁴ Further studies comparing NGF to current standards of care are needed to establish NGF as a treatment for dry eye syndrome.

Retinal Disease

In the retina, NGF is expressed by retinal pigment epithelium, Müller cells, and retinal ganglion cells, while NGF receptors are found on retinal pigment epithelium, photoreceptors, Müller cells, and retinal ganglion cells.^{49,65,66} Significantly reduced NGF mRNA expression and protein levels were found in the eyes of rats affected by an inherited retinal degeneration causing photoreceptor cell loss (Royal College of Surgeons rats),⁶⁷ suggesting a potential role for NGF in certain forms of retinopathy. Indeed, in the same rat model, retrobulbar injections of NGF resulted in improved photoreceptor cell survival and increased retinal vascularity compared to controls.⁶⁸ Similarly, a study performed on C3H mice found that the photoreceptor degeneration typically seen in this mouse model was mitigated by a single time intravitreal injection of NGF or multiple retrobulbar NGF injections.⁶⁹

NGF has also been studied in diabetic animal models. Streptozotocin-induced diabetic rats are found to have increased expression of retinal NGF and NGF receptor initially, which is thought to have a protective effect.^{70,71} Over time, however, NGF expression decreases in this diabetic rat model, and this is followed by retinal ganglion cell and Müller cell apoptosis. These rat studies have demonstrated mitigation of retinal ganglion cell and Müller cell loss with topical NGF treatment.

Clinically, NGF has not yet been extensively studied for retinal disease. In one case report, an elderly patient with advanced non-neovascular age-related macular degeneration

had improvement in visual acuity and electroretinogram amplitude after one month of topical administration of 200 µg/mL NGF, with further improvement after 3 months of treatment.⁷² This improvement was not robust, as acuity and ERG parameters worsened upon discontinuation. However, the testing parameters again improved after NGF was restarted. A pilot, non-controlled study has also been performed in patients with retinitis pigmentosa.⁷³ In this study, patients received 10 days of topical NGF, which was generally well tolerated. The results suggested a possible improvement in functional ERG and visual field testing in the study patients. However, larger, controlled, and masked trials have yet to be completed.

Optic Nerve Disease

In the visual pathway, NGF receptors are found in retinal ganglion cells and Muller cells⁶⁶ and in the visual cortex,⁷⁴ as well as oligodendrocytes of the optic nerve.⁷⁵ The possibility of cells in the optic nerve being receptive to exogenous NGF as a therapy has been tested in several experimental and clinical studies. Several early studies confirmed the neuroprotective effect of NGF on neurons in the optic nerve. Intraocular injection of NGF was found to mitigate the loss of myelinated optic nerve fibers after optic nerve transection in a rat study.⁷⁶ More recently, topical administration of rh-NGF was found to prevent RGC apoptosis, promote axonal survival, and inhibit astrocyte-driven optic nerve degeneration.⁷⁷ Similarly, intraocular NGF administration significantly reduced functional retinal ganglion cell damage in a rat model of retinal ischemia.⁷⁸ The neuroprotective effects of NGF were corroborated in subsequent animal studies of glaucoma. In a rat model of glaucoma, achieved by injection of hypertonic saline into the episcleral vein, topical administration of NGF significantly reduced retinal ganglion cell loss.^{79,80} Similarly, a rabbit model of glaucoma, achieved by intracameral injection of 2% methylcellulose, resulted in increased aqueous humor NGF levels.⁸¹ The ensuing retinal and optic nerve damage was reduced in rabbits pretreated with retrobulbar injections of NGF, whereas intraocular injection of an inhibitory anti-NGF antibody resulted in worsened damage.

Limited attempts have been made to translate the animal model studies into clinical trials. In an uncontrolled study, three patients with advanced glaucoma treated with topical NGF experienced improvements in various clinical and electrophysiological parameters, including visual field mean deviation, contrast sensitivity, and pattern electroretinogram conduction delays.⁸⁰ These effects appeared to persist after discontinuation. An open-label pilot study, and subsequently a double-masked clinical randomized control trial were performed investigating the use of topical NGF for the treatment of vision loss from optic pathway gliomas.^{82,83} Patients receiving topical NGF were found to have improved electrophysiological testing (e.g. visual evoked potential) and improved visual field testing when compared to controls. Importantly, no significant concurrent tumor growth was noted during this study, and no significant adverse effects were seen.

CHALLENGES OF OCULAR NGF THERAPY

Production

For most of its experimental history, NGF has been used in its murine form (mNGF), derived from the salivary gland of adult male mice as initially described by Bocchini and Angeletti in 1969⁸⁴. NGF is highly evolutionarily conserved, and high sequence homology exists across diverse species.^{84–86} For this reason, mNGF has been used not only in benchtop research but also in human clinical trials, with success. However, the isolation process is time-intensive and technically complex, making adaptation to mass production for clinical use difficult.

Advances in genetic recombination techniques have enabled the development of recombinant human NGF (rhNGF). These recombinant forms maintain the bioactivity of mNGF while allowing scalability of NGF as a therapy for different clinical targets.^{87,88} Of note, recent biochemical studies of mNGF and rhNGF suggest that significant differences exist between murine and human forms, and that these two molecules should no longer be considered equivalent.⁸⁹

Drug Development

Given that NK – the principle application of ocular NGF therapy – is rare, drug development has been limited by scientific and financial interest. Development interest in rh-NGF was advanced when the company currently producing rh-NGF in clinical trials (Dompé Farmaceutici S.p.A., Italy) secured orphan drug status from the European Medicine Agency (EMA) in 2013 and the United States Food and Drug Agency (FDA) in 2018. Clinical trials for rh-NGF in the treatment of NK proceeded soon after orphan drug status was granted, and the drug was approved by the EMA and the FDA soon thereafter.

Stability

NGF, and the growth factor family in general, suffers from poor protein stability and a short biological half-life.⁹⁰ NGF in particular has less conformational stability than the other neurotrophins.^{91,92} NGF found within autologous serum tears, along with other growth factors, experiences a significant decrease in concentration after one day at room temperature.⁹³ For this reason, drug storage is an important concern for NGF therapeutics. Current recommendations are for autologous serum tears to be prepared in single-day vials, stored frozen, and thawed but kept refrigerated for the day of use.⁹³ The storage and handling instructions of the current FDA approved NGF eye drop, Oxervate™, reflect the logistical complexity required to maintain NGF stability, and are similar to those for autologous serum tears.⁹⁴

Poor stability also affects the ability of NGF to exert effects on target tissue. rhNGF becomes unstable in plasma at physiologic temperatures after approximately four days.⁹⁵ Pharmacokinetic studies of rhNGF have found that after subcutaneous injections, the molecule achieves wide distribution, preferentially to highly perfused and neuronal cell types throughout the body.⁹⁶ Because of extensive protein redistribution as well as enzymatic degradation, even delivering the drug directly to the target tissue may be limited

in long-term effect. For example, in a study of an intracranial polymeric rhNGF delivery system, drug concentration in brain tissue decreased exponentially with distance from the device, and tissue half-life was estimated at approximately 1.7 hours.⁹⁷ NGF in topical eye drops permeates into various ocular tissues, including the cornea, retina, and optic nerve, and even reaches tissue as distant as neurons in the forebrain.^{30,98} However, peak levels of NGF are seen six hours after administration, necessitating frequent administration to achieve a desired local effect.⁹⁹ Stability and duration of effect are reflected in the dosage instructions for Oxervate™, which require eye drop administration six times daily.

Drug stability becomes an especially important concern when considering that one of the primary target effects of NGF, neuronal regeneration, is a chronic process. Subbasal nerve fiber regeneration after keratorefractive surgery, for example, occurs over the course of years.^{100,101} Thus, for maximal benefit of NGF, long-term application may be necessary. The current commercially available formulation, Oxervate™, an eight-week treatment duration is recommended. Whether a second or otherwise extended treatment course is beneficial has yet to be determined.

Efficacy

Questions of efficacy arise from the first clinical trials involving NGF in NK. While preclinical studies suggest efficacy in corneal nerve regrowth after keratorefractive surgery,⁵⁷ no preclinical studies have been published on an animal model of NK – the drug's only current indication. In clinical trials, while corneal nerve regrowth was not directly assessed, patients did not experience significant changes in corneal sensitivity.⁶³ In the case of NK, this finding may hamper the drug's long-term efficacy. Moreover, due to the cost of the current formulation, long-term rh-NGF use may be cost-prohibitive. Still, it is relatively early in rh-NGF development, and long-term data is forthcoming.

Off-target Effects

Given the diversity of cell types responsive to NGF and its complex signaling mechanisms, off-target effects need to be minimized. In phase I studies of intravenous rhNGF in healthy patients, generalized myalgia was a common adverse effect.³⁶ Similarly, in clinical trials of topical rhNGF, ocular pain is the most common adverse effect.⁶³ This adverse effect may be related to the neurotrophin's involvement in various pain pathways, or related to the sensitivity of regenerated sensory neurons. In fact, anti-NGF antibody therapy has been used to treat inflammatory and neuropathic pain.¹⁰²

FUTURE DIRECTIONS

Expanded Indications

To date, NGF has been investigated as an ocular therapy in the treatment of ocular surface disease, age-related macular degeneration, retinitis pigmentosa, optic pathway glioma, and advanced glaucoma. NGF therapy in most of these applications is nascent, and further clinical studies are needed to demonstrate clinical benefit. Given the myriad cells that are responsive to NGF – namely, corneal epithelium and endothelium, epithelium of the conjunctiva, lens, iris, and ciliary body, as well as retinal pigment epithelium, Müller

cells, and retinal ganglion cells^{49,,65,,66,,103,,104}— NGF has the potential to impact numerous ocular structures. Further study into the impact of exogenous NGF on different ocular cell populations may reveal off-target effects or indeed new clinical targets.

Sustained Drug Delivery

Because neuronal regeneration is a slow process, NGF treatments require long-term application. In most experimental and clinical trials to date, NGF has been given with a saline or similar aqueous vehicle and requires frequent applications for an extended duration. In non-ocular applications, various methods have been employed to deliver a controlled amount of NGF to target tissue over an extended period of time. For example, NGF has been incorporated into microspheres of polymers like poly(L-lactide)co-glycolide^{105,106} for sustained release to peripheral nerves in a rodent model.¹⁰⁷ Alginate microbeads containing NGF along with other growth factors (VEGF, IGF-1, FGF-1, PDGF, and HGF) were successfully used to deliver growth factors for over four weeks *in vitro*¹⁰⁸ for a proposed treatment of stress urinary incontinence. A collagen/hydroxyapatite composite material provided sustained NGF release and was efficacious in promoting bone formation in a rodent model.¹⁰⁹

While not yet investigated for ocular NGF, drug delivery of other therapeutics have been studied extensively in the eye.¹¹⁰ Current drug delivery solutions for other ocular therapeutics may be applied to NGF to address issues with NGF stability and target tissue delivery.

Gene Therapy

Some research has focused on circumventing issues with NGF delivery to target tissue by using gene therapy to allow native cells to express the therapeutic molecule. So far, this technology has primarily been investigated in CNS pathology. In one early study, autologous fibroblasts were cultivated and genetically modified to produce NGF. These cells were introduced as grafts into a region of degenerated forebrain in a monkey model, and were shown to significantly reduce degeneration of a subset of neurons in this model.¹¹¹ More recently, devices have been developed which contain genetically modified cell lines growing within an implantable, encapsulated device, known as encapsulated cell biodelivery. This technology has been implemented in the treatment of Alzheimer's disease with the NsG0202/NsG0202.1 device – a device containing cells engineered to produce NGF implanted in the basal forebrain.^{112,113}

While ocular applications of gene therapy have so far focused on the treatment of inherited genetic conditions, such as Leber congenital amaurosis¹¹⁴ and retinitis pigmentosa,¹¹⁵ future directions may exploit this technology for the production of therapeutic molecules in native tissue.

Alternate Modalities and Targets

Udonitrectag (Recordati S.p.A.), a low molecular weight compound that mimics NGF, aims to address the issue of NGF stability. Another novel drug, NRO-1 (Neuroptika), targets an alternative neurotrophin pathway – glial cell line-derived neurotrophic factor – rather than

NGF, and aims to avoid the concerns of NGF and pain.¹¹⁶ Both agents are currently in phase II clinical trials.

Autologous serum tears and plasma rich in growth factors are two blood-derived therapies containing NGF and other growth factors that are commonly used in various ocular surface diseases.¹¹⁷ The concentration of NGF in serum is orders of magnitude lower than that used in Oxervate™; however, serum tears have demonstrated efficacy in the treatment of NK.¹¹⁸ Rigorous, prospective clinical trials have not yet been completed.

An alternative mechanism has been targeted with substance P and insulin-like growth factor-1, which work in conjunction to promote epithelial wound healing¹¹⁹ and neuronal function.¹²⁰ This combination therapy has been tested successfully in animals¹²¹ and in an open-label clinical trial for stages I and II NK.¹²² Further research of the clinical efficacy of this treatment has not yet been completed.

CONCLUSIONS

NGF is a bioactive molecule that influences various systems throughout the body via complex molecular pathways. In the eye, NGF exerts beneficial effects on degenerative conditions such as NK and glaucoma. NGF represents the first FDA approved treatment directed at the underlying pathophysiology of NK. Treatment of other ocular conditions may follow the success in NK. Understanding the challenges associated with NGF as an ocular therapy may help hasten the development and more widespread use of this treatment modality.

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