Nerve growth factor: basic studies and possible therapeutic applications

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Abstract

The nerve growth factor (NGF) belongs to a family of neurotrophic factors called neurotrophins. It was discovered as a molecule that stimulates the survival and maturation of developing neurons in the peripheral nervous system and has later been shown to protect adult neurons in the degenerating mammalian brain. Basic and clinical studies have been undertaken to use NGF as a therapeutic agent aimed at restoring and maintaining neuronal function in the central nervous system and to determine the mechanisms to safely deliver the molecule into the brain. Recent studies have also recognized that the role of NGF extends far beyond the horizon of nerve cells and even beyond the peripheral and central nervous system. Studies published from our laboratory have shown that topical application of NGF possesses a protective action on human pressure ulcer, corneal ulcer and glaucoma. Here, we will review these studies, supporting the therapeutic potential of NGF.

Keywords

Alzheimer’s disease, corneal neurotrophic ulcers, glaucoma, nerve growth factor, neurodegeneration, pressure ulcers, psychiatric diseases

The nerve growth factor

The nerve growth factor (NGF) was discovered by R. Levi-Montalcini nearly 60 years ago after transplantation of a malignant mouse sarcoma into the body wall of 3-day-old chick embryos (Levi-Montalcini et al., 1954). A few years later, larger amounts of NGF were found and purified from snake venom and from the mouse submaxillary glands (Levi-Montalcini, 1987; Levi-Montalcini & Angeletti, 1968). In the early 1980s, three NGF homologous growth factors were identified, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and NT-4/5, each with distinct and/or overlapping activities within the developing peripheral and central nervous systems, which were collectively indicated as neurotrophins (Barde, 1990). They stimulate morphological differentiation, regulating neuronal gene expression, through interaction with specific cellular receptors, and are required in mature neurons for maintaining neuronal function and phenotype (Huang & Reichardt, 2001). It has been shown that the failure of trophic interaction between the target cells and their innervation may result in nerve dysfunction and neuronal degeneration (Conner & Varon, 1994; Sofroniew et al., 2001).

Neurotrophins are all synthesized as large precursor proteins and cleaved to physiologically active molecules (Teng et al., 2010). The mature form of NGF descend from proteolytic cleavage of a precursor form (ProNGF), which has important roles during development and in adult life (Fahnestock et al., 2004a, 2004b). The mature NGF exerts its biological action by challenging the specific receptor tropomyosin kinase receptor A (TrkA), which is a typical tyrosine kinase receptor (Huang & Reichardt, 2003). Stimulation of TrkA is promoted by TrkA, forming a complex with the p75 pan-neurotrophin receptor (p75NTR) (Friedman & Greene, 1999; Reichardt, 2006; Schor, 2005). The major intracellular signaling pathways activated by the TrkA/p75NTR complex are Ras-mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK), phosphatidylinositol 3-kinase (PI3K)-Akt and Phospholipase C (PLC)-γ (Chao et al., 2006; Klesse & Parada 1999; Reichardt, 2006). The binding of NGF to p75NTR alone also activates nuclear factor-κB (NFκB) pathways, independent of TrkA (Mamidipudi & Wooten, 2002). All of the above pathways promote cell survival and/or differentiation. The ProNGF, on the other hand, binds to a sortilin–p75NTR complex and activates cell death through the stimulation of Jun N-terminal kinase (JNK) (Teng et al., 2010).

Until early 1980s most of the studies on NGF were directed to analyze its role on growth, differentiation and survival of peripheral sensory and sympathetic neurons. This narrow experimental approach was due to the general belief that NGF and/or NGF-receptors were absent in the central nervous system. This belief was challenged in 1984, after the evidence published by Seiler and Schwab (Seiler & Schwab, 1984) demonstrating that radiolabeled NGF injected in the frontal cortex of rodents was transported directly to forebrain...
NGF and peripheral neuropathies

In vivo and in vitro findings have shown that NGF promotes survival, differentiation and function of peripheral sensory and sympathetic nerve cells. These findings lead to the hypothesis that the purified NGF might be useful to prevent and/or protect peripheral nerve degeneration, such as those occurring in diabetes, leprosy, HIV infection or surgical traumas (Aloe et al., 2012; Rask, 1999). The history of the trials conducted in the 1990s on diabetic patients is paradigmatic in respect to the potentiality of NGF in the care of peripheral neuropathies. Diabetes is often characterized by degeneration of PNS neuron/fibers (Apfel, 1999; Apfel et al., 1994), associated with altered local levels of NGF/NGF receptors and with deregulation of the NGF signaling pathway. It was reported that NGF administration in animal models of diabetic neuropathies reversed the neurodegenerative signs and normalized the activity of PNS neurons (Apfel, 1999; Apfel et al., 1994). The results of a phase II trial on patients with diabetic neuropathy raised some optimism (Apfel et al., 1998); however, a subsequent phase III trial failed, despite the fact that some previous parameter were partially confirmed (Apfel, 2002; Apfel et al., 2000). Why the results of the first and second trial were different is not clearly known. The interpretation given by the authors include: different biological preparations and/or properties of the NGF used in the two trials; the non-homogeneous patient populations in terms of age; the onset and severity and clinical history of the neuropathy; different selection of the placebo group of patients; the occurrence of undesired side effects, such as hyperalgesia, with consequent interruption of NGF administration (Apfel, 2002; Apfel et al., 2000). The most plausible cause leading to the final negative result, the failure of the study and the interruption of clinical investigations on NGF and diabetic neuropathies should, however, be identified with the need for using a low dosage of NGF, in comparison with animal studies, due to the occurrence of side effects (Apfel, 2002). Moreover, it was also correctly hypothesized that the efficacy of NGF treatment in human diabetic neuropathy was limited to small sensory neuron functions, thus leaving large diameter fibers function unaffected (Apfel, 2002). Unfortunately, the trials were discontinued and it was impossible to have further insight into the real efficacy of NGF treatment in diabetic neuropathy. In light of these controversial results, possible alternative strategies to further investigate the role of NGF on human peripheral neuropathy could be: using small NGF mimetic molecules, yet under investigation for central neurodegeneration (Longo et al., 2007); using molecules able to promote the endogenous synthesis and release of NGF in proximity of damaged tissue, without eliciting the undesired NGF-related effects (Manni et al., 2000; Riaz et al., 1999; Samina Riaz & Tomlinson 2000).

NGF and the central nervous system

Alzheimer’s disease

The first study suggesting the presence of NGF and/or NGF-receptors in the CNS was published in 1984 (Seiler & Schwab, 1984). Subsequent investigations confirmed these observations and demonstrated that NGF administration directly into the brain can improve cholinergic dysfunction observed in age-related memory disturbances (Bartus et al., 1982; Fischer et al., 1987). Because the degeneration of basal forebrain cholinergic neurons (BFCN) and the decline of cognitive abilities are hallmarks of the AD (Cuello et al., 2010; Schaeffer & Gattaz, 2008), it was hypothesized that NGF might be of therapeutic value for AD patients. However, a major obstacle encountered in these studies was the difficulty to deliver NGF directly to brain neurons, due to the poor permeability of NGF to the blood–brain barrier when injected systemically (Pan et al., 1998), while the intra-cerebro-ventricular (ICV) delivery results rather invasive. Anyhow, the first clinical trials performed in patients with AD were published in the 1990s by Swedish scientists (Eriksdotter Jonhagen et al. 1998; Olson et al., 1992). The results showed partial beneficial effects after chronic ICV administration of NGF, such as a marked transient increase in uptake and binding of $[^{11}C] $-nicotine in the frontal and temporal cortex, a persistent increase in cortical blood flow, a progressive decrease of slow wave EEG activity and the improvement of verbal episodic memory tests, but also negative side effects: a reversible weight loss during the NGF infusion period and the development of back pain symptoms after the beginning of ICV infusion. Such symptoms most probably reflect the NGF-mediated hyperactivation of nociceptive transmission system in the spinal cord, after NGF spreading through the cerebrospinal fluid. These side effects were considered to outweigh the positive outcomes and lead to discontinuation of trials in AD patients. Other studies using ICV infusion and/or grafting of NGF-producing cells directly into the patient brain (Tusznyski et al., 2005) reached the conclusion that this experimental approach might entail serious surgical risks and high costs, suggesting that less invasive strategies for delivering NGF into the brain of patients with AD should be tested. However, the success of this NGF-based therapeutic strategy depends on a careful optimization of delivering the molecule directly into the brain and, more importantly, on the absence of undesired and invasive effects.

Studies published in recent years and carried out on laboratory animals reported that NGF can be safely delivered into the brain by the olfactory pathway (Chen et al., 1998; Koevary et al., 2003; Zhao et al., 2004) or by ocular administration (Di Fausto et al., 2007; Lambiase et al., 2007b) and that the molecule reached the NGF target cells into the brain relatively intact and biologically active. These observations suggested that both the olfactory and the ocular pathways may represent promising noninvasive route for NGF delivery to NGF-responsive brain neurons (see Aloe et al., 2012 for a more detailed discussion about this topic).
Stress, anxiety and psychiatric-like disorders

Studies published in the 1980s showed that aggressiveness induced in adult male mice produces a massive release of NGF in the bloodstream (Aloe et al., 1986) and in the CNS (Spillantini et al., 1989) (Figure 1). Moreover, it has been shown that not only aggressive behavior per se causes the release of NGF, but also fear and anxiety stimulates synthesis and release of endogenous NGF (Alleva et al., 1993; Aloe et al., 1994) (Figure 1). To assess whether this hypothesis was correct, the level of circulating NGF was measured after alcohol consumption or withdrawal, or heroin abstinence, showing a consistent variation of circulating levels of NGF (Aloe et al., 1996; Heberlein et al., 2008) and that drug inducing sedation, such as haloperidol lowered the circulating NGF presence (Aloe et al., 1997), further suggesting that anxiety causes synthesis and release of NGF.

These observations suggest to test whether the circulating and brain NGF changes in mechanisms related to depression (Gioiosa et al., 2009). We measured the presence of NGF in laboratory animals and in humans showing depression-like behavior and found significant changes in both circulating and brain NGF content (Angelucci et al., 2000; Cirulli et al., 2009). Moreover, brain NGF was selectively affected by electroconvulsive treatment in an animal model of depression (Angelucci et al., 2003).

Changes of circulating and brain NGF levels have been also observed in an animal model of schizophrenia and in schizophrenic subjects (Aloe et al., 1997, 2000; Angelucci et al., 2004). Why the levels of NGF are affected in these subjects is not known. It is known that NGF plays a critical role in maintaining functional connections in adult brain neurons and low synthesis of NGF in developing brain can negatively affect neurogenesis and neuroplasticity (Conner & Varon, 1994; Sofroniew et al., 2001). It has been hypothesized that schizophrenia develops as a result of abnormal neuronal migration and/or differentiation during development (Fiore et al., 1999; Weinberger, 1987). Thus, it might be possible that structural brain deficits observed in schizophrenic post-mortem brain (Weinberger et al., 1983; Weinberger, 1987) might be linked to deficits of NGF synthesis and/or utilization during crucial development stages (Fiore et al., 1999, 2004).

To further investigate this hypothesis, Bersani et al. measured the circulating NGF levels in 26 male inpatients meeting DSM-IV criteria for Schizophrenia (Bersani et al., 1999). The patients received a standard hospital diet, the same for all patients, and had previously taken neuroleptics and benzodiazepines for variable periods. The control group was made up of 29 healthy male volunteers. The psychopathology of the patients was assessed using the Positive and Negative Syndrome Scale (PANSS). It was found that NGF plasma levels in schizophrenic patients were significantly lower than in healthy controls. The possibility that this effect might be due to stress induced by hospitalization seems unlikely, because stress both in animal models and in humans is known to enhance rather than lower blood NGF (Aloe et al., 1994). Because patients were neuroleptic-free for at least 15 d, the hypothesis of an effect induced by neuroleptic treatment was also not considered.
Topical NGF for epithelial and ocular diseases

Studies published during last 15 years in collaboration with clinicians, demonstrated that topical NGF application could promote healing effects on human ocular ulcers, skin ulcer induced by diabetes or rheumatoid arthritis, as well as on glaucoma and age-related retinal maculopathy. These findings will be briefly described and commented below.

Skin ulcers

The evidence showing that topical NGF exerts healing effect on human pressure ulcers was published around the beginning of the new century (Bernabei et al., 1999; Landi et al., 2003). In total 19 patients were treated with purified murine NGF. It was reported that daily topical application of NGF resulted in a significant acceleration of the healing process, in comparison with patients treated with conventional available therapies. Briefly, the lesions in the NGF-treated patients displayed a marked peripheral scar indicating that the healing process was taking place and that the rate of recovering was not related to the severity of the ulcer, the age of the patient or the site of the ulcer (Bernabei et al., 1999; Landi et al., 2003).

Moreover, the healing action of NGF on cutaneous ulcer, was not limited to pressure ulcer, since chronic vasculitic ulcers secondary to rheumatoid arthritis (RA) can heal after NGF application (Tuveri et al., 2000). The RA-associated skin ulcers were treated with topical NGF in four patients and they showed a rapid improvement after the first 2–3 weeks of NGF application. This healing action was followed by a significant reduction of local pain and inflammation. Thereafter, the ulcer improves progressively, reaching complete healing after eight weeks of NGF treatment (Tuveri et al., 2000).

Likewise, leg cutaneous ulcer induced by diabetes (Figure 2A–B) responds positively to topical NGF treatment (Generini et al., 2004). Even chronic ulcers with deep damages close to the clearly underneath bone responds to the NGF application by healing few weeks after the treatments. In brief, three diabetic patients that developed severe foot or leg ulcers were treated with topical NGF. The first clear reduction of the size of the ulcer was noted after 8 weeks of NGF administration, and the follow-up study indicated that the NGF stimulated the proliferation and/or differentiation of local immune cells, blood vessel and even neurite outgrowth. Skin healing continued to progress

Figure 2. Panels A–B: Human calcaneal ulcers before (A) and 6 weeks after daily NGF administration (B). The treatment with NGF does not only cause the reduction of ulcer size but also the growth of damaged blood vessels and peripheral neurites. Panels C–F: Photographs of the eye of a patient with a corneal neurotrophic ulcer before (C, E) and after (D, F) treatment with NGF. The healing action of NGF is particularly visible when comparing picture in panels E and F, where pictures of the eye have been taken after fluorescein eye drop application. The ulcer area is evidenced as a white-yellow area in panel E and was completely disappeared (F) after 4 weeks of daily topical treatment with NGF.
thereafter with the NGF application and in some patients the ulcer displayed complete healing in 5–6 weeks. No relapse was observed after over 2 years of follow-up (Generini et al., 2004).

**Corneal neurotrophic ulcer**

The human corneal neurotrophic ulcer is an ocular pathology caused by diverse endogenous and exogenous insults that can lead to blindness (Lambiase et al., 2003). A consistent number of patients (more than 200) with severe corneal ulcer who had received conventional treatment, including artificial tears, antibiotics, eye patching and soft contact lens bandaging, with little or no benefit at all, have been treated so far, starting from the end of the 1990s, with NGF administered as eye drops (Aloe et al., 2012; Lambiase et al., 1998, 2003, 2007b). A positive action of topical NGF administration has been observed in almost all of the patients treated so far, a few days after the beginning of NGF treatment, and patients have always displayed a complete resolution of the corneal ulcer after 10 d to 6 weeks of treatment (Figure 2C–D). Structurally, the first signs of healing are an advancement of epithelial cells from the margin to the center of the ulcer associated with mild to moderate conjunctiva hyperemia. Almost all patients show photophobia and the burning of their eyes during slit lamp examinations, and most patients had improvement of corneal contact sensitivity that suggested functional recovery of corneal innervation. During the first week of treatment, some patients complained of a transient burning sensation. The rate of healing was not associated with the severity of the ulcer or to its depth in the stromal layer, or with the age of the patients or the cause of the ulcer (Lambiase et al., 1998, 2003, 2007b). The evidence that NGF restored corneal integrity and sensitivity lead to the hypothesis that the progressive corneal damage that occurs in patients with corneal sensory nerve deficits was due to a deficit of endogenous NGF synthesis, release and/or utilization. Moreover, while over 200 patients have been successfully treated so far for corneal ulcers, the occurrence of serious side effects linked to the action of NGF on pain system was almost absent, neither were developed circulating anti-NGF antibodies (Lambiase et al., 2007a).

**Glaucoma**

After the observation on the human corneal ulcer, we decided to carry out studies on laboratory animals using labeled NGF to trace the route of the molecule when applied to the ocular surface. These pharmacokinetics studies revealed that NGF application to the corneal surface could reach the retina and optic nerve (Lambiase et al., 2005). Previous studies demonstrated the presence of NGF receptors on retinal cells (Hallbook et al., 1996; Vecino et al., 1998) and also indicated that NGF could rescue retinal ganglion cells after optic nerve lesion (Carmignoto et al. 1989; Turner & Delaney, 1979). Using animal models of experimentally induced glaucoma, we demonstrated that the eye NGF application can indeed protect retinal cells damaged by glaucoma (Lambiase et al., 2009a). Based on these findings, we tested the effect of topical application of NGF in three patients with advanced glaucoma, with imminent risk of loss of visual function (Lambiase et al., 2009a). We found a progressive improvement in the functionality of the inner retinal layer and in the parameters of the post-retinal neural conduction, evident during the treatment period. After discontinuation of treatment: electrophysiological parameters were significantly improved in parallel with the clinical ones; the defect of visual field was improved gradually after discontinuation of treatment; contrast sensitivity was significantly improved in all patients and improvement was maintained 3 months after cessation of treatment; visual acuity improved significantly in all patients where it remained unchanged for the 3-month follow-up. The treatment showed absence of undesired side effects, except for the development of local burning during the first week of treatment in a single patient.

Because age-related macular degeneration (ARMD) is an ocular disease affecting visual retinal function, we tested the effect of NGF on an aged patient affected by this ocular disorder. We treated a 94-year-old woman affected by ARMD, whose visual acuity was progressively worsening in spite of previous surgical and medical treatments, with NGF eye drops (Lambiase et al., 2009b). We found a significant improvement in visual acuity and electrofunctional parameters as early as 3 months after initiation of treatment. These results are in line with previous reports on a neuroprotective effect of NGF on retinal cells and on NGF eye drops bio-availability in the retina and optic nerve (Lambiase et al., 1997).

**Modulating endogenous NGF by physical therapies**

A number of studies have shown that the basal NGF levels, both in the blood stream and in the brain, can be significantly affected by physical exercise, environmental determinants and, as we have demonstrated, by electroacupuncture (Manni et al., 2005; Manni et al., 2010). Traditional Chinese acupuncture and its western derivate electroacupuncture (EA) have been proven effective in the therapy of neuropathic pain (Abruisha et al., 1998; Andersson & Lundeberg, 1995) and the neurophysiological correlates of these techniques are actually subjects of extensive investigations (Abruisha et al., 1998; Andersson & Lundeberg, 1995; Manni et al., 2010; White, 2009). Acupuncture is a potent form of sensory stimulation. This perspective is the key point from which Western scientific community has studied the mechanisms of acupuncture treatment, first on pain relief and then in a wide spectrum of diseases, including infections and inflammations, dysfunction of the autonomic nervous system, peripheral and central nervous system diseases, metabolic disorders (Andersson & Lundeberg, 1995). Needles insertion in the skin and deeper tissues results in particular patterns of afferent activity in peripheral nerves. It has been suggested that EA with repetitive muscle contraction results in the activation of physiological processes similar to those resulting from physical exercise (Andersson & Lundeberg, 1995). It has recently been proposed that at least some of the effects attributed to acupuncture are mediated by neurotrophins and in particular by NGF (Manni et al., 2010). The first convincing evidence of the correlation between NGF and acupuncture has been produced in our collaborative studies on the estrogens-induced polycystic ovary syndrome (PCOS) model in rat. We demonstrated that low-frequency electroacupuncture decreased the ovarian NGF and NGF-receptor...
content and modulated the ovarian adrenergic responsiveness, normalizing some of the pathological features of the disease (Manni et al., 2005; Stener-Victorin et al., 2003). More recently, we demonstrated that EA increased NGF synthesis in the retina of a rat strain affected by an inherited retinopathy (Pagani et al., 2006). We also applied EA in an experimental model of diabetic neuropathy (Manni et al., 2011), demonstrating that the early increase of spinal and peripheral NGF, associated with the development of thermal hyperalgesia, could be corrected by EA. These effects were associated with the normalization of pain mediators, which are known to be under NGF control (Manni et al., 2011). Notably, works from our group demonstrated that EA was able to modulate NGF levels in the brain of diabetic rats (Rocco et al., 2013), and of stressed mice, with improvement of stress-affected cognitive behavior (Manni et al., 2009), and that EA could be useful in reducing pain-related NGF effects, when systemic NGF was administered to healthy rats (Aloe & Manni, 2009). The overall data on the link between EA and NGF point to EA as a possible supportive tool that can be useful in the modulation of endogenous NGF or to reduce NGF-induced side effects, such as hypersensitivity and hyperalgesia, when clinical treatment with NGF is necessary.

Other experimental approaches, such as, spontaneous, not-forced physical exercise has been also proven effective in modulating NGF content and NGF activity in both the CNS and peripheral field. Physical activity in experimental rats is obtained by placing the animals in cages equipped with free running wheels, where rats can spontaneously perform a pleasant running activity, or with the experimenter-controlled rotating treadmill, where animals are moderately forced to run (Ang & Gomez-Pinilla, 2007). Such experimental paradigms induce elevation of NGF mRNA in healthy rats (Neepher et al., 1996) and are able to increase brain NGF with parallel improvement of behavioral tasks as well as oxidative stress and apoptosis/neuroprotective factor expression in models of brain damage (Ang et al., 2003; Chung et al., 2010; Ding et al., 2004; Matsuda et al., 2011) and aging (Chae & Kim, 2009; O’Callaghan et al., 2009; Um et al., 2011). Overall these experimental evidences point to a beneficial effect of moderate and aerobic exercise, linking them to the exercise-driven regulation of brain and peripheral neurotrophic milieu.

Conclusion

Nerve growth factor (NGF) is the founding member of a neurotropic factor family produced by and acting upon a number of developing, adult and damaged NGF-receptive neuronal and non-neuronal cells. Several studies have been performed to investigate the potential role of this molecule in the treatment of neurodegenerative diseases of the PNS and of the CNS, including AD. Recent studies have shown that NGF can be safely delivered into the brain by nasal or ocular administration, prospecting potential therapeutics action on cell degeneration in the central nervous systems. We have also reported recent and ongoing clinical data supporting the potential therapeutic properties of NGF for human skin and ocular ulcers. In summary, we believe that the discovery of NGF and of other neurotrophins has provided illuminating insights into most areas of contemporaneous neuroscience research, which will most likely provide in the future effective therapies for neurodegenerative and epithelial diseases.

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Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

References


