Neurotrophic Factors in Neurodegeneration

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Conflict of interest statement: The author is a scientific advisor of Ceregene, Inc.

Neurotrophic factors (NTFs) have the unique potential to support neuronal survival and to augment neuronal function in the injured and diseased nervous system. Numerous studies conducted over the last 20 years have provided evidence for the potent therapeutic potential of NTFs in animal models of neurodegenerative diseases. However, major obstacles for the therapeutic use of NTFs are the inability to deliver proteins across the blood-brain-barrier, and dose-limiting adverse effects resulting from the broad exposure of nontargeted structures to NTFs. Two recent developments have allowed NTFs' promise to be truly tested for the first time: first, recent improvements in viral vectors that allow the targeted delivery of NTFs while providing a long-lasting supply and sufficient therapeutic doses of NTFs; and second, improved animal models developed in recent years. In this review, we will discuss some of the potential therapeutic applications of NTFs in neurodegenerative diseases and the potential contribution of disturbed neurotrophic factor signaling to neurodegenerative diseases.

Brain Pathol 2006;16:295–303.

INTRODUCTION

Substantial progress has been made over the last two decades in elucidating the neurobiological function of neurotrophins and other neurotrophic factors (NTFs) during development and adulthood. Since the discovery of nerve growth factor (NGF), the first NTF described, it has become clear that these naturally produced neuron survival-promoting factors are vital not only for nervous system development but also for the maintenance and functioning of the adult nervous system. The accompanying reviews focus on various aspects of neurotrophins in human neurological disorders (88, 127, 130, 150). In this review, I will also discuss other NTFs that have promise for treatment of neurodegenerative disorders.

The discovery that NTFs support neuronal survival and function in the adult central nervous system (CNS) generated broad interest in the use of these factors to intervene in neurodegenerative diseases. Numerous *in vitro* studies and *in vivo* studies in animal models of neuronal degeneration have provided proof-of-concept and preclinical data that have led to several clinical trials starting in the early 1990s using peripheral or intracerebroventricular (i.c.v.) protein administration, and continuing to date using more sophisticated means of NTF delivery. Indeed, it has become increasingly clear that the successful implementation of NTF therapy requires a targeted, localized delivery of NTFs to avoid unwanted adverse effects resulting from widespread receptor activation. These insights have led to the first promising clinical trials of NTFs in Alzheimer's Disease (AD) and Parkinson's Disease (PD). This review will highlight some of the preclinical studies conducted to date with NTFs in models of AD, PD and other neurodegenerative disorders, and summarize current and previous clinical trials.

More recently, several lines of research have indicated that changes in neurotrophin signaling may also contribute to neuronal degeneration in some CNS disorders (see accompanying review by Twiss et al) (150). Although defects in NTFs or their receptors have not been found to be the underlying cause of neurodegenerative disorders, recent studies suggest that changes in retrograde neurotrophin transport, decreased neurotrophin synthesis, altered processing of proneurotrophins or signaling through $p75^{NTR}$ may contribute as a secondary event to neuronal degeneration in some CNS disorders.

NGF AND AD

NGF was the first neurotrophin that was discovered in the search for neuron survival-promoting factors in the nervous system. Although initial reports focused on its effects in development and in the peripheral nervous system (PNS), studies indicating expression of NGF in the adult neocortex and hippocampus (32, 84, 137) have concluded that NGF also has important activities in the adult CNS. In the mid-80's several groups reported concomitantly that i.c.v. infusions of NGF can prevent the lesion-induced degeneration of cholinergic neurons in the medial septum (53, 57, 87, 157). As cholinergic neurons in the basal forebrain undergo severe degeneration in AD (122, 154, 155), and this degeneration is likely to contribute to the cognitive decline in AD (13, 123), it was speculated that NGF might have therapeutic potential in preventing or slowing the cognitive decline in AD by targeting the cholinergic component of neuronal degeneration in AD (58).

Subsequent studies confirmed the potent effects of NGF in primate models of lesion-induced degeneration (40, 76, 79, 145, 146), and importantly also indicated that NGF infusions can ameliorate cholinergic neuronal atrophy and memory deficits in aged rodents, and increase cholinergic activity (49, 50, 99, 100).

Taken together, these reports indicate that NGF is highly potent in preventing

cholinergic neuronal degeneration and in augmenting cholinergic function by increasing acetylcholine production. These studies led to a small clinical trial using i.c.v. infusions of NGF, but treatments had to be discontinued because of the development of a pain syndrome in some patients (46, 116). Several animal studies indicated similar adverse effects, including hypophagia, weight loss, Schwann cell hyperplasia, sprouting of sensory and sympathetic neurons, and pain syndromes (69, 92, 129, 156, 158) resulting from the broad exposure of nervous system structures to NGF.

In parallel studies, a means of localized, intraparenchymal NGF delivery was developed using cells genetically modified to express NGF. Using the same models of cholinergic neuronal degeneration described above, cellular grafts serving as biological minipumps next to cholinergic cell bodies were found to be equally effective in preventing lesion-induced or neurotoxin-induced degeneration in rodents (37, 71, 102, 126) and primates (40, 79, 147, 148). Cellular NGF delivery was further shown to prevent age-related neuronal degeneration in rodents (26) and primates (28, 139), and to ameliorate memory deficits in aged memory-impaired rats (26, 101, 104). Additional safety and dose-escalation studies in primates confirmed that localized NGF delivery to the basal forebrain by genetically modified fibroblasts is safe and well tolerated (M.H. Tuszynski, unpub. data).

On the basis of these results, a phase I study of *ex vivo* NGF gene therapy was initiated, enrolling eight subjects with mild AD (149). The aim of this study was primarily to determine whether NGF gene transfer is safe, but secondary outcome measures included fluoro-deoxy-glucose positron emission tomography (PET) scans and cognitive testing. The rationale for the enrollment of patients in early to mid-stage of AD was twofold: first, patients need to able to give informed consent to an invasive experimental treatment; and second, NGF needs to be administered at a time when neurons are still alive to be therapeutically effective. Primary autologous fibroblasts were cultivated from a skin biopsy from each patient and genetically modified to express NGF. The study design included a staggered entry with a 3-month surgery delay between patients, and a dose escalation, with the first patients receiving only unilateral injections, followed by higher cell doses and bilateral cell injections. Cells were stereotactically implanted into the basal forebrain adjacent to the nucleus basalis of Meynert (NBM), which provides cholinergic input throughout the neocortex. Initially, surgeries were performed while patients were only sedated, and abrupt movements during the cell injection resulted in hemorrhages in two patients. General anesthesia in subsequent subjects allowed the safe completion of the study and no adverse events related to NGF delivery were observed. Cognitive testing indicated an improvement in the rate of cognitive decline, in particular after longer time periods post surgery. PET scans in bilaterally treated subjects also indicated an increase in metabolic activity throughout the neocortex, consistent with the widespread modulation of cortical activity from the NBM. In addition, histological analysis of the brain of one of the subjects, who died 5 weeks after the surgery, showed sprouting of cholinergic neurons from the NBM into NGF-secreting grafts, to a similar extent as previously observed in the primate brain.

These data indicated for the first time that basal forebrain cholinergic neurons (BFCNs) in the Alzheimer's brain remain responsive to NGF. Although definitive conclusions about the effectiveness of NGF cannot be drawn from this small open label trial, if effects of similar magnitude can be observed in placebo-controlled, blinded trials, this would represent a significant improvement over current symptomatic treatments.

Since the initiation of the phase I trial described above, substantial improvements in gene therapy and vector design have eliminated the need for labor-intensive preparation of autologous cells, *in vitro* gene transfer and cell characterization. Direct *in vivo* injection of replication-incompetent viral vectors, such as adeno-associated virus (AAV) or lentivirus, allows for the localized production of trophic factors in the CNS. Studies using *in vivo* NGF gene transfer in animal models of cholinergic neuronal degeneration have confirmed the neuroprotective effects of NGF on BFCNs (19, 21, 74, 75, 97, 159, 160). These studies, together with additional safety and toxicology studies (23), have led to a second phase I study sponsored by Ceregene, Inc. to evaluate the safety of AAV–NGF gene transfer in AD. Should this study, conducted at Rush University, Chicago, indicate that NGF delivery by *in vivo* gene transfer is safe, additional phaseII/III trials will likely provide an answer as to whether NGF gene therapy is a valuable means of reducing cholinergic neuronal degeneration, and whether targeting the cholinergic component of AD can delay the cognitive decline in AD patients.

GDNF FAMILY LIGANDS IN PD

Although a large number of trophic factors have been shown to enhance the *in vitro* survival of dopaminergic neurons affected in PD (10, 63, 66, 67, 86, 94, 107), recent studies have focused on glial cell-line derived neurotrophic factor (GDNF) and other members of the same family, as these factors appear to have the most robust effects on dopaminergic neuronal survival (94).

GDNF, the first member of the GDNF family ligands (GFLs) was initially characterized as a highly specific NTF for midbrain dopaminergic neurons (15, 143). Since the discovery of GDNF, several other highly homologous members of this family have been discovered, including neurturin (85), persephin (107) and artemin (10). Each of these molecules signals through the receptor tyrosine kinase ret after binding preferentially to one specific GDNF family receptor of the four family members discovered to date (GDNF family receptor (GFR)-alpha1-4). Signal transduction of GDNF is mediated by binding to GFR-alpha-1 and to a smaller degree to GFR-alpha-2, followed by ret-induced intracellular phosphorylation events.

GDNF has been tested in a significant number of animal models of PD [reviewed in (73)]. Starting in the mid-1990s, GDNF injections had been shown to protect dopaminergic neurons in the substantia nigra (SN) from axotomy and from 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced lesions in rodents (15, 143) and in nonhuman primates (54). Similar effects were obtained using encapsulated GDNF-producing cells: dopaminergic neurons were rescued and amphetamine-induced rotational abnormalities were normalized (42, 144).

A subsequent phase I trial injecting GDNF i.c.v. turned out to be not only ineffective but resulted in severe adverse effects, including anorexia, and severe nausea hours to several days after injections; weight loss occurred in the majority of subjects (115). Adverse effects encountered in this trial are not surprising, and clearly a result of the exposure of nontargeted structures to GDNF. As mentioned above, similar adverse effects have been observed with i.c.v. infusions of NGF in rodents and in AD patients. The lack of efficacy resulted from insufficient diffusion of GDNF from the lateral ventricle to the actual target area, the striatum. Thus, the majority of GDNF resided in the cerebrospinal fluid without ever reaching the affected neurons in the SN or their projections in caudate and putamen.

To achieve sufficient concentrations in the striatum, clinical trials of intrastriatal GDNF infusions were initiated showing much more promising outcomes (56, 95, 119, 138), including improvements in the Parkinson's rating scale, increased dopamine uptake indicated by PET scans and morphological responses in one patient who had died, while adverse effects were mild or absent. A subsequent larger placebo-controlled trial did not replicate these initial findings (89). However, the mode of GDNF delivery was changed, the dose was lower than in the previous trials, potentially too low to see any clinical benefit, and the patient population was atypical for PD (12). Thus, additional trials are needed to determine whether GDNF will benefit PD patients.

The relatively focused loss of dopaminergic neurons in the SN makes PD an ideal candidate disease for NTF gene therapy. Promising results in models of PD using GDNF delivery by recombinant adenoviral (18, 27, 91), adeno-associated viral vectors (38, 96, 98) and lentiviral vectors (17, 83) support the view that GDNF gene therapy as a valuable alternative means to localized infusions, which are complicated by shifts in catheter positions, infections, stability of trophic factors and the need to refill infusion pumps.

In addition to GDNF, neurturin, a trophic factor with similar properties as GDNF (85), has been tested in animal models of PD showing efficacy comparable to GDNF (63, 128). Rodent and primate efficacy and toxicology data (14, 24, 34, 61) have led to a phase I trial to test the safety of AAV-2 mediated neurturin delivery to the striatum of PD patients. Should the phase I trial currently underway at the University of California, San Francisco, and Rush University, Chicago indicate that neurturin gene therapy is safe, blinded, placebo-controlled phase II/III trials will determine whether neurturin gene therapy can protect dopaminergic neurons from neuronal degeneration and improve motor dysfunction in PD.

NTFS IN HUNTINGTON'S DISEASE (HD)

A large number of NTFs have also been tested as neuroprotective agents in animal models of HD to prevent striatal degeneration of medium spiny neurons. Many studies have been conducted using striatal neurotoxic lesions that do not fully replicate the pathological changes of HD resulting from an autosomal dominant disorder with progressive motor, cognitive and psychiatric disturbances. Since the identification of the genetic defect causing HD (142), transgenic mouse models and viral expression of huntingtin with variable length of CAG poly-glutamine repeats have allowed a better replication of the pathological changes underlying the disease.

In excitotoxic lesion models, all NTFs tested have indeed been reported to be neuroprotective to a variable degree, including NGF (6, 35, 39, 51, 52, 80, 81, 103, 151), BDNF (52, 103, 121, 152), NT-3 (6), NT-4/5 (3), GDNF (7, 120), transforming growth factor-β (3) and the neuropoetic cytokine CNTF (6, 41, 44). The mechanism of some of the neuroprotective effects observed is not fully established and might be indirect (82). It might be necessary to re-evaluate some NTFs reported to be neuroprotective after excitotoxic lesions in improved animal models that more closely resemble the human disease. For example, GDNF reported to be effective in excitotoxic lesion models (7, 120) appears to be ineffective in transgenic mouse models overexpressing mutant huntingtin (124).

More recent studies have also evaluated NTF gene transfer using AAV or lentivirus as a means to provide long-term, localized NTF support in animal models of HD. Overexpression of BDNF, GDNF and CNTF using AAV (72), adenovirus (16, 112) or lentivirus (36, 106, 125) were found to be protective after excitotoxic lesions.

The only NTF tested in a clinical trial in HD patients is CNTF (9, 20). This trial was based on animal studies using CNTF protein delivery (6) or encapsulated CNTF-producing cells in rodent (41, 43, 45) and primate models (44, 111). The phase I study delivered CNTF into the lateral ventricle of six patients using encapsulated CNTF-secreting cells. After retrieval of the capsules, low cell survival was observed in about 60% of all retrieved capsules and no clinical benefit was observed in any of the treated subjects. The lack of any clinical benefit might partially be a result of the limited diffusion of CNTF through the ventricular wall into the adjacent putamen (82) similar to the limited diffusion of GDNF after intraventricular injection.

As mentioned earlier, BDNF has also been tested in several animal studies for its neuroprotective effects after neurotoxic lesions. Recent evidence points toward a role of huntingtin in influencing BDNF transport and BDNF expression, providing additional rationale to investigate BDNF as a potential therapeutic molecule in this devastating polyglutamine disease (see below).

NTFS IN ALS

Several NTFs have been found to have potent effects on motor neuron survival *in vitro*, during development, after injury to motor neuron systems and in genetic models of motor neuron degeneration, providing a rationale to develop NTFs as treatment for ALS, in which ventral motor neuron degeneration is extensive. BDNF, CNTF, insulin-like growth factor-1 (IGF-1) and GDNF have been evaluated in animal models of motor neuron disease or ALS using direct protein delivery, with encouraging results (60, 65, 68, 77, 78, 93, 109, 110, 117, 118, 134–136, 161–163). Based on these studies, clinical trials with CNTF (5, 108, 140), BDNF (141) and IGF-1 (22, 90) have been conducted. To date, these clinical trials have essentially failed, an outcome that is at least partially attributable to the mode of NTF delivery and/or the instability of the administered molecules. The mode of administration

likely led to subtherapeutic levels in the CNS or dose-limiting adverse effects caused by broad distribution centrally or peripherally, including weight loss, severe coughing, fever and muscle wasting.

Intrathecal infusions of CNTF or intrathecal delivery of CNTF using encapsulated heterologous cells producing CNTF in patients with ALS did not lead to the same adverse effects previously reported but also failed to deliver significant clinical benefits, potentially because of inefficient penetration of the spinal cord parenchyma (1, 2).

More recently, improvements in gene therapy vectors and the ability of viral vectors to be retrogradely transported to motor neurons after injection into peripheral muscle targets have shown some promise in transgenic mouse models of ALS. Expression of vascular endothelial growth factor (VEGF) in motor neurons via retrograde transport of a VEGF-coding lentivirus from muscle (8) and expression of IGF-1 in motor neurons after AAV-2 injection into muscle (70) improved animal survival and delayed motor neuron death in transgenic mice overexpressing mutant superoxide dismutase-1 (SOD-1). GDNF expressed in muscle after AAV gene transfer was also shown to be retrogradely transported to motor neurons, to delay motor neuron degeneration and to prolong the lifespan of SOD-1 expressing mice (153).

Re-evaluation of some previously tested factors such as IGF-1 in clinical trials with site-specific delivery may therefore allow for therapeutic doses to be reached in ventral spinal cord motor neurons without adverse effects from widespread CNS and PNS exposure.

NEUROTROPHIN PROCESSING, SIGNALING AND TRANSPORT IN NEURODEGENERATIVE DISORDERS

As mentioned earlier, NTFs, including neurotrophins and GFLs, are vital for nervous system development, indicated by the lethality of many homozygous knockout mice for NTFs or severe developmental abnormalities in the nervous system and other organs of homozygous and heterozygous knockout animals [see (150)].

Although loss of NTF signaling during development and adulthood has clearly been shown to result in developmental deficits and neuronal degeneration, no direct evidence has been provided to link the loss of neurotrophin signaling to the etiology of any specific neurodegenerative disease. Despite the lack of any direct evidence for a role of NTF deficiency as the underlying cause for neurodegenerative diseases, impairment of target-derived signaling by retrograde transport of NTFs may contribute to neuronal dysfunction and neurodegenerative diseases such as AD, ALS or Huntington's disease (64, 131) (Figure 1).

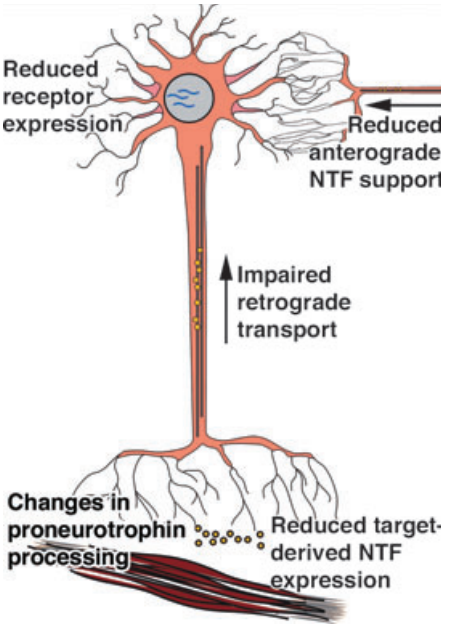

Figure 1. Schematic outline of potential changes in neurotrophic factor (NTF) signaling that could contribute to neuronal degeneration. Reduced NTF expression by peripheral targets or central nervous system target neurons, dysfunctional proneurotrophin processing, diminished receptor expression by affected neuronal populations, or decreased anterograde and retrograde transport of NTFs could impair NTF support and further aggravate neuronal dysfunction.

NGF is expressed in neocortex and hippocampus, and normally retrogradely transported to cholinergic cell bodies in the nucleus basalis and medial septum, respectively. In AD, several studies have indicated that cortical NGF levels cortex are stable (4, 114) or increased (33, 59, 133), whereas NGF levels in the nucleus basalis are decreased, providing evidence for a deficit in retrograde NGF transport from cortical targets back to the basal forebrain. Further support for this hypothesis comes form mouse models of Down's syndrome, which demonstrate a marked age-related atrophy of BFCNs, one that can be reversed by NGF administration (62). The degeneration of cholinergic neurons is strongly associated with a highly diminished retrograde transport of NGF in these animals (30), which can be directly linked to the presence of an extra copy of amyloid precursor protein (APP) (132). Diminished retrograde transport of NGF has also been shown in aged rodents (29), one correlational animal model for the cholinergic atrophy observed in AD. Furthermore, decreases in NGF receptor expression by BFCNs precede cholinergic neuronal loss in AD [reviewed in (31)]. Thus, early decreases in receptor expression could result in diminished retrograde NGF transport from the cortex to the nucleus basalis (29, 30, 113) and contribute to neuronal degeneration. Current strategies aimed at augmenting cholinergic function by NGF gene therapy in AD therefore provide NGF directly to cholinergic cell bodies, bypassing the need for long-distance retrograde transport. One potential concern is that NGF delivery to the cell soma instead of the normal target (cortex) could result in the withdrawal of cortical cholinergic projections. However, data in aged primates indicate that NGF delivery at the cell soma in the basal forebrain increases cholinergic innervation density in the cortex (28).

In HD, recent studies point toward a role of mutant huntingtin in disrupting BDNF expression and transport. Reduced BDNF levels have been found in brain regions most affected in HD patients (caudate and putamen) (48) and in transgenic mice that express human mutant huntingtin (164). Two possible mechanisms underlying these changes have been suggested [reviewed in (105)]. Reduced BDNF gene transcription (164) caused by differential interaction of mutant huntingtin (containing expanded CAG repeats) with the transcription machinery (165) has been reported as one possible explanation. However, it is also possible that mutant huntingtin interferes with the anterograde transport of BDNF from cortex to striatum, contributing to BDNF depletion in the striatum (55). Supporting the hypothesis that defects in BDNF availability in the striatum could contribute to neuronal degeneration also comes from studies in conditional BDNF knockout animals which have shown that depletion of cortical BDNF results in neuronal atrophy of medium spiny neurons, followed by neu-

ronal loss with aging (11). In addition, decreases in BDNF advance the onset of motor dysfunction and neuronal degeneration in a mouse model of HD (25). Taken together, these studies provide a rationale to deliver BDNF into caudate and putamen to prevent or slow the degeneration of medium spiny neurons in HD, but additional animal studies are needed.

Proneurotrophins may also play a role in neurodegeneration. As discussed in more detail in the previous review (150), proneurotrophins appear to have antagonistic functions compared with the mature form of neurotrophins. The significance of proneurotrophins in neurodegenerative diseases remains to be determined. It appears that the majority of NGF found in cortex is pro-NGF and increased levels of pro-NGF have been found in AD (47). Whether this increase in pro-NGF is a result of decreased processing of pro-NGF, changes in pro-NGF transport or whether changes in expression underlie the increased levels is unknown. The possibility that proneurotrophins could induce cell death in neurodegenerative diseases following binding to $p75^{NTR}$ is a theoretical possibility that remains to be proven.

CONCLUSIONS

NTF delivery continues to be an attractive neuroprotective treatment strategy for neurodegenerative disorders. As outlined in this review, the means of targeted delivery is one key for the successful implementation of NTF therapy. Advances in viral vectors now allow for a localized, long-term delivery of NTFs, thereby avoiding adverse effects from the broad exposure of CNS, PNS and other organ systems. Clinical trials to be conducted over the next years will allow us to truly determine whether NTFs are efficacious in delaying or slowing neuronal degeneration, thereby affecting some of the associated cognitive, psychiatric and motor dysfunctions.

ACKNOWLEDGMENTS

Supported by the California Roman Reed SCI Research Fund, International Spinal Research Trust, Wings for Life and the NIH (NS46466; NS047101). Thanks to Ron Alfa for his help in designing Figure 1.

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