POTENTIAL USE OF NEUROTROPHIC AGENTS IN THE TREATMENT OF NEURODEGENERATIVE DISORDERS

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Abstract. The use of neurotrophic agents as therapy for neurodegenerative disorders was originally proposed several years ago (1). Since then, several lines of evidence have converged to support the consideration of neurotrophic factor therapy for clinical disorders. In particular, the cholinergic neuronal atrophy that occurs in Alzheimer's disease has been identified as a strong potential candidate for the first clinical trials of nerve growth factor (NGF) in a human neurodegenerative disorder. In the current communication, we review issues related to potential trials of NGF in Alzheimer's disease.

INTRODUCTION

Degeneration of specific populations of neurons is a feature of neurodegenerative disorders. For example, Parkinson's disease is associated predominantly with degeneration of dopaminergic and noradrenergic neurons of the brainstem (41), amyotrophic lateral sclerosis with degeneration of motor neurons of the anterior horn cell and central motor projections (31), and Huntington's disease with degeneration of neurons primarily in the caudate/putamen complex (23). Alzheimer's disease (AD) is associated with degeneration of multiple populations of neurons, including cholinergic, noradrenergic and serotonergic neurons, among others (for review see 4, 7). Cholinergic neuronal degeneration in AD is particu-
larly apparent in the region of the basal forebrain, and the degree of cholinergic degeneration in AD has been correlated with both the presence of pathological markers of AD in the brain (i.e., density of senile plaques in the brain) and with the severity of dementia that comprises the predominant clinical manifestation of this disease (4, 7, 29). The etiology of AD is unknown, and thus far attempts at treatment have met with little success. Mild improvement in some features of cognitive dysfunction have resulted from augmentation of cholinergic function in the brain (e.g., treatment with anticholinesterases and acetylcholine precursors (4, 7, 33, 34), but limiting side effects combined with marginal benefit have precluded routine treatment with these agents.

Cognitive dysfunction also occurs in animal models of cholinergic deficiency. For example, impairments on memory tasks occur after pharmacological blockade of the cholinergic system in the rat and monkey (4, 7, 34), and are partially reversed after restoration of cholinergic influence (e.g., by administration of anticholinesterases). Further, certain strains of aged rats (e.g., Fisher, Sprague-Dawley) demonstrate age-related degeneration of cholinergic basal forebrain neurons and deficits on behavioral mnemonic tasks (4, 7).

Recently it was demonstrated that basal forebrain cholinergic neurons in the adult are responsive to nerve growth factor (NGF) (11, 16, 20, 39). Following transection of the septohippocampal projection in the rat, 65% to 90% of cholinergic neurons of the medial septum and vertical limb of diagonal band undergo retrograde degeneration (2). This degeneration is entirely prevented by infusions of mouse submaxillary gland-derived NGF into the lateral ventricle (11, 16, 20, 39), leading to the hypothesis that NGF is required to sustain normal function of basal forebrain cholinergic neurons. Also, rats with age-related (as opposed to lesion-induced) cholinergic degeneration of the basal forebrain show improvement in both morphological and behavioral indices of the septohippocampal projection after NGF infusions (8).

The relationship between memory, cholinergic neurons, and NGF has led to the hypothesis that NGF may be of therapeutic benefit in AD (1). To further assess this possibility, several additional questions must be addressed.

**ARE NGF AND NGF RECEPTOR LEVELS AFFECTED IN THE BRAINS OF AD PATIENTS?**

Initial studies of NGF changes in AD patients are conflicting (13, 17, 26). While one study suggested no loss of NGF mRNA levels in brains of AD patients, two other reports stated that immunolabeling
for NGF receptor is diminished in the basal forebrain of AD patients (13, 17, 26). Studies of NGF receptor mRNA levels in AD patients also suggested diminished levels. Further studies are needed to sort out these differences. Theoretically, NGF protein levels and NGF receptor levels could be affected independently in AD, and deficiencies of either alone could lead to cholinergic neuron dysfunction. Indeed, even if alterations in NGF synthesis, receptor binding or signal transduction are lacking in AD, NGF therapy might still be beneficial by augmenting the function of remaining, intact (or mildly dysfunctional) cholinergic neurons. Augmentation of existing cholinergic circuits could, in turn, promote functional improvement.

ARE PRIMATE CHOLINERGIC NEURONS RESPONSIVE TO NGF?

We have conducted preliminary investigations into this question and have found that continuous intracerebroventricular delivery of NGF into the brains of primates that have undergone fornix lesions will prevent the retrograde degeneration of cholinergic neurons that otherwise occurs in control animals (35). Monkeys that undergo unilateral fornix lesions and infusions of artificial CSF into the ventricular system for a four-week period show retrograde degeneration of medial septal cholinergic neurons, with only $45 \pm 5\%$ of neurons visible immunocytochemically at a four-week timepoint. However, primates receiving 180 $\mu$g/ml of mouse submaxillary gland-derived NGF after unilateral fornix lesions show a persistence of $80 \pm 6\%$ of neurons. Thus, injured primate neurons are responsive to NGF. It remains to be determined whether an NGF molecule of higher sequence homology to human NGF will be more efficacious than mouse-driven NGF in primate model systems (work is currently in progress).

COULD NGF HAVE DELETERIOUS EFFECTS ON THE BRAIN?

Although NGF has been unequivocally shown to prevent retrograde degeneration of cholinergic neurons, NGF may also affect other neuronal systems in a manner that could be deleterious to overall cognitive function. For example, intracerebroventricular NGF infusions promote aberrant sympathetic neurite sprouting at the base of the brain (19). Also, NGF appears to upregulate the expression of $\beta$-amyloid precursor protein, a precursor to one of the pathological components of plaques in the brains of AD victims (24). $\beta$-amyloid precursor protein also has
trophic and trophic properties (38), and may promote aberrant neurite sprouting in the brain. By promoting expression of β-amyloid precursor protein, NGF infusions could exacerbate an already dysfunctional response in some neuronal systems. However, there exist several forms of the β-amyloid precursor protein, and it is not known which form it is that NGF promotes. One form of the β-amyloid precursor protein, APP751, contains a protease inhibitor domain that may promote aberrant neurite sprouting. Another form of the β-amyloid precursor protein, APP695, lacks the protease inhibitor domain and may not promote aberrant sprouting. Interestingly, the protease inhibitor-containing form of the β-amyloid precursor protein appears to be selectively increased in AD (27). If NGF differentially promotes expression of the form of β-amyloid precursor protein lacking the protease inhibitor, APP695, then it may actually prevent expression of a deleterious molecule in AD while promoting cholinergic neuron survival. Indeed, studies in rats suggest that NGF increases the ratio of APP695 to APP751 (18). Further studies in aged animal and non-human primate models are needed to clarify these issues.

MIGHT NGF HAVE UNDESIRABLE SYSTEMIC SIDE EFFECTS?

The degree to which NGF administered into the brain actually gains access to extracerebral structures is unknown. Since NGF has a variety of non-neurological effects including modulation of immune function and macrophage activation, it is possible that NGF may have untoward systemic effects. Adverse systemic effects of NGF have not been noted in rat or primate studies, however. In 1970, NGF was peripherally administered to three children with neuroblastomas in an attempt to induce differentiation of neuroblastomas into more benign tumors (21). Mouse submaxillary gland-derived NGF was injected intramuscularly in a saline solution. All three children experienced untoward effects with this peripheral administration including sweating, mild rise in blood pressure, and severe headaches in two of the three patients. These may have been direct toxic effects of the NGF, but more likely were related to antigenic impurities contaminating the NGF samples produced 20 years ago. It is also possible that use of an NGF molecule with higher sequence homology to human NGF than the mouse NGF used in the study might reduce adverse reactions. Thorough toxicity studies of currently available NGF, including escalating dose studies and peripheral injections of NGF, should be conducted in animal models. Future human trials, if performed, will provide further information.
IS A SOURCE OF HUMAN NGF AVAILABLE, AND IS IT AVAILABLE IN SUFFICIENT QUANTITIES FOR HUMAN CLINICAL TRIALS?

Human NGF has been sequenced (37). Gene manipulation techniques have resulted in the construction of a recombinant human NGF molecule that will soon be broadly available and is currently being tested in both in vitro and in vivo model systems (3, 5, 12). This should ultimately provide NGF in sufficient quantities for use in human trials. However, human NGF should be tested extensively in both rat and non-human primate models before human use to verify safety and biological activity.

CAN NGF BE DELIVERED INTO THE BRAIN FOR PROLONGED PERIODS?

Potential therapy of human neurodegenerative disorders may require chronic NGF delivery to the brain for several years. Chronic delivery systems would require stability of the NGF protein molecule for prolonged periods of time, or else ready access to an NGF reservoir that could be repeatedly replenished without trauma to the recipient or risk of infection. In addition, hardware to chronically deliver NGF to the brain would have to be developed, and would have to be tolerated by the patient. Clinical trials with intraventricular administration of cholinergic agonists or acetylcholinesterase inhibitors have been conducted for periods of several months without significant complications (14), but infusions for periods beyond months have not been done. On the other hand, chronic drainage cannulas that permit egress of CSF from the brain have functioned in humans for decades. Similarly, implantable cardiac pacemakers driven by nuclear power sources, and more recently chronically implanted subcutaneous pumps for the delivery of insulin, have functioned in humans for prolonged periods. Over the long-term, one might anticipate greater problems with chronic CNS cannulation devices than with chronic peripheral devices since the CNS possesses fewer defenses against inflammatory processes and is therefore more vulnerable to damage and infection.

Potential alternatives to chronic cannulation of the CNS to deliver trophic factors exist, including the use of implanted, sustained-release polymer matrices that could deliver diffusible substances for months (9). More recently, we demonstrated the ability of genetically modified cells to deliver NGF to the brain. Fibroblasts were genetically modified using a retroviral vector to produce NGF that could be readily detected in vitro. When grafted to the brain, these genetically modified NGF-producing cells prevented lesion-induced retrograde cholinergic neuron de-
generation (30). Viability of these grafts for up to three months has been shown, and we are currently working to extend this period. If successful, this approach would eliminate the need for any hardware to chronically deliver NGF to the brain. This approach could also be used to deliver other molecules that might be deficient in a variety of CNS diseases. For example, the gene for tyrosine hydroxylase (TH) has been inserted in fibroblasts and grafted to the brains of rats afflicted with a model of Parkinson's disease (40). These TH-secreting cells reduced rotational asymmetries in lesioned rats. Thus gene modification combined with intracerebral grafting offers potential for the chronic delivery of neuroactive compounds to the CNS.

WHAT DOSE OF NGF IS REQUIRED FOR SPARING OF CHOLINERGIC NEURONS?

Experimental studies in rats have not yet established an optimal NGF concentration for sparing of septal cholinergic neuron function after fimbria-fornix lesions, despite the relative ease with which such an experiment could be performed in this model. In previous studies we have found that using osmotic pump flow rates of 0.45 μl/h, concentrations of 50 μg NGF/ml will spare virtually 100% of medial septal cholinergic neurons from retrorgrade degeneration, while 25 μg NGF/ml will spare fewer neurons (12). Thus, pharmacological NGF doses in the range of 25-50 μg/ml are effective in the rat brain, but extrapolation to primate models cannot be confidently made given the differing sizes of the brain, differing rates of CSF volume and flow, and differing neuronal characteristics. Further studies addressing these issues are needed.

INTO WHICH PART OF THE BRAIN SHOULD NGF BE INFUSED?

Rat studies have employed infusions of NGF into the CSF-containing lateral ventricles. In rats, however, the object of study of most NGF infusions has been medial septal neurons, which populate a small and densely packed region of the basal forebrain that is anatomically close to the ventricular system. Cholinergic neurons in the AD brain that are affected by degeneration are situated over a broad expanse of the subcortical basal forebrain, and are not tightly situated in a well-defined anatomical region that is close to the ventricular system. Thus it may be somewhat misleading to project NGF effects on a tightly packed cholinergic nucleus to a more diffuse cholinergic nucleus. Some studies in the rat have addressed NGF effects in the nucleus basalis, a broader
anatomical region than the medial septum. After cortical lesions in rats, cholinergic neurons in the nucleus basalis undergo atrophy (28, 32). The benefit of NGF in preventing nucleus basalis neuron atrophy is less obvious than results observed in the medial septum after fimbria-fornix lesions (6, 15, 22). Features of these different neuronal populations other than anatomical location probably account in large part for this difference in NGF-responsiveness, but generalizations from one model to another should still take into account anatomical dissimilarities.

Several related issues may be important in determining where in the brain NGF should be infused. First, to what extent does the NGF molecule penetrate the ependymal lining of the intact ventricular system and diffuse into more distant parenchyma? The NGF molecule has a MW of 13,250 daltons, and its diffusion may be impeded by its size. Studies are needed that measure NGF levels in parenchymal regions located various distances from both the intact NGF-infused ventricular system and the lesioned NGF-infused ventricular system. Secondly, the required distance of NGF diffusion may vary between species as a function of brain size, with greater distances required in larger brains to reach a location of NGF deprivation. If NGF penetration into larger brains is poor over their greater distance, then intraparenchymal rather than intraventricular infusions of NGF may be required. If intraparenchymal NGF infusions are required, multiple infusions points might be necessary to reach the relatively diffuse population of human basal forebrain cholinergic neurons. A need for multiple infusion sites might be excessively cumbersome and prohibitive on a practical level. Ideally, a single infusion site of NGF should probably be first attempted in clinical trials, even if preliminary animal studies indicate marginal NGF diffusion distances.

An indirect, preliminary indication that NGF may adequately diffuse in larger mammalian brains has been described above: NGF administered to primate brains at a concentration of 180 μg/ml spared the majority of medial septal cholinergic neurons from retrograde degeneration after fornix lesions (35). However, it is possible in the primate model that severed axons may have had direct access to NGF molecules in the ventricular system through the cut fornix and NGF need not have diffused over long intraparenchymal distances. The same may be true of results obtained in rat fimbria-fornix lesion studies. Breakdowns in the ventricular epithelium occurring in animal lesion models are probably repaired over the four-week period following the fornix lesion, however. Further, NGF infusions in aged rat models have shown beneficial effects on medial septal cholinergic neurons in the absence of fimbria-fornix lesions or ependymal disruption (8), and NGF infusions in unlesioned adult rats have resulted in elevated choline acetyltransferase activity
in both septum and hippocampus (10). In AD, although there is continual neuronal degeneration, a large scale breakdown of the ventricular ependyma is not present. The possibility remains that axotomized neurons in the fornix of experimental animals are physically closer to the ventricular system and thus have better access to an intraventricular supply of NGF than would basal forebrain cholinergic neurons and their axons in the brains of patients with AD.

ARE THERE ALTERNATIVES TO CNS DELIVERY OF NGF?

Penetration of molecules beyond the blood brain barrier (BBB) depends on molecule size, lipid solubility, and the presence of specific transport mechanisms including active and passive transport or endocytosis. NGF administered to the periphery does not cross the blood brain barrier because of its size and lack of lipid solubility. However, the successful CNS administration of other molecules with poor BBB penetration has been achieved in some cases by the following procedures: (1) binding the molecule to a lipid carrier that permits penetration of the BBB without compromising the activity of the transported molecule, (2) peripheral administration of a BBB-diffusible precursor molecule that is converted into an active final molecule once past the BBB, or (3) peripheral co-administration of a second molecule that indirectly assists crossing of the first molecule into the CNS. An example of the latter two approaches is levodopa therapy for Parkinson's disease. In this case, the lipophilic precursor to dopamine, levodopa, is administered orally and absorbed in the GI tract where it enters the circulation and gains access to the CNS by diffusing across the BBB. However, levodopa is rapidly destroyed in the periphery by decarboxylases unless carbidopa, a peripheral decarboxylase inhibitor, is co-administered with the levodopa to impede its peripheral breakdown. It is possible that manipulations of the NGF molecule could be made in a similar way to effect CNS penetration and eliminate the need for direct CNS drug delivery. At the present time, however, success with this approach has not been reported.

WHAT SHOULD BE THE DURATION OF NGF THERAPY IF CLINICAL TRIALS IN AD ARE BEGUN?

It is possible that a relatively brief period (e.g., months) of NGF support may be sufficient to restore cholinergic function for a prolonged period in the brains of AD patients. Alternatively, continual NGF infusions may be required in a manner analogous to chronic requirements for levodopa therapy in Parkinsonian patients or antiepileptic therapy
in patients with seizures. Existing data suggest that prolonged prevention of cholinergic cell degeneration is not achieved by transient NGF infusion after fimbria-fornix lesions (25, 36). The duration of NGF therapy in initial clinical studies should be guided by assessment of clinical responsiveness. If patients improve or if worsening is delayed after a relatively short treatment period, crossover periods of no drug treatment can be used to assess whether deterioration occurs in the absence of NGF. If no improvement is seen early during the course of NGF infusion, infusions should probably be continued for at least one year barring significant experimentally-induced complications.

An additional question to consider is the point in the patient's course of AD that therapeutic trials of NGF should be initiated. Patients afflicted early in the course of AD may be more responsive to therapy aimed at preventing further cholinergic degeneration than patients afflicted later in the course of the disease. However, it may be difficult to ethically justify initial experimental trials in individuals still possessing a relatively intact quality of life.

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