Growth factor gene therapy for Alzheimer disease

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The capacity to prevent neuronal degeneration and death during the course of progressive neurological disorders such as Alzheimer disease (AD) would represent a significant advance in therapy. Nervous system growth factors are families of naturally produced proteins that, in animal models, exhibit extensive potency in preventing neuronal death due to a variety of causes, reversing age-related atrophy of neurons, and ameliorating functional deficits. The main challenge in translating growth factor therapy to the clinic has been delivery of growth factors to the brain in sufficient concentrations to influence neuronal function. One means of achieving growth factor delivery to the central nervous system in a highly targeted, effective manner may be gene therapy. In this article the authors summarize the development and implementation of nerve growth factor gene delivery as a potential means of reducing cell loss in AD.

**KEY WORDS** • Alzheimer disease • basal forebrain cholinergic system • gene therapy • nerve growth factor

Alzheimer disease is the most common neurodegenerative disorder, afflicting approximately 4 million people in the United States. This number will exceed 14 million by the year 2050. At a current cost of $100 billion per year, AD is an imminent epidemic that threatens to severely strain the healthcare system unless effective therapies are developed. Alzheimer disease accounts for the majority of dementia cases, with chief features of memory loss and dysfunction of at least one other higher cortical function. In the late stage of the disease, profound loss of memory and executive function occurs, rendering individuals incapable of self care. With disease progression, there is profound degeneration of cortical neurons in frontal, parietal, and temporal neocortices but relative sparing of primary sensory and motor cortices.

Extensive degeneration of subcortical neuronal populations also occurs in AD, including cholinergic, serotonergic, and noradrenergic systems. By the midstages of AD, for example, significant degeneration of the subcortical BFC system has occurred, a cell population that includes the nucleus basalis of Meynert. This degeneration of the cholinergic system, which projects extensively throughout the entire neocortex and hippocampus, leads to the loss of acetylcholine, which in turn is thought to exacerbate significantly the “cortical” dementia in AD. Cholinergic systems modulate activity in target hippocampal and cortical regions, a mechanism that is thought to be responsible for the impairment in cognition when these cholinergic inputs are lost. Indeed, cholinergic neuronal degeneration has been shown to correlate with clinical severity, density of amyloid plaques, and the loss of synapses in AD. Thus, therapies targeted at ameliorating or preventing dysfunction of cholinergic neurons could affect cognitive decline in AD. To date, the only therapeutic agents approved by the Federal Drug Administration for the treatment of AD are the AChE inhibitors, which augment acetylcholine levels in the brain. Cholinesterase inhibitors, however, exert only modest effects on symptoms of AD. It is thought that this occurs because the degeneration of the BFC system continues unabated during treatment with AChE inhibitors, eventually leading to inadequate cholinergic compensation. Further, the doses of orally administered cholinesterase inhibitors that can be administered are limited because of the development of peripheral side effects. Hence, the therapeutic potential of fully augmenting cholinergic function in AD cannot entirely be tested with currently available oral cholinesterase medications.

Novel therapies that target neuronal loss in AD should not only aim to augment neuronal function but ideally
should also prevent cell loss. Nervous system growth factors, or NTFs, long known to promote survival and function of distinct neuronal populations during nervous system development, are a class of molecules that might achieve these objectives in neurodegenerative disorders. To date, more than 100 NTFs have been identified in the CNS. Most can be grouped into distinct “families” of related growth factors based on common structures and signaling mechanisms. The first NTFs to be discovered, and the most thoroughly studied to date, is the “classic neurotrophin” family consisting of NGF, brain-derived NTF, neurotrophin-3, and neurotrophin-4/5. Nerve growth factor is the prototypical member of the classic neurotrophin family, and is a specific survival factor for BFC neurons. It is produced throughout life by neurons in the hippocampus and neocortex, secreted extracellularly, and then bound by specific receptors on cholinergic axon terminals in target regions. It is then retrogradely transported to the cholinergic cell body. Nerve growth factor also acts locally on the axon terminal via NGF receptor–mediated tyrosine kinase signaling pathways. Collectively through these receptor mechanisms, NGF modulates the function of BFC neurons during development and throughout adult life in a manner that is as yet incompletely understood. Notably, levels of NGF protein are diminished in BFC neurons in the brains of patients with AD, although its production by the cortex and hippocampus is not altered, suggesting that deficient NGF transport to cholinergic neurons may cause or contribute to the loss of this cell population. The finding in animal models that NGF delivery to the adult brain can ameliorate cholinergic cell loss and reverse cholinergic atrophy with aging has led to a focus on NGF delivery as a potential therapy for AD. For reasons to be presented, however, safe NGF delivery requires specific targeting to the BFC system. Gene therapy is one means of achieving localized, targeted, and restricted delivery of NGF into the brain and is the subject of a current Phase I clinical trial in AD.

NERVE GROWTH FACTOR EFFECTS IN THE ADULT BRAIN

Nerve growth factor was serendipitously discovered in 1951 by Levi-Montalcini and Hamburger in the course of in vitro experiments in which they examined properties of a sarcoma cell line. Subsequently NGF was discovered to be an essential survival factor for peripheral sensory and sympathetic neurons during development. Nerve growth factor was purified and characterized in the early 1970s, and by the mid-1980s it was shown to be normally produced during adulthood by neurons in both the hippocampus and neocortex. This discovery raised the possibility that NGF continued to play a role in the CNS into maturity. Indeed, Hefti reported that injections of NGF into the adult animal CNS prevented the degeneration and death of BFC neurons after axotomy. In the following year, NGF was also shown to reverse atrophy of BFC neurons and ameliorate deficits in learning and memory in aged rats. These findings were extended to the nonhuman primate brain, where NGF infusions were also shown to rescue lesion-induced cholinergic degeneration. Human NGF was cloned, and infusions of human NGF were reported to prevent degeneration of BFC neurons in primates.

Thus, by 1991 substantial evidence derived from rodent and primate models suggested that NGF delivery might be a logical means of attempting to ameliorate cholinergic cell loss in AD. Indeed, ICV infusion of NGF was attempted in three AD patients in Sweden. Whereas Mini-Mental Status Examination scores (a simple measure of cognitive function) did not improve after NGF infusions, transient increases in scores were seen on some episodic memory tests and there was some evidence of improved physiological parameters of brain function, including nicotine binding, cortical blood flow, and electroencephalographic activity. The trial, however, was discontinued after the onset of debilitating side effects due to NGF infusion. In particular, back pain developed in patients within 2 to 14 days after the onset of ICV NGF infusions. Pain was hypothesized to result from NGF diffusing throughout the cerebrospinal fluid and reaching nociceptive NGF-responsive cell bodies in the dorsal horns and dorsal root ganglia. In addition, weight loss occurred, likely due to NGF-mediated activation of satiety centers in the hypothalamus. Notably, the authors of previous animal studies reported that whereas ICV NGF infusions successfully rescued degenerating cholinergic neurons, these infusions also induced weight loss, sympathetic axon sprouting around cerebral vasculature, and migration and expansion of Schwann cells into a thick cellular layer surrounding the medulla and spinal cord.

These findings prohibited the initiation of further clinical trials involving ICV infusions of NGF. Thus, whereas NGF treatment offered substantial potential as a means of reducing cholinergic cell loss in AD, an alternative method of delivery was required to deliver sufficient NGF doses to basal forebrain regions while restricting NGF spread to other sensitive neuronal populations of the hypothalamus, brainstem, and spinal cord.

GENE DELIVERY OF NGF

One approach to the specific delivery of NGF to cholinergic neurons of the basal forebrain is gene therapy. In general, there are two forms of gene therapy: ex vivo and in vivo gene therapy. In ex vivo gene therapy, cells are genetically modified in vitro to express a gene of interest, and are then implanted into the brain where they in effect act as biological micropumps for secretion of the desired protein. In in vivo gene therapy, a gene delivery vector is injected into the brain to modify directly host brain cells to increase NGF production. In early studies investigators focused on ex vivo gene delivery because the techniques for this form of gene delivery were developed first. More recently investigators have focused largely on in vivo gene delivery because more efficient gene delivery systems for in vivo gene therapy have been developed. Ex vivo and in vivo gene therapy have distinct individual advantages and disadvantages. The advantages of in vivo gene therapy include the following. 1) Simplicity: gene delivery is accomplished by the single step of direct vector injection into the desired target organ, as opposed to the considerable cell processing necessary to perform ex vivo gene therapy. Deactivated adenovirus, adenoassas-
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associated virus, herpes virus, and lentivirus have been used successfully to deliver genes of interest in experimental animal models.23,42 2) Minimal invasiveness: injections of in vivo vectors deliver several microliters of vector particles in an injection fluid solution, a procedure that is simple and safe. 3) Repeatability: the same location can be injected more than once using in vivo gene delivery approaches. There are also, however, relative potential disadvantages of in vivo gene therapy including the following. 1) Nonspecificity of target cell infection: many different cell types can be infected when in vivo vectors are injected in the CNS, including neurons, glia, and vascular cells. 2) Toxicity: some in vivo vectors are toxic to host cells (for example, herpes virus and rabies virus) and elicit immune responses (such as adenovirus).23 Lentiviral and adenoassociated viral vector systems have not shown adverse effects, and newer-generation herpes virus and "gutless" adenoviruses without deleterious properties are being developed.

The relative advantages of ex vivo gene delivery include the following. 1) It has the ability to target selectively specific cell types for production of the gene of interest before engrafting of cells into the host brain. 2) Immunocompatibility: host cells are obtained via biopsy sampling, grown in vitro, genetically modified, and then implanted into the same host. Thus, no foreign cells are introduced, eliminating any need for immunosuppression. 3) Safety: because infectious virus particles are not made by genetically modified host cells in vitro, there is little risk of inadvertently introducing wild-type virus into a host with ex vivo gene therapy, and little risk of recombination of the vector with wild-type viruses that may exist in the host body. The potential disadvantages of ex vivo gene delivery include the following. 1) To be maintained and genetically modified in vitro, host cells must be capable of dividing, thus certain postmitotic cell populations such as neurons cannot be targets of transduction by ex vivo gene therapy. Current ex vivo gene therapy approaches target primary fibroblasts, stem cells, tumor cells, Schwann cells, or endothelial cells. 2) Invasiveness: grafting of cells is an intrinsically more invasive process than injection of suspensions of in vivo gene therapy vectors. 3) Although tumor formation has not been observed with more than 200 grafts of primary fibroblasts into the primate CNS, delivery of dividing cells bears the risk of tumor formation. Tumors have been observed when grafting immortalized cell lines; however, more recent derived conditionally immortalized cell lines do not form tumors when grafted.

NERVE GROWTH FACTOR GENE THERAPY FOR AD

Ex vivo gene delivery techniques were better refined than in vivo gene therapy vectors at the time that we began NGF gene therapy experiments in animal models several years ago. Thus, our current clinical trial of ex vivo NGF gene therapy for AD is based on an ex vivo gene delivery approach, established after several years of efficacy and safety studies in rodents and nonhuman primates. In vivo gene therapy, however, will likely become commonly performed in the near future in clinical trials of gene delivery for a variety of nervous system diseases.

In rodent and then primate experiments, ex vivo NGF gene delivery has been shown to prevent cholinergic neuronal degeneration after lesions in the adult brain. Cholinergic neurons were induced to degenerate by transecting their projections from the septal nucleus to the hippocampus. After transections of cholinergic axonal projections to the hippocampus, 60 to 90% of cholinergic neurons undergo atrophy and eventually die. Ex vivo NGF gene delivery, however, was shown to rescue most cholinergic neurons in both rodent and primate brains.13,26,35,40

In a second set of experiments, the ability of ex vivo NGF gene therapy to prevent age-related neuronal atrophy was studied. Aged rats and, in separate experiments, aged monkeys received grafts of NGF-secreting fibroblasts to another component of the BFC referred to as the basal nucleus of Meynert, or Ch4 region. In aged rats, ex vivo NGF gene therapy reversed age-related cholinergic neuronal atrophy and improved spatial memory function.7 Subsequent experiments in aged primates also demonstrated that ex vivo gene therapy reversed spontaneous atrophy of BFC neurons,38 and reversed cholinergic terminal degeneration in widespread cortical areas.8 Indeed, as a normal function of aging in primates there is atrophy (but not death) of 40% of BFC neurons, and ex vivo NGF gene delivery restored the number of healthy cholinergic neurons to values within 7% of those observed in young monkeys.38 Aging in the primate brain is also associated with an approximate 25% reduction in cholinergic axon density in the cortex; ex vivo NGF delivery completely restored cholinergic axon density in the aged monkey brain to values observed in young monkeys. Thus ex vivo NGF delivery effectively prevented and reversed cholinergic neuronal degeneration in the animal brain and did so without eliciting adverse effects. Further, genetically modified fibroblasts grafted into the brain sustained NGF gene expression for at least 1 year in primates, the longest time point that we examined. Effects of ex vivo NGF gene therapy on cognitive decline with aging in primates are being examined.

Cholinergic neuronal degeneration is an integral component of generalized cell loss in the brains of individuals with AD, and loss of cholinergic function correlates strongly in AD with dementia severity and synapse loss.28,39 Indeed, to date in the only effective treatments for AD cholinergic systems are targeted.11,30 The studies summarized in the preceding paragraphs indicate that ex vivo gene therapy offers the potential, for the first time, to prevent ongoing cholinergic neuronal degeneration in AD. Before proceeding with clinical trials, however, we sought to determine whether ex vivo NGF gene therapy was safe. Thus, in a dose-escalation design, adult rhesus monkeys received steadily increasing volumes of autologous, NGF-secreting fibroblasts. Over a 20-fold dose range, no adverse effects of ex vivo NGF gene delivery were observed in primates.

Based on this extensive set of efficacy and safety data in primates, we proposed a Phase I clinical safety trial of ex vivo NGF gene delivery in humans, which involved a dose-escalation design in eight patients with AD. Serial safety parameters are being monitored in the individuals in this trial, as are neuropsychological measures. Overall, there are two objectives of this clinical program of ex vivo NGF gene therapy in AD after the Phase I safety testing.
has been completed: 1) to determine whether NGF will significantly reduce the rate of cognitive decline in AD and 2) to determine whether NGF will augment the activity and function of remaining cholinergic neurons in the brains of individuals with AD. If the Phase I trial is successful, subsequent Phase II and III trials are planned. This clinical program will eventually address two key questions that cannot be answered in animal models. First, will NGF prevent cholinergic neuron loss in AD, given that the precise cause of cell loss in the AD brain is unknown? Second, will preventing or delaying degeneration of the cholinergic system in AD be sufficient to ameliorate cognitive decline?

REGULATING GENE EXPRESSION

An important need in gene therapy applications is the capacity to regulate gene expression. The ability to increase the level of gene expression with progression of the disease or, conversely, to downregulate gene expression in the event of adverse effects is a highly desirable feature of gene therapy approaches. Thus, exogenous control over the expression of transferred genes might improve the safety and efficacy of gene therapy.

Several different systems have been developed to control the expression of genes in viral vectors, including tetracycline regulatable systems. In the originally described vector system, administration of tetracycline (or its analog, doxycycline) reduces expression of the gene of interest. Such a system is preferential in circumstances in which prolonged gene expression is likely to be necessary in disorders such as neurodegenerative disease. Tetracycline would only be administered if the gene expression needed to be downregulated by using the so-called “tet-off” system.

Other investigators and our group have adapted the tet-off system and incorporated all the elements necessary for regulated gene expression into a single retroviral vector. In vitro experiments, we have shown that the expression of NGF can be regulated by doxycycline. Amounts of doxycycline as low as 1 ng/ml can repress gene expression nearly completely within 24 hours. Gene expression can also be regulated in grafts of genetically engineered fibroblasts in vivo. Expression of the reporter gene green fluorescent protein under the control of the tetracycline regulatable promoter can be turned on and off, depending on the administration of doxycycline in the animals’ drinking water for up to 3 months in vivo, the longest time point examined. More importantly, NGF-dependent neuronal rescue and axonal sprouting can also be controlled in vivo. Significant rescue of cholinergic neurons after development of fimbria–fornix lesions was only evident in animals with NGF–expressing grafts that received no doxycycline in drinking water (that is, gene expression was turned “on”). Animals that received doxycycline (NGF expression was therefore turned “off”) failed to show cholinergic neuronal protection after lesioning. Furthermore, significant growth of cholinergic axons into NGF-secreting fibroblast grafts in the brain was present only in NGF-grafted animals that did not receive doxycycline. Animals that received control grafts and those treated with doxycycline and grafts of cells with tetracycline-regulated NGF vectors showed very sparse axonal growth. Thus, we were able to control completely the biological effects of NGF gene expression in the brain by using a regulatable vector system.

More studies are required to allow investigation of whether long-term regulation of gene expression can be maintained as well as to improve the available regulatable systems. The studies conducted to date, however, provide the basis for the future application of controlled gene delivery in the CNS to enhance the safety and effectiveness of neurological gene therapy.

OTHER GENE DELIVERY STUDIES

As noted previously, multiple neuronal populations degenerate in AD, and NGF therapy for basal cholinergic neurons may or may not be sufficient to reduce progression of the disorder. In future approaches to AD investigators may target other neuronal populations with other growth factors. The concept of growth factor gene delivery may also be relevant to the treatment of other neurodegenerative disorders including Parkinson disease, Huntington disease, and amyotrophic lateral sclerosis.

References


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