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Therapeutic gene transfer to the nervous system using viral vectors

Joseph C Glorioso,[2] Marina Mata,[1,3] and David J Fink[1,2,3]

Departments of [1]Neurology and [2]Molecular Genetics and Biochemistry, University of Pittsburgh, Pittsburgh, Pennsylvania, USA; and [3]GRECC, VA Pittsburgh Healthcare System, Pittsburgh, Pennsylvania, USA

The past few years have been marked by substantial progress in preclinical studies of therapeutic gene transfer for neurologic disease using viral-based vectors. In this article, the authors review the data regarding (1) treatment of focal neuronal degeneration, exemplified by Parkinson disease, ischemia, and trauma models; (2) treatment of global neurologic dysfunction, exemplified by the mucopolysaccharidoses and other storage diseases; (3) peripheral nervous system diseases including motor neuron disease and sensory neuropathies; and (4) the use of vectors expressing neurotransmitters to modulate functional neural activity in the treatment of pain. The results suggest that a number of different viral vectors may be appropriate for gene transfer to the central nervous system for specific disease processes, and that for the peripheral nervous system herpes simplex virus–based vectors appear to have special utility. The results of the first human gene therapy trials for neurologic disease, which are just now beginning, will be crucial in defining the next step in the development of this therapy. *Journal of NeuroVirology* (2003) **9,** 165–172.

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Introduction

It has been 30 years since gene therapy was first formally proposed as a treatment for genetically determined inherited disorders (Friedmann and Roblin, 1972). Despite the setback caused by the well-publicized death of one patient in a gene therapy trial in 1999 (Carmen, 2001), the first successful human gene therapy, for X-linked severe combined immunodeficiency in children, has been reported (Cavazzana-Calvo *et al*, 2000). In recent years, several proposed human gene therapy protocols for neurologic disease have been reviewed by the recombinant DNA advisory committee (RAC) of the National Institutes of Health (NIH) and a number of these are now in clinical trial. It is thus an apt time to consider the progress of gene therapy for neurologic disease, and the prospects for future advances in the field.

There are several reasons that therapeutic gene transfer or "gene therapy" might be particularly appropriate for treating conditions affecting the nervous system. More unique RNA sequences are expressed in brain than in any other tissue and a large proportion of the identified genetic diseases display a neurologic component to the phenotype. The blood-brain barrier limits the penetration of systemically administered macromolecules into brain, and macromolecules injected directly into the ventricles penetrate only a short distance into brain parenchyma. In many cases, the regional specialization of brain function dictates that a therapeutic intervention may be best achieved by the local expression of a transgene product such as a neurotrophic or antiapoptotic factor. In addition, the widespread and redundant use of a limited repertoire of neurotransmitters and receptors in diverse pathways in the nervous system means that the local production of neurotransmitters achieved by therapeutic gene transfer may be used to achieve desired outcomes while avoiding unwanted adverse side effects that would result from activation of the same receptors in other pathways by a

Address correspondence to David J. Fink, MD, S-520 BST, 200 Lothrop Street, Pittsburgh, PA 15213, USA. E-mail: dfink@pitt.edu

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systemically administered drug. Nonviral means of gene transfer, such as liposomes, have generally proven ineffective for gene transfer to the nervous system. On the other hand, a number viral-based vectors, including those based on viruses such as lentivirus (LV) or herpes simplex virus (HSV) that naturally infect the nervous system, or developed from viruses like adenovirus (Ad) or adenoassociated virus (AAV) that are not naturally neurotropic, have proven effective in different model systems.

In this review, we summarize the published data to date regarding therapeutic gene transfer using viral vectors in animal models of neurologic disease, and describe several human trials of therapeutic gene transfer for neurologic disease that have been approved by regulatory agencies, some of which are now enrolling patients. The review is focused on preclinical studies in animal models of neurologic disease, and their translation to human therapy. Progress in four different specific applications relevant to neurologic disease will be reviewed: (1) treatment of focal neuronal degeneration, exemplified by Parkinson disease, ischemia, and trauma models; (2) treatment of global neurologic dysfunction, exemplified by the mucopolysaccharidoses and other storage diseases; (3) peripheral nervous system diseases including motor neuron disease and sensory neuropathies; and (4) the use of vectors expressing neurotransmitters to modulate functional neural activity in the treatment of pain. The use of gene transfer to modify cells that are subsuquently implanted into brain or spinal cord (Blesch *et al*, 2002; Tuszynski, 1997), and the reports regarding the use of gene transfer in the treatment of glioblastoma, either by direct cell killing, immunologic effects, or suicide gene therapy (Andratschke *et al*, 2001; Markert *et al*, 2001), will not be considered in this review. The basic biology of the principal vectors that are used in these applications has been reviewed elsewhere (Kennedy, 1997).

Treatment of focal neurodegeneration: Parkinson disease, stroke, and trauma

Focal neurodegeneration would appear to be an ideal target for therapeutic gene transfer. Despite the fact that the pathogenic mechanisms underlying progressive cell death in neurodegenerative disease are incompletely understood, several peptides that act either as trophic factors or to interrupt the apoptotic cascade intracellularly have been identified. It is unlikely that such potent substances delivered either systemically or intrathecally would not cause serious adverse effects (Apfel, 2001). Because gene transfer offers the possibility of local production of such factors to prevent neurodegeneration, a number of investigators have focused on this possibility. Idiopathic Parkinson disease (PD), a condition characterized by degeneration of dopaminergic (DA) neurons in the substantia nigra (SN), has the advantage of a very restricted anatomic target (the SN) and well-characterized animal models. The first studies of gene transfer in PD, employing the model of 6-hydroxydopamine (6-OHDA)–induced degeneration of DA cells in the SN, demonstrated that intrastriatal injection of an Ad vector expressing the glial cell–derived neurotrophic factor (GDNF) prevented the degeneration of DA neurons, resulting in both histologic and behavioral correction of the disease phenotype (Bilang-Bleuel *et al*, 1997). Subsequent studies have confirmed these results using AAV-based vectors (Mandel *et al*, 1997, 1999), other Ad vectors (Choi-Lundberg *et al*, 1998; Connor *et al*, 1999; Bjorklund *et al*, 2000), replication-defective HSV vectors (Yamada *et al*, 1999), and LV vectors (Bensadoun *et al*, 2000). Protection of DA neurons from 6-OHDA toxicity *in vivo* has also been reported in experiments in which the antiapoptotic peptide Bcl-2 was expressed using an HSV vector in the rat (Yamada *et al*, 1999). Both the LV (Kordower *et al*, 2000) and AAV (Bjorklund *et al*, 2000) experiments have been shown to protect DA neurons in primates. No human trials to prevent cell death in PD based on the preclinical data generated have been proposed to date.

An alternate gene transfer approach to the treatment of PD utilizes gene transfer designed to enhance neurotransmitter production in the striatal circuitry damaged in PD. The most obvious candidate is tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine synthesis. Injection of an AAV vector expressing TH into striatum was first demonstrated to reverse one behavioral abnormality in the 6-OHDA model of PD (Kaplitt *et al*, 1994), and similar results were obtained with an HSV-based amplicon vector expressing TH (During *et al*, 1994). However the size of the human striatum, the likely requirement that dopamine production will need be closely regulated to avoid adverse effects, combined with the complexity and variability of PD symptomatology, make this type of therapy problematic. Modulation of neurotransmitter effect can be achieved by enhancing prodrug conversion. It has been demonstrated that transfer of the gene coding for the aromatic acid decarboxylase (AADC) enhances the conversion of DOPA, administered systemically, to dopamine (Sanchez-Pernaute *et al*, 2001). The first human PD gene transfer trial, on the other hand, has proposed to transfer the gene coding for glutamic acid decarboxylase (GAD) in order to increase γ-aminobutyric acid (GABA) expression in the extrapyramidal pathway (During *et al*, 2001). In the phase I trial that has been proposed, the vector will be inoculated along with the placement of a deep brain stimulator into the subthalamic nucleus.

Therapeutic results of focal gene transfer has been demonstrated in models of ischemic brain injury in rodents using a variety of vectors. Expression of

interleukin-1 receptor antagonist from an Ad vector (Betz *et al*, 1995), Bcl-2 from an HSV amplicon vector (Lawrence *et al*, 1997) or from an AAV vector (Shimazaki *et al*, 2000), GDNF from an AAV vector (Tsai *et al*, 2000), and heat shock protein (HSP) 72 from an HSV amplicon (Hoehn *et al*, 2001) have all been shown to attenuate the amount of cell loss in a variety of models of transient and permanent ischemia. Although these "proof-of-principle" studies, demonstrate a biological activity of gene transfer, not all of the studies have been correlated with behavioral outcomes that would be required to support the clinical use, and in all of these studies, the vectors have been injected prior to the ischemic insult, which would severely limit the clinical situations for which such gene transfer would be applicable. Similar results have also been demonstrated in models of nervous system trauma. Injection of HSV vectors expressing Bcl-2 or GDNF up to 30 min after spinal root avulsion improves motor neuron survival and preserves expression of choline acetyltransferase in lesioned motor neurons (Natsume *et al*, 2002; Yamada *et al*, 2001). Intraspinal injection of a plasmid encoding Bcl-2 complexed in a lipsome immediately following spinal cord section has been demonstrated to protect neurons of Clark's nucleus and the red nucleus from injury-induced degeneration (Shibata *et al*, 2000; Takahashi *et al*, 1999), and intraspinal application of vascular endothelial growth factor (VEGF) using an Ad vector appears to ameliorate the effect of a corticospinal tract injury in rodents (Facchiano *et al*, 2002). Injection of an Ad vector expressing neurotrophin-3 (NT-3) into spinal cord after dorsal root injury enhanced the regeneration of a subpopulation of dorsal root axons (probably myelinated A fibers), into and through the CNS environment (Zhang *et al*, 1998). Injection of Ad vectors expressing fibroblast growth factor-2 (FGF2) or nerve growth factor (NGF) 16 days after dorsal root injury induced robust axonal regeneration into normal as well as ectopic locations within the dorsal spinal cord, resulting in near-normal recovery of thermal sensory function (Romero *et al*, 2001). Fewer unwanted adverse effects were seen with FGF2 than with NGF.

Correction of global brain disease: Mucopolysaccharidoses and other storage diseases

Gene transfer has also been applied to the treatment of diseases that affect the central nervous system globally. In these cases, the aim of gene transfer is a diffuse distribution of the corrective gene product throughout the nervous system. It was originally demonstrated that administration of a recombinant Ad vector expressing beta-glucuronidase directly into the lateral ventricles of mutant mice increased the beta-glucuronidase activity in crude brain homogenates to 30% of heterozygote activity. Histochemical demonstration of beta-glucuronidase activity in brain revealed that the enzymatic activity was found principally in ependymal cells and choroids plexus (Ohashi *et al*, 1997). An adenovirus vector expressing aspartylglucosaminidase (AGA) injected intraventricularly into the brain mice with aspartylglucosaminuria (AGU) resulted in AGA expression in the ependymal cells lining the ventricles and diffusion of AGA into the neighboring neurons. One month after administration of the wild-type Ad-AGA, a total correction of lysosomal storage in the liver and a partial correction in brain tissue surrounding the ventricles was observed (Peltola *et al*, 1998). Similar results have been demonstrated in the mucopolysaccharidosis (MPS) VII mouse injected with an Ad vector expressing beta-glucuronidase, with the distribution of enzyme activity and phenotypic correction increased by mannitol-induced disruption of the brain–cerebrospinal fluid (CSF) barrier (Ghodsi *et al*, 1999). Using the same models, others have shown that AAV vectors expressing beta-glucuronidase injected directly into brain parenchyma can result in phenotypic correction (Sferra *et al*, 2000; Skorupa *et al*, 1999). Wolfe and coworkers reported that the AAV vector not only produced the normal enzyme from infected cells at the injection sites, but that the secreted enzyme was also disseminated along most of the neuraxis, resulting in widespread reversal of the hallmark pathology. The extensive area of correction surrounding the transduction sites suggested that a limited number of appropriately spaced sites of gene transfer may provide overlapping spheres of enzyme diffusion to cover a large volume of brain tissue (Bosch *et al*, 2000a, 2000b; Skorupa *et al*, 1999). AAV-mediated correction has been reported to improve cognitive function in the murine model of MPS VII as measured by the Morris water maze test (Frisella *et al*, 2001). More recently, Davidson and coworkers have demonstrated that injection of a feline immunodeficiency virus (FIV)-based vector expressing beta-glucuronidase into striatum unilaterally resulted in bihemispheric correction of the characteristic cellular pathology and that treatment of beta-glucuronidase–deficient mice with established impairments in spatial learning and memory resulted in a dramatic recovery of behavioral function (Brooks *et al*, 2002).

In the mouse model of MPS IIIB resulting from a defect in alpha-*N*-acetylglucosaminidase (NaGlu), an NaGlu-expressing AAV vector injected into brain resulted in 6 months of expression of recombinant NaGlu (rNaGlu) in multiple brain regions of adult MPS IIIB mice. The vector transduced an area of 400 to 500 microns surrounding the infusion sites, but after 6 months, the correction of glycose aminoglycan storage involved neurons of a much larger area (Fu *et al*, 2002). In a mouse model of metachromatic leucodystrophy, Naldini and coworkers demonstrated that a lentiviral vector encoding a functional

arylsulfatase A (ARSA) gene injected into the brain of adult mice with germ-line inactivation of the mouse gene encoding ARSA resulted in sustained expression of active enzyme throughout a large portion of the brain, with long-term protection from development of neuropathology and hippocampal-related learning impairments (Consiglio *et al*, 2001).

Correction of phenotypic deficits in both histology and behavior in MPS mice using gene transfer has been impressive, and the reversal of established deficits (Brooks *et al*, 2002) represents an important clinical feature in consideration of the development of a practical treatment. Several features of this model should be kept in mind. The relevant gene product is taken up by cells throughout the brain by binding to mannose-6-phosphate receptors. Thus, global correction of these diseases can be achieved by transduction of a fraction of cells within the brain as long as the gene product released from the cells is adequately distributed through the brain. In other models using enzyme replacement, it has been noted that replacement of as little as 10% of the normal enzyme activity may be sufficient to correct the phenotype. Regarding the application to human disease, issues of volume of distribution need to be explored. Even though correction of an animal model has not yet been demonstrated, a human trial of gene transfer to treat Canavan disease using liposomes to transfer aspartoacylase has been reported (Leone *et al*, 2000), and the same group has now begun a similar study in children using an AAV vector.

Diseases of the peripheral nervous system: Polyneuropathy and motor neuron disease

The peripheral nervous system presents a number of challenges that are distinct from the central nervous system, but the underlying rationale for the use of gene therapy is similar. Studies with recombinant peptides have demonstrated that a number of neurotrophic factors, including NGF, NT-3, insulin-like growth factor (IGF), and vascular epithelial growth factor (VEGF) can prevent the degeneration of peripheral sensory axons that results in polyneuropathy (Apfel, 1999). But these potent short-lived peptides cannot be administered to patients in the same doses that are effective in the animal models because of unwanted adverse systemic effects (Apfel, 2002). One approach to this problem is to selectively transduce dorsal root ganglion neurons to express a neurotrophic factor in order to achieve local (autocrine or paracrine) protective effect while avoiding systemic side effects. In this regard, HSV-based vectors are particularly well suited because of the natural tropism of the wild-type virus that affords efficient uptake into dorsal root ganglion (DRG) neurons from peripheral inoculation of the vector (Mata *et al*, 2001).

Using transduction of DRG neurons by peripheral inoculation of an HSV vector, we have demonstrated a protective effect against the development of neuropathy in three different models of polyneuropathy. Selective large fiber nerve degeneration caused by overdose of pyridoxine (PDX) can be prevented by subcutaneous inoculation of an HSV-based vector containing the coding sequence for NT-3, measured by the amplitude and conduction velocity of the evoked sensory response, as well as preservation of H-wave amplitude (Chattopadhyay *et al*, 2002). Treated animals show preservation of a population of large myelinated fibers that otherwise degenerate in this condition, and the preservation of electrophysiologic and histologic parameters is reflected in behavioral testing of treated animals (Chattopadhyay *et al*, 2002). Inoculation of an HSV-based vector expressing NGF under the control of the human cytomgalovirus promoter (HCMV) prior to the start of PDX intoxication provides a similar protective effect (Chattopadhyay *et al*, 2003). Similarly, injection of an replication-incompetent HSV vector expressing NGF under the control of the HCMV promoter 2 weeks after the induction of diabetes (by injection of streptozotocin) prevents the development of neuropathy, measured by reduction in evoked sensory nerve amplitude, and also increases expression of neuropeptides in the DRG (Goss *et al*, 2002a). Similar results have been obtained in a model of drug-induced sensory neuropathy resulting from administration of cisplatin (Chattopadhyay *et al*, personal communication). Iatrogenic neuropathies caused by chemotherapy for cancer are models that may be tested in human disease. A similar protective effect has been observed by transfer of VEGF using a plasmid injected into muscle in models of ischemic and diabetic neuropathy (Schratzberger *et al*, 2000, 2001), although one must assume that the protective effect in those models results from circulating levels of VEGF achieved by muscle transduction and thus may not avoid the potential for systemic side effects.

Motor neuron disease is a serious and fatal affliction without currently effective treatment. Like polyneuropathies, administration of trophic factors appears to slow the progression of the disease in rodent models, but a human trial of ciliary neuronotrophic factor (CNTF) in motor neuron disease had to be abandoned because of the cytokine-like side effects of the systemically administered trophic factor (Apfel, 2002). An AAV-based vector expressing GDNF has been demonstrated to protect a motor neuron-like cell line from apoptotic cell death *in vitro* (Keir *et al*, 2001). After intramuscular injection of the NT-3 adenoviral vector, pmn mice (a model of motor neuron disease) showed a 50% increase in life span, reduced loss of motor axons, and improved neuromuscular function as assessed by electromyography. These results were further improved by coinjecting an adenoviral vector coding for CNTF (Haase *et al*, 1997). Administration of an adenoviral vector expressing cardiotrophin 1 (CT-1) to newborn pmn

mice led to sustained CT-1 expression in the injected muscles and bloodstream, prolonged survival of animals, and improved motor functions. CT-1–treated mice showed a significantly reduced degeneration of facial motor neurons and phrenic nerve myelinated axons. The terminal innervation of skeletal muscle, grossly disturbed in untreated pmn mice, was almost completely preserved in CT-1–treated pmn mice (Bordet *et al*, 1999). This approach relies on systemic release from injected muscle, and thus may not avoid the problems of systemic administration. Achieving adequate systemic levels from muscle transduction in larger animals may prove difficult. To date, no vectors have been created from viruses that would naturally target motor neurons in a manner similar to the targeting of DRG neurons by HSV-based vectors, and efforts to construct vectors that would target to motor neurons have to date been unsuccessful.

Gene transfer for the treatment of pain

In a manner analagous to the correction of PD by using gene transfer to achieve focal neurotransmitter release (transduction with a TH vector to produce DA, transduction with a GAD-expressing vector to produce GABA), several studies have demonstrated that gene transfer may be used to provide an analgesic effect in the treatment of pain. Opiate drugs are exceptionally potent analgesic agents, but the action of these drugs on central and peripheral opioid receptors resulting in nausea, sedation, respiratory suppression, and constipation or urinary retention, respectively, limit the dose that may be used. Continued use of opiate drugs in chronic pain leads to tolerance, and addiction is also a problem. Several different gene transfer approaches have been taken to the treatment of pain.

Iadarola and coworkers demonstrated that a recombinant Ad encoding a secreted form beta-endorphin injected intrathecally into lumbar CSF transduced meningeal cells, and that beta-endorphin secretion attenuated inflammatory hyperalgesia, without affecting basal nociceptive response (Finegold *et al*, 1999). HSV-mediated gene transfer to deliver and express opioid peptides to be released from primary afferent terminals may be used to alter the physiology of postsynaptic neurons, affecting nociceptive transmission in the spinal dorsal horn. An HSV vector containing the human proenkephalin gene injected subcutaneously in the foot produces an antihyperalgesic effect in rodents (Wilson *et al*, 1999), and a 50% reduction in the spontaneous pain behavior during the delayed phase of the formalin test of inflammatory pain (Goss *et al*, 2001). The naltrexone-reversible analgesic effect in inflammatory pain is maximal 1 week after vector inoculation, and can be reestablished by reinoculation of the vector after the initial effect has waned (Goss *et al*, 2001). In the spinal nerve ligation (SNL) model of neuropathic pain, injection of the vector 1 week after SNL produced a naloxone-reversible antiallodynic effect that was continuous, persisted for several weeks, and could also be reestablished by reinoculation of the vector after the original effect had waned. In the neuropathic pain model, vector-mediated enkephalin expression enhances the effect of morphine, reducing the ED_{50} of morphine from 1.8 mg/kg to 0.15 mg/kg, and the vector continues to provide an antiallodynic effect in the face of tolerance to morphine induced by repeated injection of the drug (Hao, personal communication). A similar analgesic effect for HSV-mediated expression of proenkephalin has been demonstrated in a model of polyarthritis (Braz *et al*, 2001), and in a rodent model of pain caused by cancer in bone (Goss *et al*, 2002b). We have presented a proposal for a phase I human trial of the proenkephalin-expressing vector in the treatment of pain resulting from cancer metastatic to bone to the RAC in June, 2002.

Summary and conclusion

In the last 5 years, substantial progress has been made in moving gene transfer for neurologic disease from a hypothetical possibility to a real treatment. The data considered in this review suggest that a number of different vectors (Ad, AAV, LV, HSV) may be used for focal gene transfer to the central nervous system. The choice among these vectors will ultimately be decided by the results of the human trials, and practical aspects of manufacturing. For global distribution within the brain, it would appear that the smaller vectors (AAV and LV) may be advantageous, but the problem of delivering a gene product to the entire human brain from focal injections would appear to be daunting. For peripheral sensory nervous system applications, including the prevention of neuropathy and the treatment of pain, HSV, because of its natural tropism to sensory neurons, would appear to be the vector of choice. No vectors with similar tropism to motor neurons have yet been demonstrated.

As outlined in this review, potent therapeutic effects of gene transfer have now been demonstrated in several relevant models of different neurologic diseases. A human trial of gene transfer for Canavan disease (using liposomes and AAV vectors) is underway, and trials for Parkinson disease (using an AAV vector expressing GAD) and for the treatment of pain (using an HSV vector expressing proenkephalin) have passed through the RAC to the Food and Drug Administration (FDA). Although novel vectors that may extend the range of therapeutic options continue to be developed, the observations from the first human trials will be crucial in defining the next step in the development of this therapy.

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