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Strategies of Gene Therapy for Parkinson's Disease

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Parkinson's disease (PD) is a progressive neurodegenerative condition primarily involving the loss of dopaminergic neurons of the substantia nigra pars compacta (SNpc). This leads to a variety of symptoms and signs, including the primary motor deficits of tremor, bradykinesia, and rigidity, as well as other nonmotor problems such as cognitive, affective, and autonomic disturbances that may have pathology outside of this dopaminergic pathway degeneration. Of all neurological diseases, PD has several features that make it one of the most promising targets for clinical gene therapy. The internment of the major pathology to a compact localized neuronal population and the anatomy of the basal ganglia circuitry demonstrate that global gene transfer is not required, and there are well - defined sites for gene transfer. This review summarizes the studies of gene transfer using viral vectors and three separate gene transfer strategies currently being pursued for PD.

KEY WORDS: Parkinson's disease · Gene therapy · Dopamine.

INTRODUCTION

Parkinson's disease (PD) is one of the most-common neurodegenerative disorders, and is characterized by rigidity, tremor, akinesia and gait disorder. The pathoanatomical basis for the predominant motor symptoms constitutes the loss of catecholaminergic neurons, especially dopaminergic neurons of the substantia nigra pars compacta (SNpc), in the adult brain at a pace far more rapid than that of normal aging.[14] This cell death can take place over a period of 20 years or more, and the clinical symptoms of PD are not exhibited until a loss of - 80% of striatal dopamine has occurred, representing a loss of - 50% of the dopaminergic cell bodies within the SNpc.[20)24)]

Traditional pharmacological treatments for PD focus on the direct replacement of dopamine by the administration of the dopamine precursor levodopa (L-dopa), or dopamine agonists, in combination with agents that act to prolong the action of dopamine at the synapse or prevent breakdown of dopamine. However, treatment with L-dopa becomes less efficacious over time and can lead to debilitating side effects such as dyskineasia, hallucinations and disorientation. Direct augmentation of dopamine does nothing to halt the progress of the disease, and addition of exogenous dopamine to compromised systems may contribute to the ongoing pathology.[19)] Thus, gene therapy strategies involving the transfer of gene encoding factors that increase dopaminergic cell phenotype and survival represent an innovative approach in attacking this disease.[34)]

PD is particularly amenable to treatment using a gene therapy strategy for several reasons : 1) the identification of an active and preventable cell death process occurring within the substantia nigra (SN) ; 2) the confinement of the initial pathology of the disease to discrete locations within the brain ; 3) the progression of the disease over a long time-frame ; and 4) neurodegenerative diseases like PD are, by definition, chronic, so any treatment options must be long-lasting or permanent. This makes PD particularly suited to treatment with viral vectors, in which a single application of a vector can result in prolonged, stable transgene expression, with the production of physiologically relevant levels of enzymes involved in the dopamine synthesis pathway, or prolonged growth factor production over several months to promote reinnervation of the damaged nigrostriatal pathway.[34)]

Efficient vector systems for transduction of neurons have been developed, such as recombinant adeno-associated (AAV) virus,[41)] adenovirus[3)] and lentivirus.[33)] This has been coupled with the optimization of manufacturing methods for these vectors to produce pure, high-titer vector stocks suitable for use in the human brain,[5)] thus enabling infusion of a small volume into brain tissue to transduce a maximal number of cells. However, there are

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at least three separate gene transfer strategies currently being pursued in an effort to combat PD. This review provides a description of viral vectors and the progress of research on these various gene therapy strategies.

Viral vectors for PD gene therapy

Virus vectors can efficiently deliver genes to neurons and other neural cells *in vitro* and *in vivo*. These vectors allow us to monitor neurobilogical functions, replace, correct, express or block expression of target genes, tag cells for fate determination, and change the physiological state of specific cell populations. Gene transfer to the brain using viral vectors offers the advantage of being less invasive than transplantation techniques, leaving the striatal circuitry undisturbed by cellular implants and eliminating the risk of unwelcome host immune responses or tumor formation.

Adeno-associated virus vectors

Adeno-associated viruses (AAVs) are small, non-enveloped, single-stranded DNA viruses of the parvovirus family.[41] The wild type AAV genome is 4.7kb and integrates into the genome at a specific site on chromosome 19. No human disease has been associated with AAV infection. There are at least seven serotypes known. Recently, additional types were recovered from mammalian tissue samples and some are currently being analyzed for their potential use as vectors.[21] Different serotypes infect tissues with varying efficiency. The serotype most commonly used for AAV vectors is serotype 2, which infects the brain and retina very well, and thus has been used for a wide spectrum of applications targeting these organs.[4][11] AAV1 infects muscle well. As more and more AAV serotypes are being characterized and different serotype capsids are combined to generate novel tropisms, there is the potential that tissues that have not been easily infected with the current AAV serotypes may become amenable to AAV-based gene transfer in the future. Recent work supports the use of other AAV serotypes in brain-directed gene transfer. The properties of AAV4 and AAV5 differ from those of AAV2 ; AAV5 diffuses more widely, and AAV4 primarily transduces ependymal cells.[12] In the cerebellum, AAV5 transduces purkinje cells, but not granule cells, with high efficiency.[1] This is probably due to the selective expression of the AAV5 receptor on specific neuronal types (J. A. Chiorini, unpublished data). Other serotypes of AAV may also show distinct tropisms when injected into the brain. Although AAV vectors are highly effective for gene delivery and are non-toxic, they have a relatively small transgene capacity (4 - 5kb). This can be overcome by the infection of cells simultaneously with two AAV vectors, which can recombine to generate a larger genome.[13] Studies in peripheral tissues show that transgenes are typically retained as extrachromosomal elements, but they can also integrate randomly into the genome.[6] A number of clinical trials evaluating the use of AAV vectors for genetic and acquired diseases are currently underway. The brain is the target for monogenetic metabolic disorders, such as canavan disease[26] or degenerative disorders such as Parkinson's disease.[28]

Adenovirus vectors

Adenoviruses (Ad) are non-enveloped icosahedral DNA viruses with a 36-kb genome. With over 50 different human adenoviral serotypes, current vectors are primarily derived from those known as 2 and 5 - the most common serotypes to which most adults have been exposed.[29] The deletion of essential genes, typically E1, renders the adenovirus replication deficient and makes room for the insertion of an expression cassette. Adenovirus-based vectors have been most extensively evaluated in clinical studies. The advantages of utilizing adenovirus vectors for gene therapy include : 1) they are easy to propagate in high titers ; 2) they can infect most cell types ; and 3) they can be manipulated to accommodate large DNA inserts.

First-generation Ad vectors have significant potential in therapies where only a limited duration of gene expression is necessary and permanent expression of the transferred gene, which may lead to undesired effects, is not required. The induction of angiogenesis is an application that meets these criteria. A vaccine vehicle is another application for adenovirus vectors, in which long-term expression is not desirable and the induction of immune responses is wanted.[51] Various attempts have been made to change the immunogenicity of first-generation Ad vectors by deleting more genes from their adenovirus genome by creating helper-dependant vectors (gutless or gutted vectors).[32] Expression from gutless vectors has been observed for up to 6 months without inflammation *in vivo*. However, there are no *in vivo* data, thus far from parkinsonian models.[47] Efficient *in vivo* regulation of transgene expression has been shown with a Tet-off adenovirus system.[10] Transient rotational behavior has been induced after unilateral striatal transduction with an adeovirus-dopamine

D2 receptor ; this might be applicable for parkinsonian syndromes with concurrent striatal degeneration.[48]

Lentivirus vectors

Lentiviral (LV) vectors can infect dividing and non-dividing cells, broadening their potential application to target cell types unable to be infected with retroviral vectors.[27] The vector integrates its DNA into the host genome at active gene loci, raising concerns of the possible transactivation of oncogenes or disruption of host genes.

Studies in animals using gene transfer to hemaopoietic stem cells have shown the successful correction of genetic hematological disorders such as thalassemia, as well as successful gene transfer to the brain and liver.[27] Persistent gene expression *in vivo* has been reported for at least 5 - 13 months.[45] The safety of LV transfection has been improved by further deletion of the HIV-1 envelop and virulence genes, the segregation of viral components on distinct plasmids and the development of self-inactivating vectors.[2] Recently, a self-inactivating Tet-on TH LV showed regulatory capability *in vivo* over two orders of magnitude in 6-OHDA-depleted striatum, however, no biochemical or behavioral consequences were examined.[49]

Strategies for gene therapy in PD

Three separate gene therapy strategies that may be used to attack this disease (Fig. 1) exist. First, the transfer of genes involved in dopamine biosynthesis may relieve the immediate motor symptoms of the disease through sustained local production of dopamine in a manner similar to systemic administration of oral L-dopa (dopamine replacement therapy). Second, transfer of growth factor genes such as glial-cell-line-derived neurotrophic factor (GDNF) might prevent further dopaminergic cell death (neurotrophic factor therapy). Third, the transfer of genes involved in inhibitory neurotransmission could be used to reduce the activity of brain nuclei that become overactive in PD (GAD gene therapy).[34]

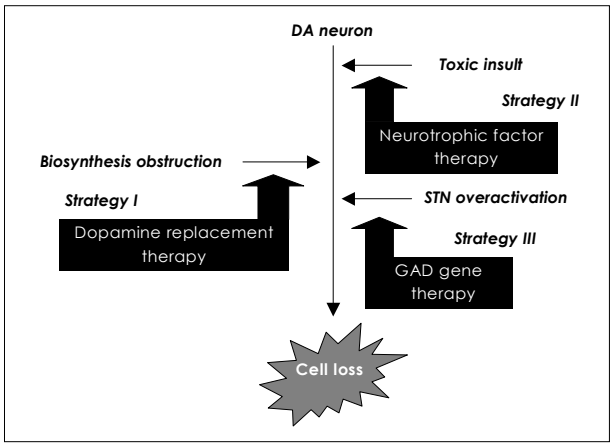

Fig. 1. Gene therapy strategies for PD. The putative event and functional consequences involved in cell loss of DA neurons and depicted. There are three separate gene therapy strategies, which are dopamine replacement therapy, neurotrophic factor therapy and GAD gene therapy.

Dopamine replacement therapy

The rate-limiting enzyme in dopamine (DA) production is tyrosine hydroxylase (TH), which converts the amino acid tyrosine to L-dopa (Fig. 2). L-dopa is then metabolized to dopamine by aromatic amino acid decarboxylase (AADC).[43] Another factor influencing this pathway is the essential TH cofactor 6-tetrahydrobiopterin (BH_4), the level of which is limited by availability of the enzyme GTP-cyclohydrolase I (GCH1).[42] In a 2000 study, TH expression in the striatum of 6-OHDA lesioned rats was achieved by AdV- or HSV-mediated transduction.[36] This decreased apomorphine-induced rotation by 30 - 65% but was not corroborated in later studies with adenoviral gene transfer.[10] HSV-TH transfer also led to higher potassium-induced striatal dopamine levels. Effects seemed

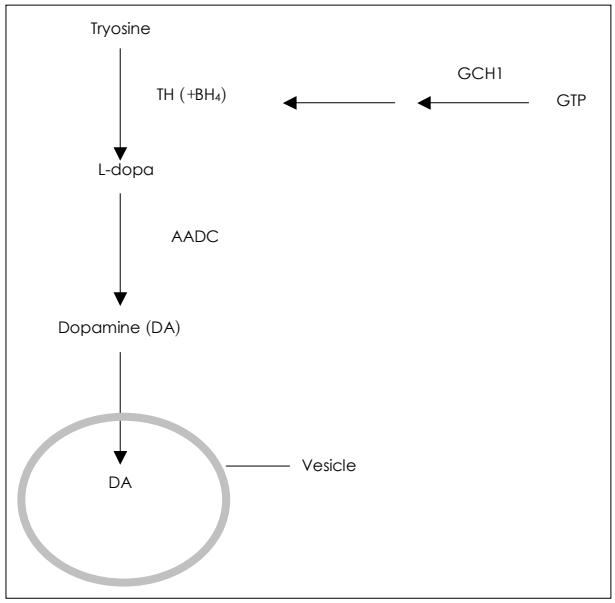

Fig. 2. The dopamine synthesis and storage pathway. Tyrosine is converted to L-dopa by the enzyme tyrosine hydroxylase (TH), a reaction that also requires the TH cofactor 6-tetrahydrobioptein (BH_4). Guanosine triphosphate cyclohydrolase I (GCH1) is the first and rate-limiting enzyme involved in BH_4 synthesis. Conversion of L-dopa to dopamine requires the enzyme aromatic amino acid decarboxylase (AADC).

to abate after 2 weeks in Ad-treated rats but were preserved after delivery of the herpes simplex virus (HSV) for up to 16 months, despite TH immunoreactivity in only 5 - 300 striatal cells.[16] Thus, the functional connection between TH transfer and rotational improvement in the latter study was cast in doubt.[39] The AAV-mediated delivery of AADC to the striatum after stable 6-OHDA lesions, allowed for L-Dopa-regulated dopamine increases, as measured by striatal microdialysis. Expression was stable for 6 - 12 months.[35]

Various vector systems have been used in recent preclinical studies to deliver different combinations of these enzymes. These studies include multicistronic LV simultaneously encoding GCH1, TH and AADC,[2] combinations of AAV vectors separately encoding GCH1 and TH[30] or GCH1, TH and AADC[40]; and HSV vectors coexpressing AADC and TH.[46] In all cases, the coexpression of enzymes and functional recovery of the experimentally lesioned animals was observed.

Neurotrophic factor therapy

Various neurotrophic factors with putative effects on the nigrostriatal dopamine system have been evaluated for their therapeutic potential in PD. The most potent trophic factor for midbrain dopaminergic neurons is GDNF, which is a homodimeric member of the transforming growth factor beta (TGF-β) superfamily.[37] The GDNF receptor components GDNFR-α/GFR-α and c-Ret are both expressed in adult SN but not, or only weakly, in the striatum.[23] GDNF may mediate at least three different effects[9][31]: 1) an increase in striatal dopamine synthesis and release in normal and lesioned individuals, partly mediated by transcriptional effects; 2) protection of dopaminergic neuronal cell bodies against some (but not all) toxic insults; and 3) local sprouting and outgrowth of dopaminergic fibres with possible reinnervation of target regions. These effects may dissociate from each other depending on the dose, site of application and time-point after injury.[17]

A 1998 study regarding adenoviral-mediated transfer of GDNF showed protection from 6-OHDA-induced cell death following infusion of the vector adjacent to the cell bodies of the SN[7] and into the striatum.[8] A direct comparison between the benefits of infusing GDNF-encoding vectors into either the SN or striatum resulted in greater prevention of behavioral deficits and greater preservation of striatal innervation. A potential problem with the use of adenoviral vectors is the production of an inflammatory response and the consequent downregulation of transgene levels following injection into the brain over time. Other groups have reported the use of AAV- or LV-mediated delivery of GDNF to ensure long-term transgene expression and have demonstrated both the neuroprotection[22] and restoration of function and innervation in the rat model of PD, with transgenic GDNF still at maximal levels six months after gene transfer.[50]

GAD gene therapy

In the current model of basal ganglia dysfunction in Parkinson's disease, nigrostriatal degeneration renders the subthalamic nucleus overactive with ensuing hyperactivity in the SN pars reticulata (SNr) and internal globus pallidus (Gpi). This, in turn, dampens motor output structures. Deep brain stimulation in the subthalamic nucleus (STN) curbing excitatory outflow from the STN is the most successful in treating end-stage complications related to Parkinson's disease therapy. A similar down-regulation of non-physiological hyperactivity of the STN might be achieved pharmacologically by enhancing the level of the inhibitory neurotransmitter GABA, which is synthesized by GAD. This hypothesis has been tested by the overexpression of AAV-GAD65/67 in the STN of rats later lesioned with 6-OHDA in the MFB.[38] Ad-GAD67-mediated transduction of cultured glial cells or LV transfer of GAD65/GAD67 in cultured astrocytes has previously been proven to enhance GABA release.[18][44] Expression *in vivo* is stable for 4 - 5 months.[38] Whereas baseline GABA levels of the STN are unchanged, local GABA release following the electrical stimulation of the STN is increased four-fold and a shift in the majority of target neurons in the SNr from an excitatory to an inhibitory phenotype is elicited.[38] AAV-GAD65 is much more effective than AAV-GAD67; this appears to be related to lower expression levels of AAV-GAD67. AAV-GAD65-treated animals exhibit less apomorphine-induced rotation and less behavioral side bias (head position bias, forelimb use). Even more important, 35% of dopaminergic cells in the SNpc survive following the 6-OHDA lesion in GAD65-expressing animals (as opposed to 1%).[38] Neuroprotection by GAD65/67 transfer has also been observed in chronically lesioned, aged rats.[15] Combining neuroprotection with functional compensation is attractive; a Phase I clinical trial has been approved to begin soon.

CONCLUSION

PD is a debilitating, neurodegenerative disorder arising from the loss of dopaminergic neurons in the SNpc and subsequent depletion of striatal DA levels, resulting in distressing motor symptoms. Current mainstay treatments for PD, such as administration of L-dopa, treat the motor symptoms but do little to alter the ongoing pathology. Accordingly, alternative long-term treatment strategies such as gene therapy are being pursued. The replacement of missing or defective genes may be considered in the rare cases of hereditary PD with loss-of-function mutations.

Viral vector systems capable of efficiently transducing neurons, such as AAV, have been developed, along with protocols for the manufacture of pure vector stocks suitable for application to the human brain. A clinical trial, based on GAD gene transfer to the subthalamic nucleus using an AAV vector, is poised to recruit patients soon.[15] The trial is a dose-escalation safety study in which three cohorts of patients will receive between 10^{11} and 10^{12} particles of rAAV-GAD at the time of STN stimulator implantation[25] as approved by the US Food and Drug Administration.

Newer approaches include attempts to influence detrimental cell signaling pathways and to restrain overactive basal ganglia structures. Nevertheless, current models of PD do not address issues regarding long-term protein expression ; the choice of target structures, transgenes and safety remain to be solved.

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