

The current status of gene therapy for Parkinson's disease

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ABSTRACT

The recent development of viral vectors, especially vectors derived from adeno-associated virus (AAV), has translated gene therapy for Parkinson's disease (PD) from animal experiments into clinical trials. The current gene therapy protocols used are based on three major strategies. The first protocol involves local production of dopamine via the introduction of dopamine-synthesizing enzyme genes into the putamen. The aromatic L-amino acid decarboxylase (AADC) gene has been transferred in this manner with the aim of efficiently converting orally administered L-dopa. The delivery of triple genes including tyrosine hydroxylase (TH), guanosine triphosphate cyclohydrolase I (GCH) and AADC is also being undertaken, and is aimed at continuously supplying dopamine into the putamen. The second protocol involves the protection of nigrostriatal projections via the production of neurturin, a trophic factor for dopaminergic neurons in the putamen. The final method includes the modulation of neural activity along the output pathway of the basal ganglia by transducing the subthalamic nucleus with vectors expressing glutamic acid decarboxylase (GAD-65, GAD-67), a key enzyme required for the synthesis of the inhibitory transmitter γ -aminobutyric acid (GABA). The initial results of phase 1 studies using AAV vectors have not only confirmed the safety of these vectors, but have also revealed the alleviation of motor symptoms associated with PD.

KEY WORDS: Adeno-associated virus, Aromatic L-amino acid decarboxylase, Neurturin, Glutamic acid decarboxylase, Positron emission tomography
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Introduction

Almost two decades have passed since the first gene therapy clinical trial was conducted for the treatment of adenosine deaminase deficiency at the National Institute of Health in the United States in 1990. Although gene therapy appeared to be a ground breaking form of medical treatment at the time, it has not proven to be as successful in treating disease as initially anticipated. A patient with ornithine transcarbamylase deficiency died of systemic inflammatory response syndrome

(SIRS) following the administration of a large quantity of adenoviral vector into the hepatic artery.¹ In addition, some children with X-linked severe combined immunodeficiency (X-SCID) developed leukemia due to the activation of an oncogene after gene therapy using a retroviral vector.^{2,3} These reports describing the severe, adverse effects of gene therapy dampened the excitement underlying the success of gene therapy treatment to some extent. However, encouraging results have been obtained more recently with clinical studies for Parkinson's disease (PD).^{4,7}

Table. Gene therapy clinical trials for Parkinson's disease.

| Gene | AADC | | TH/GCH/AADC | Neurturin | | GAD | |
|--------------------|------------------------------------|--------------------|-------------------------------------|--|------------------------------|---|----------------------------|
| Function | Convert L-dopa to dopamine | | Synthesis of dopamine from tyrosine | Neurotrophic factor for dopaminergic neurons | | Synthesis of inhibitory neurotransmitter GABA | |
| Vector | AAV | | EIAV | AAV | | AAV | |
| Phase | I | | I | I | II | I | II |
| Institute | UCSF ⁷ | JMU | Henri Mondor Hospital ²² | UCF ⁶ | Multi-center ²⁶ | NYP Hospital ⁵ | Multi-center |
| Dose | 9x10 ¹⁰ | 3x10 ¹¹ | 1x 2x | 1.3x10 ¹¹ 5.4x10 ¹¹ | 5.4x10 ¹¹ Sham | 3.5x10 ⁹ 1x10 ¹⁰ 3.5x10 ¹⁰ | 1x10 ¹¹ Sham |
| Number of subjects | 10 | 6 | 6 | 12 | 54 | 12 | 40 |
| Target | Putamen (Bilateral) | | Putamen (Bilateral) | Putamen (Bilateral) | | STN (Unilateral) | STN (Bilateral) |
| PET tracer | ^[18F] fluoro-m-tyrosine | | | ^[18F] fluoro-DOPA | | ^[18F] fluoro-deoxyglucose | |

AADC, aromatic L-amino acid decarboxylase; AAV, adeno-associated virus; EIAV, equine infectious anemia virus; GABA, γ -aminobutyric acid; GAD, glutamic acid decarboxylase; GCH, guanosine triphosphate cyclohydrolase I; TH, tyrosine hydroxylase; STN, subthalamic nucleus. Dose of AAV vectors are represented as vector genome/patient.

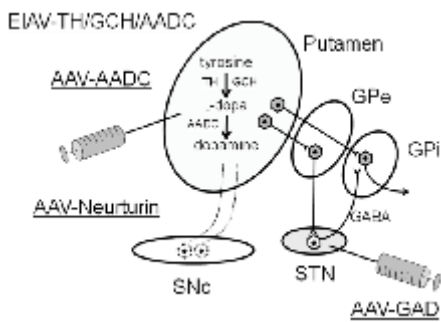


Figure. Current gene therapy strategies for the treatment of Parkinson's disease

Three gene therapy strategies are currently available for the treatment of (PD). 1) production of dopamine. AAV vectors expressing AADC or EIAV vector expressing TH, GCH and AADC are infused into the putamen. 2) Protection of the nigrostriatal pathway. The neurotrophic factor neurturin is produced continuously in the putamen. 3) Modification of neuronal activity in the STN by introducing the GAD gene.

AADC, aromatic L-amino acid decarboxylase; AAV, adeno-associated virus; EIAV, equine infectious anemia virus; GABA, -aminobutyric acid; GAD, glutamic acid decarboxylase; GCH, guanosine triphosphate cyclohydrolase I; GPe, globus pallidus external segment; GPi, globus pallidus internal segment; TH, tyrosine hydroxylase; SNc, substantia nigra pars compacta; STN, subthalamic nucleus.

Adeno-associated virus vectors

Technology that efficiently introduces a therapeutic gene into target cells is essential for successful gene therapy. Viral vectors, in particular vectors derived from adeno-associated virus (AAV), have been shown to be suitable for the transduction of neurons in the mammalian brain using stereotaxic surgery.⁸ AAVs are small (25 nm), single stranded DNA viruses that belong to Parvoviridae.⁹ No clear pathogenicity in humans has been reported, and many adults synthesize antibodies against AAVs following latent infection in childhood.^{10,11} The AAV genome exists in episomes in the nucleus and is rarely incorporated in the chromosomes. To date, more than one hundred genotypes have been defined for primate AAVs. The

vast majority of vectors used in clinical applications are derived from serotype 2 (AAV2).

AAV2 has a 4.7-kb genome, both ends of which contain hairpin structures called inverted terminal repeats (ITR). A region encoding the non-structural protein Rep and capsid protein VP are also present. These regions for Rep and VP can be substituted with a therapeutic gene when constructing vectors. AAV vectors express an exogenous gene in the brain for long term use (more than seven years) and do not produce any significant inflammatory or immunological reactions.¹² Although the size of the genes that can be inserted into the AAV2 genome is limited to less than 4.5 kb, most of the therapeutic genes fit into this range. It has also been shown that several AAV vectors are able to infect one cell at the same time and can express plural genes.¹³ AAV vectors have been applied in clinical trials for numerous disorders including hemophilia,¹⁴ cystic fibrosis¹⁵ and retinal degeneration.¹⁶ To date, adverse effects due to the administration of the vectors themselves have not been found. In addition to gene therapy, AAV vectors have also been recently used as a genetic tool in the neurosciences.^{17,18}

Gene transfer of dopamine-synthesizing enzymes

Dopamine is synthesized in the brain from diet-derived L-tyrosine. Three enzymes are essential in this production process.¹⁹ Tyrosine hydroxylase (TH) is the rate-limiting enzyme that converts L-tyrosine to L-3, 4-dihydroxy-phenylalanine (L-dopa). Guanosine triphosphate cyclohydrolase I (GCH) is the rate-limiting enzyme that synthesizes the essential TH co-factor tetrahydrobiopterine (BH₄), while aromatic L-amino acid decarboxylase (AADC) converts L-dopa to dopamine. These three enzymes are transported from the substantia nigra in an anterograde manner to the striatum. A severe loss of the nerve terminals in advanced Parkinson's disease (PD) is associated with an 80-95% depletion of striatal enzyme activity. Gene transfer of TH, GCH and AADC,^{13,20} or AADC alone,^{12,21} into the striatal neurons has led to behavioral recovery in primate models of

PD.

Two phase I clinical trials were conducted at the University of California San Francisco (UCSF) and Jichi Medical University (JMU) to evaluate the safety and potential efficacy of AAV vector-mediated gene delivery of AADC to the bilateral putamen. Alleviation of motor symptoms associated with PD was observed in both trials. In the UCSF study,⁷ a low dose cohort of five patients received 110¹¹ vector genome (vg) and a high dose cohort of five patients received 310¹¹ vg of AAV-AADC. The mean improvements of the ten patients in the total score of unified Parkinson's disease rating scale (UPDRS) were 31% (10.5 points) in the off-state and 32% (10 points) in the on-state. The mean improvements in the motor score of UPDRS were 36% (12 points) in the off-state and 28% (4.3 points) in the on-state. Positron emission tomography (PET) using [¹⁸F]fluoro-m-tyrosine (FMT), a tracer for AADC, revealed a 30 and 75% increase in Kⁱ values in the putamen of the low-dose and high-dose cohort, respectively, at 6 months after the gene delivery. In the JMU study, six patients received 310¹¹ vg of AAV-AADC. Motor function in the off-state improved to a mean of 46% (11.6 points) based on the UPDRS scores at six months after surgery, without any apparent changes in the short-duration response to levodopa. PET revealed a 56% increase in FMT activity, which persisted for up to 96 weeks (manuscript in preparation). Phase 2 trials of AAV-AADC are currently in the planning stages.

Using a vector derived from the equine infectious anemia virus (EIAV), a type of lentivirus, a phase I/II trial involving the triple gene transfer of TH, GCH and AADC into the bilateral putamen has been initiated at the Henri Mondor Hospital in France.²² Two dose levels have been evaluated in cohorts of three patients per dose. All patients treated at the second dose level have completed their six-month assessments and have shown improvement in motor function in the off-state when evaluated on the motor score of UPDRS. The mean improvement was 34% relative to the patients' pre-

treatment motor function.²² Studies into the high dose (5 times the lower dose) cohort are planned. Using this triple gene transfer, dopamine will be continuously produced in the putamen and may reduce “wearing-off” effects by avoiding pulsatile stimulation of dopaminergic receptors.

Gene transfer of neurturin

An alternative approach to gene therapy for PD is the protection of the nigrostriatal pathways from progressive degeneration by providing genes encoding for neurotrophic factors. Neurturin is a natural analog of glial cell line-derived neurotrophic factor (GDNF).²³ GDNF is a small glycoprotein that provides strong trophic support for the dopaminergic neurons. However, GDNF protein has limited usefulness as a therapeutic agent due to its short duration of activity and poor ability to cross the blood-brain barrier. In animal models of neurotoxin induced PD, viral vector-mediated gene delivery halts ongoing degeneration of the nigrostriatal pathway, resulting in functional recovery, even after substantial numbers of dopaminergic cells have been depleted.^{24,25}

A phase I gene therapy trial that introduced the neurturin gene into the bilateral putamen was conducted at the UCSF.⁶ The first six patients entered into the study receiving a dose of 310¹¹ vg, while the next six patients received a dose of 5.410¹¹ vg of AAV vector. A mean improvement of 36% (14 points) in the UPDRS motor score in the off-state and a mean increase of 25% (2.3 h) in on time without troublesome dyskinesia were observed one year after surgery. Subsequently, a phase II double-blind control study was undertaken at nine academic institutions in the United States, and in which one-third of a total of 54 patients received sham surgery (partial burr hole without infusion of vectors). Significant differences were not obtained in terms of the degree of motor performance between the gene transfer group and the control group at 12 months, although some treatment effects have since been observed in 30 subjects followed for 18 months under

blind conditions.²⁶ Analysis of post-mortem brain tissue from two patients treated with AAV vectors demonstrated that neurturin was expressed in the putamen, but not in the substantia nigra. Earlier intervention may be required for success in future trials of this kind of neuro-protective approach, as most of the nigrostriatal fibers have already been lost when the PD symptoms appear.

Gene transfer of glutamic acid decarboxylase

In PD, depletion of dopamine in the striatum leads to an increase in the activity of the subthalamic nucleus (STN).²⁷ The increased excitatory drive of the STN to the internal portion of the globus pallidus (GPi) and to the substantia nigra pars reticulata (SNr) then exerts inhibitory effects on the thalamo-cortical projection and brainstem nucleus, resulting in motor symptoms such as bradykinesia and rigidity. During the last two decades, deep brain stimulation of the STN, which is thought to modify STN output by high-frequency stimulation, has become routine treatment for advanced PD patients and has resulted in the improvement of motor function. Gene transfer of glutamic acid decarboxylase (GAD-65 and GAD-67), a rate-limiting enzyme required for the synthesis of inhibitory transmitter γ -aminobutyric acid (GABA), into the STN is aimed at converting excitatory output to inhibitory output, thus, obtaining a similar effect to electrical stimulation.²⁸

An open-label phase I clinical trial has been conducted at the New York Presbyterian Hospital.⁵ A total of 12 patients in three dose-escalation cohorts received AAV-GAD into the unilateral STN contra-lateral to more severe motor symptoms. At 12 months after the vector infusion, the mean improvement on motor score of UPDRS was 27% in the off-state and 24% in the on-state.⁵ A PET scan using [¹⁸F]fluorodeoxyglucose (FDG) as a tracer revealed a decrease in uptake into the ventrolateral nucleus (VL) and dorsomedial nucleus (MD) of the thalamus on the operated side, and an increased uptake in the ipsilateral premotor and motor cortical regions.²⁹

The underlining physiological changes in PD include, in addition to increases in the firing rate of the STN, the tendency of pallidal neurons to fire in more irregular patterns, as well as abnormal oscillatory synchronization in the basal ganglia.³⁰ Thus, mechanisms underlying how DBS and AAV-GAD gene therapy is effective remain to be defined.³¹ A double-blinded phase II study of AAV-GAD infusion into the bilateral STN is currently underway for 40 subjects, including 20 subjects that received sham surgery.

Future prospects

Owing to the development of efficient viral vectors, gene therapy for PD has been tested in clinical trials, with the initial results of phase I studies proving encouraging. In contrast to cell transplantation, immunosuppressive drugs are not necessary for the current gene therapy strategies. If the primary purpose of treatment is the supplementation of dopamine into the striatum for improving motor performance, then gene therapy appears to be simpler than cell transplants. Cell therapy may prove useful in treating Parkinsonism including cerebral ischemia, striato-nigral degeneration and cortico-basal degeneration, where neurons in the striatum are damaged. However, several PD symptoms that L-dopa is not effective at rescuing including cognitive dysfunction, depressive state, frozen gait, posture reflex disturbance and sleep disturbance have also been reported.³² For the treatment of these symptoms, effective therapeutic genes must be identified and delivered into the appropriate areas of the brain. Development of vector constructs that avoid over-expression is also required for increasing safety.³³ Although AAV and EIAV are reported to be non-pathogenic for humans, the long-term safety must be confirmed. It is expected that in the near future gene therapy will become the general choice for the treatment of PD.

Competing interests – None

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