Chapter 5
GAD Gene Therapy for Parkinson's Disease

Michael G. Kaplitt and Matthew J. During

Abstract The ability to directly modulate intracellular processes through genetic manipulation has long been felt to have great potential for treating intractable neurological and psychiatric diseases. To date, the largest number of clinical trials of central nervous system gene therapy has been in patients with Parkinson's disease (PD). Most of these have used adeno-associated virus (AAV) as a vehicle, which we demonstrated to be safe and effective for stable gene therapy in the brain more than 20 years ago. Here, we describe the development and results of the first human gene therapy for PD, which used AAV to transfer the gene for glutamic acid decarboxylase (GAD) into the subthalamic nucleus (STN). The STN is in the basal ganglia circuitry which is dysfunctional in PD, and human therapy for drug resistant PD has focused upon either lesioning or electrical stimulation of the STN for many years. In an initial open label pilot study, unilateral injection of AAV-STN into the STN of the more symptomatic hemisphere demonstrated safety and suggested evidence of efficacy based upon both motor improvements and reversal of functional imaging abnormalities up to 1 year. This led to a randomized, double-blind phase II clinical trial of bilateral AAV-GAD into the STN compared with patients receiving bilateral sham surgery. This confirmed the effectiveness of AAV-GAD, as the treated patients showed significantly greater improvements than the sham patients throughout both the 6-month blinded phase and full 12-month study phase, again with a very good safety profile. Functional imaging further supported these findings and identified a pattern of changes unique to the sham patients with improvement which was not seen in either the AAV-GAD patients or sham non-responders. These combined data support ongoing development of AAV-GAD as the only gene therapy in the CNS to date to demonstrate efficacy compared with contemporaneous sham controls and provide a stronger foundation for the further development of CNS gene therapy for a variety of disorders.

Keywords Parkinson's disease • Adeno-associated virus • AAV • Glutamic acid decarboxylase • GAD • Sub thalamic nucleus • STN • Positron emission topography • PET • Gene therapy

M.G. Kaplitt, MD, PhD (✉)
Department of Neurological Surgery, Weill Cornell Medical College,
525 East 68th St, New York, NY 10065, USA
e-mail: mik2002@med.cornell.edu

M.J. During, MD, PhD
Ovid Therapeutics, 205 E 42nd Street, New York, NY 10017, USA

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Background

Gene therapy has long held promise as a cutting edge approach to exploit the power of gene technology for improving human disease. This has been particularly true in the nervous system, where major advances for devastating degenerative disease have been limited. One disease which has been a focus of novel biological approaches, such as gene therapy and cell transplantation, has been Parkinson's disease. This devastating disorder is characterized by loss of dopaminergic neurons of the substantia nigra, as well as other neuronal populations, leading to a characteristic movement disorder including resting tremor, muscular rigidity, and difficulty initiating movements. Medical therapies are very effective early in the disease, but reduced benefit and medication-related complications often lead many patients to seek alternative treatments over time [1]. Surgical therapies, in particular deep brain stimulation, can provide great benefit to patients, but require implants of electrical devices in the brain and body which also require frequent adjustments initially and some maintenance over time, and can lead to hardware-related complications [2]. Parkinson's disease can also be modeled in both rodents and primates, traditionally through chemical destruction of nigral dopamine neurons. Although the predictive value of these models for therapeutic success in humans has been questioned, the availability of these rodent and primate models provides opportunities for testing novel therapies in systems which more closely mimic human disease than many other neurological disorders. This combination of unmet need, and models which reflect at least in part the pathophysiology of the human disorder, has made Parkinson's disease a major focus of novel biological therapies such as gene therapy.

Translation of central nervous system gene therapy into a clinical reality was first facilitated by the identification of transfer agents which would be safe and effective in the human brain. It was recognized early that modified viruses could be very efficient vehicles for delivery of genes into mammalian cells. These initial viral vectors were largely based upon viruses which naturally infected the target organ [3–5]. This included herpes simplex virus vectors, which we and others first utilized for gene transfer into neurons in culture and into brains of living rodents [3, 4, 6]. It was then recognized that many viral agents which do not normally cause disease in a particular organ could still function well as efficient gene transfer agents, which is the main goal of gene therapy. This led us to identify the adeno-associated virus (AAV) as a potentially powerful agent for gene transfer into the mammalian nervous system [7]. AAV is not naturally pathogenic in humans, and the small size of this virus permits ready manipulation of the genome such that vectors which contain only the gene of interest packaged into an AAV coat without any viral genes could easily be created and purified without contaminating helper viruses. We first demonstrated that AAV could be an effective agent for safe and long-term gene delivery in the brain, and at that time also showed that transferring a gene for tyrosine hydroxylase, the rate limiting step in the synthesis of dopamine, into the striatum could improve symptoms in a rodent model of Parkinson's disease [7]. That AAV strain, which we now know as AAV serotype 2, also had a particular preference for

neurons, which was unexpected. The combined features of a highly pure vector that results in efficient neuronal gene transfer and does not produce viral proteins, does not provoke inflammation or cell death and leads to long-term, stable gene production made AAV the first agent that could realistically be considered for a first-in-human use for brain gene therapy.

Preclinical Data

The goal of our research program from the start was to translate gene therapy into a human therapeutic. While the advent of AAV technology as a gene delivery vehicle for the nervous system facilitated an enormous amount of experimental research, we sought to identify an early opportunity for human central nervous system gene therapy that could be sufficiently safe and effective in a patient population with an unmet need that would justify proceeding with this novel technology. Our long focus on Parkinson's disease made this a clear favorite, and the rationale outlined above strengthened our interest in developing a human gene therapy for this disorder. Although our initial publication on AAV identified a potential approach, the failure of cell transplants, which were clearly synthesizing dopamine, to reliably improve patients in randomized studies raised some questions as to whether a gene therapy approach would be more likely to succeed [8–11]. Another concern was the location in the brain which would require gene therapy for dopamine replacement. In animals, the target of both cell transplants and tyrosine hydroxylase gene therapy by ourselves and others was the striatum. Since success in animal models failed to translate into success in humans for most prior advanced Parkinson's disease therapeutics, we were concerned that the introduction of too many untested variables in earlier clinical trials may have adversely influenced outcomes. To that point, there was virtually no history of operating in the human striatum. We thus felt that the lack of knowledge as to the proper method for targeting the striatum and potential for heterogeneity of the human striatum could introduce a variable that might confound any study regardless of the potential for effectiveness of the therapy itself.

In order to improve the possibility of success in humans, we began to focus on potential gene therapy opportunities that would be based upon therapies which were already beneficial in human patients. One of the more useful therapies for patients with reduced effectiveness and/or complications of medical therapy is deep brain stimulation of the subthalamic nucleus (STN) [2, 12, 13]. The STN is a key node within the basal ganglia circuitry regulating movement. This is normally controlled by structures which are indirectly regulated by dopamine, such that the loss of nigral neurons leads to STN dysfunction and corresponding abnormal regulation of STN downstream targets [14]. Lesioning of the STN (subthalamotomy) has been found to be effective in human patients, but the longevity of this effect can be limited in some patients and, while most patients requiring surgery have bilateral disease, bilateral lesioning is not well tolerated [15, 16]. STN DBS appears to modulate activity of this structure, and while it remains unclear as to whether DBS

acts by inhibiting neuronal function or modulating neuronal firing patterns, the consequence of DBS is similar to lesioning, with reduction in abnormal brain activity patterns and improvement in clinical motor symptoms. The methodology for targeting the STN, including image-based identification followed by intraoperative target refinement using microelectrode-guided electrophysiological recordings of neuronal activity, is standard for most neurosurgeons actively performing DBS [17]. Therefore, we felt that a gene therapy approach centered upon the STN would be a good candidate for a first-in-human application of in vivo gene therapy in the brain by dramatically reducing many clinical trials variables, since this would be based upon a brain target already shown to effectively improve symptoms in human Parkinson's disease, patients who are good candidates for such procedures are already identifiable and targeting of an infusion catheter would be based upon established surgical methods.

Given the abnormal physiology of the STN in Parkinson's disease, we settled upon the glutamic acid decarboxylase (GAD) gene as the therapeutic agent for AAV-mediated gene therapy in the STN. The GAD gene encodes an enzyme which catalyzes the rate-limiting step in the synthesis of GABA, the major inhibitory neurotransmitter in the brain. Conventional theories regarding basal ganglia circuit dysfunction in Parkinson's disease suggest that loss of GABAergic inhibitory tone into the STN following nigral dopamine cell loss leads to alterations in STN activity [14]. The consequent hyperactive and/or abnormal patterning of STN glutamatergic outflow leads to dysregulation of downstream STN targets, including the globus pallidus interna (GPi) and substantia nigral pars reticulate (SNr). We therefore hypothesized that providing the STN with a novel GABA synthetic capacity through AAV-mediated transfer of the GAD gene would lead to normalization of STN activity, as well as provide increased GABAergic tone to downstream STN targets which are also dysregulated. Additional support for this hypothesis derived not only from prior animal studies with infusion of GABA agonists into the STN, but from human studies which demonstrated improvement not only from lesioning but transient benefit from an acute infusion of the GABA agonist muscimol into the STN of awake Parkinson's disease patients prior to implantation of their planned DBS electrodes [18, 19]. Furthermore, the fact that STN lesioning was an acceptable if suboptimal procedure provided an unusual safety mechanism, such that any potential adverse effect of chronic GAD expression in the STN or from the presence of AAV in that region could theoretically be reversed by lesioning, thereby treating the patient's symptoms and eliminating the source of ongoing gene production.

Prior to entry into human clinical trials, preclinical data was generated to support the hypothesis that AAV-GAD gene therapy in the STN could be safe and effective in Parkinson's disease. We first demonstrated that AAV-GAD gene therapy in the STN could improve abnormal rotations following dopamine agonist treatment which are characteristic of parkinsonian rodents with unilateral 6-hydroxydopamine (6OHDA) lesions of the substantia nigra dopamine neurons [20]. AAV-GAD also improved a variety of spontaneous motor behaviors which may be more relevant to the human condition, including overall locomotor activity, limb use, and head position bias. These results were subsequently supported by an independent group using a similar

approach [21]. In order to confirm the network concept outlined above, we used in vivo microdialysis to measure GABA release in the SNr. Electrical stimulation of the STN to drive STN activity in 6OHDA rats following injection of AAV-GAD led to a significant peak in SNr GABA release which was not observed in animals treated with a control vector [20]. This suggested a potential autoregulatory function, since a greater level of abnormal STN activity would lead to a greater level of GABA release downstream of the STN. Finally, based upon some evidence that STN hyperactivity can cause excitotoxicity and exacerbate nigral dopamine neurodegeneration, we pretreated animals with STN AAV-GAD prior to lesioning and in fact found a significant reduction in nigral dopamine cell loss compared with controls.

Phase I Study

Based upon these results, we developed and proposed a first-in-human trial of AAV gene therapy in the adult brain, with the goal of treating Parkinson's disease patients with AAV-GAD in the STN. Although there was some evidence of disease modification using this approach, the goal of the trials and any eventual therapy was to improve patient symptoms, with any potential for neuroprotection being a secondary possible benefit. To support a filing for a human trial, we completed a study in MPTP primates, which demonstrated long-term safety as well as behavioral improvement in the AAV-GAD animals compared to baseline [22]. In order to provide more reliable physiological data in any human study compared with clinical observations alone, we intended to use positron emission tomography (PET) as a measure of local biological activity in various brain areas at baseline and following treatment. To test this, we used a similar paradigm in these parkinsonian primates and demonstrated significant improvements in abnormal brain metabolism following AAV-GAD compared with baseline and compared with control animals [22].

Following review by both the Recombinant DNA Advisory Committee (RAC) and the U.S. Food and Drug Administration, AAV-GAD in the STN was approved to begin a phase I study in patients with moderate to advanced Parkinson's disease. The study was designed initially to treat both hemispheres of the brain, since most patients who are no longer adequately responsive to medication have disease and symptoms bilaterally. However, since this was going to be the first time that a viral vector would be infused directly into the brain of an adult human for any nonlethal degenerative disorder, there were concerns about the potential for unknown toxicities despite a strong preclinical safety record. It was therefore decided that the phase I study would be an open-label, single-center study of unilateral AAV-GAD infusion into the STN. This was based upon the belief that should an unanticipated adverse event occur, it is less likely to be devastating if it were limited to only one hemisphere of the brain. In order to increase the possibility of observing a clinically meaningful signal, the more symptomatic hemisphere was chosen for treatment, since most patients at this stage still have asymmetry to their disease. Follow-ups of 1, 3, 6, and 12 months were chosen for safety, which was the primary endpoint, as

well as for efficacy analysis, and fluorodeoxyglucose (FDG) PET was chosen as a secondary outcome measure of biological activity. Twelve patients who met clinical entry criteria were enrolled in the study, with the first patient receiving AAV-GAD gene therapy in August, 2003.

The results of the phase I study indicated that unilateral AAV-GAD was safe in the chosen patient population over 1 year, and there was sufficient evidence of effectiveness to justify a more rigorous follow-on study [23]. Subjects demonstrated significant improvements in the motor subsection (part III) of the Unified Parkinson's Disease Rating Scale (UPDRS) over time, with a trend toward improvement at 1 month and significant improvements at subsequent time points. Breaking down the motor scores by body side indicated that most of the overall UPDRS part III effect was due to improvements in motor function of the body side opposite the treated hemisphere, as expected. There was also a suggestion of quality of life improvements and no evidence of toxicity over the course of the study.

PET analysis further confirmed the potential therapeutic benefit of AAV-GAD [24]. As indicated earlier, a consequence of nigral dopamine cell loss is a dysfunction in basal ganglia circuitry, which ultimately leads to abnormal activity of cortical brain regions as well. These can be quantified by FDG-PET, since neurons metabolize glucose proportionate to their level of activity, so uptake of radioactive glucose can be an effective measure of alterations in the activation of neurons grouped in particular brain regions [25]. Using this approach, we and our collaborators were able to demonstrate abnormalities in baseline motor circuitry metabolism in the brain which were significantly improved at 6 and 12 months following treatment with AAV-GAD. Although the clinical evaluations were unblinded and uncontrolled, as is usual for initial phase I trials, the individual analyzing the PET scans was blinded relative to side of treatment. Therefore, the PET studies were controlled, since the untreated hemisphere served as a control for the treated hemisphere, and was single-blinded, since the examiner was unaware of the treatment status of each hemisphere, and since each patient was treated based upon the more severe side clinically and were not uniformly treated in the same hemisphere. Therefore, in addition to a significant improvement over baseline, the improvement was also significant relative to the untreated hemispheres, which did not improve over time.

Phase II Study

The safety and efficacy results of the phase I study were encouraging, and the PET data provided a level of independent biological support that is unusual for a routine, open-label pilot study. Nonetheless, the history of randomized, blinded studies of otherwise promising cell or biological therapies for neurodegenerative disorders which failed to support initial pilot studies created an imperative to proceed with a more rigorous trial [8, 9, 11, 26–28]. Therefore, we developed a multi-center, randomized, double-blind protocol to compare patients treated with AAV-GAD to matched

controls treated with sham surgery. Sham surgery was performed by generating a partial-thickness burr hole in the skull, such that patients would perceive the drilling and the cap for the infusion system (see below) would be inserted, but the inner table of the skull would not be violated, thereby eliminating the risk of intracranial injury for control patients. A 6-month blind was chosen as the primary outcome, in order to reduce the time that patients would need to stay blinded, since maintaining a blind for a long period can be difficult, with the plan to follow patients for a full 12 months as part of the ongoing safety and efficacy analysis. The trial was 1:1 design, so that a relatively equivalent number of patients were randomized to each group, in order to enhance the statistical power of the study, with roughly 40 patients planned to be enrolled overall. All patients and caregivers remained blinded until the final patient reached 6 months after treatment, in order to prevent bias being introduced from serial unblinding. Although this meant that some patients were blinded for longer than 6 months, the enrollment was sufficiently robust that a relatively small number of patients reached 12 months or beyond in the blind.

One difference in this study compared with the phase I trial was the plan to treat patients bilaterally, given the likely need for bilateral surgery among most patients who might eventually be candidates for the therapy should it reach approval. Another change was based upon the concern over patient variability. While centers in our trial and in other studies were chosen based upon a track record of expertise in this area, one potential confound that could lead to great variability between studies is the confidence of the clinical diagnosis for patients entering the study. Since even multi-center studies are necessarily much smaller for neurosurgical interventions compared with drug trials, only a small number of patients who meet entry criteria but turn out not to have the clear disease pattern can destroy the statistical power of a study testing an otherwise promising therapy. Therefore, we used FDG PET as an entry criteria for the study. Patients did not have to meet a particular level of abnormality on PET, but their pattern of abnormal metabolism needed to be within established criteria from earlier PET studies in Parkinson's disease [25, 29, 30]. This in fact resulted in exclusion of several patients who might have otherwise clinically met criteria for enrollment.

Another substantial change from phase I to phase II was the method for infusing AAV-GAD into the STN. In phase I, we adapted a method that we had previously used for in vivo microdialysis in human patients, using a borosilicate glass fiber as an infusion catheter passed through a microelectrode guide tube, and attached to an external pump, with infusions were completed in the operating room. For the randomized study, we developed a system that might be more amenable to general clinical use. The catheter was flexible so that it could reside in the brain following removal of the guide tube without causing local trauma, similar to a DBS electrode. The last 1 cm tip of the catheter was steel, which would not absorb AAV based upon our testing and which was visible on CT scans. Again similar to the DBS electrode, a locking cap was created to lock the catheter in place to prevent migration during the infusion. A system for releasing the catheter was also created, so that the infusion could take place outside of the operating room following catheter insertion, with the catheter then removed at the bedside after infusion was completed without

necessitating a return to surgery. Since this was an untested system, there was the possibility that a catheter failure or migration could lead to poor or off-target infusion which could limit efficacy. Therefore, the protocol specified that only patients with documented bilateral catheter placements within a predefined zone considered to include the STN, and with greater than 50 % infusion of the vector fluid volume with confirmation of catheter patency following removal, would be included in the per-protocol analysis. Determination of catheter location was made by an expert DBS surgeon who did not perform a procedure in the study and who analyzed all of the post-infusion CT scans while blinded, without knowledge of patient outcome, prior to data analysis. Sham patients also underwent infusion of saline into the partial thickness burr hole. CT scans were either performed under an alias or were noted as study images and were not included in the main PACS system, to minimize the risk of unblinding. A blinding questionnaire performed throughout the study duration suggested that the blinding procedures were effective.

The results of this study demonstrated that AAV-GAD in the STN was effective compared both to baseline and compared with the contemporaneous sham surgery group [31]. Interestingly, as has been observed in the past, the sham group did show a significant improvement relative to baseline as well, but the AAV-GAD group had roughly twice the improvement on the UPDRS part III motor scores compared with controls. This represented the first demonstration of a significant improvement for a biological or cell-based therapy in the brain compared with a contemporaneous sham surgery control group, indicating that gene therapy can in fact be effective for neurodegenerative disorders. The magnitude of the effect was similar to the effect size in a recent large national U.S. study of DBS compared with best medical therapy, although it was somewhat less than European trials of the same therapy. There was also a significant improvement in the number of hours spent in the better or "ON" state at 3 months following surgery and a strong trend towards improvement at 1 month. While this was not significant at 6 months, likely due to variability in patient diaries in a small study, there again was a significant difference between groups at 12 months (data not shown). There was also evidence of a decrease in complications of medical therapy, with a significant improvement in the UPDRS part IV scale of therapeutic complications at 6 months relative to baseline and a trend at 3 months, with no change in control patients over that time period. Finally, there were no complications related to the gene therapy over the course of the study. The infusion device did fail in some patients, leading to exclusion from the per-protocol analysis, but a subsequent small design change appears to have addressed this issue.

FDG PET performed prior to and following treatment provided an opportunity for further exploration into the biological basis for these findings. There was evidence of improvements in particular PET patterns in treated patients compared with controls, and a pattern was identified which uniquely correlated with clinical improvements in AAV-GAD patients compared with controls (data not shown). One interesting observation was an analysis of PET patterns which could discriminate true treatment responders from sham or "placebo" responders. To do this, we and our collaborators analyzed the FDG-PET scans from sham surgery patients at the

end of 6 months, prior to unblinding, and compared these with baseline, to identify patterns that were common to sham responders as compared with either AAV-GAD responders or sham non-responders. Using unbiased mathematical modeling, a pattern was identified consisting of brain regions associated with affect and mood, which was termed the sham surgery-related pattern [32]. This pattern was generated from half of the sham surgery responders. A prospective analysis of the remaining half confirmed that this pattern was present in that group as well. This pattern was not present in sham non-responders, nor was it present in the AAV-GAD treatment responders, further suggesting that the AAV-GAD treatment response was a genuine biological effect distinct from the sham response.

Summary

The potential for gene therapy as a direct means of exploiting the power of genetic research has been and remains quite promising for a variety of neurological diseases. The development of AAV technology has greatly facilitated the potential for translating gene therapy into a successful human treatment, with many studies now having been completed or underway for diseases including Parkinson's disease, Alzheimer's disease, and various lethal pediatric neurogenetic disorders. The safety and suggestion of efficacy of AAV-GAD in the first phase I human trial provided support for many of these approaches through the demonstration of the possibilities of AAV technology in the human brain. Although as of the writing of this contribution, further studies of AAV-GAD to support final regulatory approval are in the planning stages, the success of AAV-GAD as the first CNS gene therapy to show efficacy compared with a sham group in a gold-standard randomized, double-blind clinical trial has provided and should continue to provide support for the future development of gene therapy as a useful treatment for neurological disorders.

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