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Review

Improved Delivery Methods for Gene Therapy and Cell Transplantation in Parkinson's Disease

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Abstract. A number of cell transplantation and gene therapy trials have been performed over the last three decades in an effort to restore function in Parkinson's disease. Much has been learned about optimizing delivery methods for these therapeutics. This is particularly true in gene therapy, which has predominated the clinical trial landscape in recent years; however, cell transplantation for Parkinson's disease is currently undergoing a renaissance. Innovations such as cannula design, iMRI-guided surgery and an evolution in delivery strategy has radically changed the way investigators approach clinical trial design. Future therapeutic strategies may employ newer delivery methods such as chronically implanted infusion devices and focal opening of the blood brain barrier with focused ultrasound.

Keywords: Parkinson's disease, gene therapy, cell transplantation, clinical trial, stereotactic delivery, iMRI-guided surgery

A number of cell transplantation and gene therapy trials have been performed over the last three decades in an effort to restore function in those afflicted with neurodegenerative disorders. Parkinson's disease (PD) has been the most frequently investigated disease thus far, with a number of therapeutic strategies including cell transplantation from a variety of donor sources as well as gene therapy for enzyme replacement or local expression of a neurotrophic growth factor [1–8]. These early human trials were informed by pre-clinical animal work, predominantly using the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine nonhuman primate (NHP) model of PD.

The surgical delivery of these therapeutics in the 1990s and early 2000s utilized the widely accepted tools of the day, typically a stereotactic frame such as the Leksell (Elekta, Stockholm, Sweden) or CRW (Integra, Cincinnati, OH). These systems were used to place a delivery cannula blindly into the intended target. For cell transplantation, a "withdraw and deposit" delivery strategy was employed by placing the cannula at the deepest point along the trajectory and withdrawing it several millimeters at the time to intermittently deposit cells [1, 9]. For viral vector gene transfer, the "withdraw and deposit" method with intermittent infusions delivered by hand or a single-point infusion in the center of the target with convection enhanced delivery (CED) were performed using simple cylindrical cannula designs [4, 5]. The determination of the amount of therapeutic to deliver was usually calculated by scaling up volumes used in the pre-clinical animal studies that resulted in a clinical change.

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Early phase 2 trials in both cell transplantation and gene therapy for PD had disappointing results, most with failure to reach their primary endpoints and/or emergence of bothersome side effects in placebo-controlled trials [1, 8, 10, 11]. These outcomes may have been related to the biology of the therapeutic material or local host environment (especially in cell transplantation). In gene therapy, the success of a particular therapeutic strategy depends on many factors including the properties of the vector and serotype used, whether they are capable of anterograde and/or retrograde transport (and if this is important to the biological intent), the type of promotor used, how the gene product is expressed and the viability of the therapeutic strategy in the first place. Early gene therapy trials were particularly hampered by suboptimal delivery methods [12]. We now realize that blind infusions of a viral vector using traditional stereotactic methods are subject to multiple sources of off-target delivery (infusate not going to or remaining in the intended target). These include reflux up the cannula, unintentional spread through perivascular spaces and misplacement of infusion cannulas; such events occur even in the hands of very experienced and skilled surgeons [13–17]. Moreover, hand injections with a syringe are far less efficient for delivering infusate over large volumes of tissue than convection enhanced delivery [12]. Indeed, CED for viral vectors has now become the method of choice for achieving predictable, adequate coverage of targets in the basal ganglia [11, 18–22].

For cell transplantation, the delivery considerations appear to be less complicated. Unlike viral vectors, transplanted cells do not need to be distributed over a large area during surgical delivery to potentially produce a therapeutic effect. Cells that survive after transplantation sprout neurites well beyond the localized cell deposits; in one PD patient who underwent postmortem analysis 24 years after fetal cell transplantation, cells deposited along three linear cannula tracts with an entry point near the coronal suture resulted in reinnervation throughout the entire postcommissural putamen [23]. In a double-blind trial of fetal cell transplant in the United States, a more anterior frontal approach with the entry point in the forehead was used to place the cannulas down the long axis of the putamen as close to the axial plane as possible [1]. Since the putamen is an elongated structure in the axial plane, this less-traditional angle of approach increased the efficiency of delivery while using only two needle tracts per putamen.

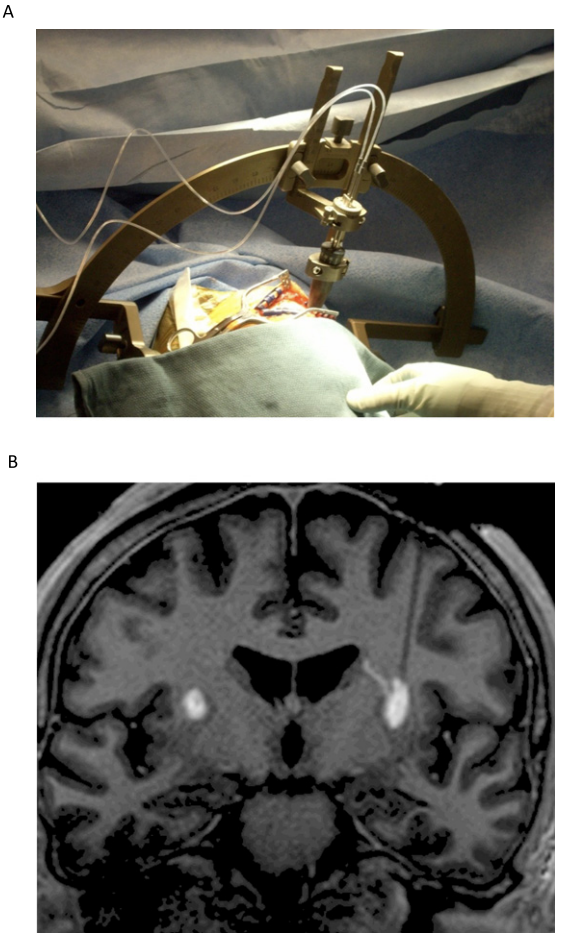

Fig. 1. A) The surgeon's view of a gene therapy infusion, circa 2010. B) The surgeon's view of a gene therapy infusion, circa 2020. A small amount of off-target delivery due to perivascular spread is starting to occur in the left putaminal infusion.

The emergence of interventional MRI (iMRI)-guided surgery using an FDA approved platform for placing devices in the human brain led to the development of co-infusion of viral vectors with gadoteridol, a gadolinium-based contrast agent (Fig. 1) [24–26]. There has been debate regarding the safety of intraparenchymal infusion of gadoteridol after several studies demonstrated radiographic evidence of contrast accumulation in certain brain regions in patients receiving repeated intravenous administration of gadolinium-based contrast agents (GBCAs) [27, 28]. However, the clinical significance and/or risk of these deposits is unknown. A subsequent *in vitro* study attempted to determine potential toxicity of such deposits by exposing a cell culture of dopaminergic neurons differentiated from a human neuroblastoma cell line to various GBCAs [29]. The

authors did demonstrate mitochondrial and cellular toxicity in the cultured cells after direct exposure to GBCAs; however, these *in vitro* experiments were quite artificial, and do not replicate the complex environment of the human brain parenchyma.

By contrast, direct intraparenchymal delivery of gadoteridol (either in liposomes or admixed directly with viral vector) has been performed numerous times in the non-human primate, an animal model that does closely replicates the local environment of the human brain parenchyma. Co-infusion of various therapeutics with gadoteridol has been performed in multiple brain targets (including striatum, thalamus, midbrain, brainstem and entorhinal cortex) with varying survival times to sacrifice and histological analysis [26, 30–34]. No tissue or cell toxicity was observed in any of these studies. Many adult and pediatric human subjects have undergone similar intraparenchymal infusions in the basal ganglia, midbrain and brainstem as well as intra-tumoral delivery [35–39]. None of these clinical trials demonstrated any known or suspected toxicity of one-time intraparenchymal administration of gadoteridol. NHP studies of AAV-based gene therapy showed this technique provided a "what you see is what you get" visualization of the infusions. That is, the area of gadoteridol enhancement seen on real-time MR images correlated highly with the area of vector transduction observed on histopathology [32]. This technique was translated to humans simultaneously in two phase 1 clinical trials of intraputaminal AAV gene therapy for PD [38, 39]. These trials started by using the traditional single-point CED infusion strategy of placing the cannula tip stationary in center of the target region. The advantages of real-time visualization during infusions quickly became apparent during the first few procedures. Off-target delivery due to reflux and perivascular spread were seen in almost all of the infusions, and effective coverage of the target was less than anticipated [14, 38, 39].

The gradual evolution in delivery methods for CNS gene therapy infusions was greatly accelerated as these two trials progressed in order to minimize off-target delivery (Table 1). The use of cannulas with two stepwise increases in diameter along the distal end of the device was previously found to resist reflux better than cylindrical or single-step cannulas. The initial dual-step cannula for these trials had a small square step 3 mm above the tip, and a second rounded step 18 mm above the tip. Real-time visualization of the infusions showed that that smaller volumes with lower flow rates usually only reflux to the first step, while increasing flow rates and larger volumes eventually cause reflux to the second step. The 18 mm distance to the second step proved to be too long for the height of some putamen in the coronal plane, so a variety of cannulas with shorter, varied step geometries were developed to tailor the cannula design to individual patients' anatomy [14].

The single-point CED strategy was found to be inefficient for achieving adequate coverage and minimizing off-target delivery. Once it starts, the only way to stop significant perivascular spread is to move the tip of the cannula away from the offending vessel. CED is most effective when the cannula tip is in contact with intact brain parenchyma, so the cannula should only be advanced during infusion, not withdrawn. If the cannula tip is already at the center of the target and perivascular spread occurs, there is little room to advance the cannula before the distal border of the target is reached. Early advancement of the cannula in this scenario may also compromise coverage in the proximal portion of the target. In addition, once the infusion rate or volume become sufficient to cause reflux beyond the first step, it is advantageous to have advanced the cannula such that the second step is at the proximal border of the target (or even within the target itself). For both of these reasons, it is better to start the infusion at the proximal end of the target and advance the cannula as the infusion is performed. These so-called stacked or progressive infusions have become the standard method for optimizing target coverage in gene therapy, and allow experienced surgeons to shape the infusions to fit the target [14, 40].

Finally, it became apparent that the volume of infusions used in the past were too conservative, and likely resulted in coverage of the target that was below what was needed to see a clinical effect. This is in part a reflection of the cautious approach taken in some earlier trials, which were first-in-human studies of these novel intracranial gene therapies. The concept of the Vd/Vi ratio (volume of distribution/volume of infusion) was developed during NHP gene therapy studies in basal ganglia targets using adeno associated virus, or AAV [26, 32]. If an infusion is optimal with minimal off-target delivery, the Vd/Vi ratio is approximately 3:1 (i.e., to cover 1500 cubic mm of tissue, one must infuse about 500 microliters of vector). As real-world infusions in the human brain frequently do suffer from off-target delivery, a more realistic Vd/Vi ratio is often closer to 2:1. In gene therapy, the Vd/Vi ratio has become a useful tool for predicting the volume of infusate needed to cover a given target.

Table 1
Summary of delivery methods in completed gene therapy clinical trials for Parkinson's disease as of the time of this writing. AAV2, adeno associated virus serotype 2; GAD, glutamic acid decarboxylase; TH, tyrosine hydroxylase; AACD, amino acid decarboxylase; CH1, GTP-cyclohydrolase-1; GDNF, glial cell-derived neurotrophic factor; STN, subthalamic nucleus; SN, substantia nigra; CED, convection enhanced delivery; iMRI, interventional MRI

Gene therapy	Phase, n	Target	Volume per target	Dual-step Cannula	CED, infusion strategy	iMRI-guided	Clinical outcome summary	Year published or completed	Reference
AAV2-GAD	Phase 1, $n=12$	STN	50 μL	No	Yes, single point	No	Improved	2007	[3]
	Phase 2, $n=45$ (22 active, 23 sham)	STN	34.5 μL	No	Yes, single point	No	Improved, but program discontinued	2011	[15]
AAV2-Neurturin	Phase 1, $n=12$	Putamen	40 μL	No	No	No	Improved	2008	[5]
	Phase 2, $n=58$ (38 active, 20 sham)	Putamen	40 μL	No	No	No	No difference active vs sham	2010	[10]
	Phase 1b, $n=6$	Putamen, SN	150 μL putamen, 30 μL SN	No	Yes, single point	No	No improvement	2013	[21]
	Phase 2b, $n=51$ (24 active, 27 sham)	Putamen, SN	150 μL putamen, 30 μL SN	No	Yes, single point	No	No difference active vs sham	2015	[11]
Lentivirus-TH, AADC, CH1	Phase 1/2, $n=15$	Putamen	[Not stated]	No	Uncertain	No	Improved	2014	[6]
AAV2-AADC	Phase 1, $n=10$	Putamen	100 μL	Yes	Yes, single point	No	Improved	2009	[4]
	Phase 1, $n=15$	Putamen	Up to 450 μL or 900 μL	Yes	Yes, single point, stacked & progressive	Yes	Improved	2019	[26]
	Phase 1, $n=8$	Putamen	Up to 1800 μL	Yes	Yes, progressive (posterior approach)	Yes	[ongoing]	2018 (follow up ongoing)	n/a
AAV2-GDNF	Phase 1, $n=13$	Putamen	450 μL	Yes	Yes, single point, stacked & progressive	Yes	No improvement	2019	[27]

For example, the human putamen has an average volume of approximately 4000 cubic mm. To approach 100% coverage of this target with AAV, one must infuse somewhere between 1333 (Vd/Vi of 3:1) and 2000 (Vd/Vi of 2:1) microliters of vector. This ratio can be used to design future trials as well as predict coverage of prior studies that utilized traditional, blind infusion techniques [14]. In recent human gene therapy trials, volumes of 1800 microliters have been safely and effectively delivered to the human putamen. This is approximately 45 times more volume than was delivered to the putamen in the earlier trials of gene therapy for PD [4, 5, 14].

Most of the recent advances in delivery methods for therapeutics to the brain in PD have focused on intraparenchymal gene therapy. This is largely due to the fact that delivery of a fluid to the brain parenchyma is more unpredictable and more dependent on surgical technique than cell transplantation, at least based on our current understanding. In the aftermath of two negative phase 2 fetal cell transplantation trials, gene therapy has also predominated the clinical trials landscape in PD over the last two decades, and the delivery methods have varied significantly. However, there are a number of exciting cell transplant trials on the horizon at the time of this writing, utilizing novel sources for transplantation such as induced pluripotent stem cells and new strategies to promote cell survival [41]. Several of these trials will be using iMRI-guided surgery for cell delivery, although it is not yet established if this technique will have the same benefits that have been seen with viral vector delivery. Since cell transplantation is not as dependent on coverage of the target as gene therapy, the only advantage that MRI-guided surgery might have is confirmation of cannula placement in the intended target. Many assumptions were made regarding infusions of fluids into the brain that turned out to be incorrect once gene therapy entered the era of iMRI-guided delivery; it will be interesting to see if there are lessons to be learned regarding surgical technique for cell transplantation in the iMRI era.

One important alternative to gene or cell-based strategies in PD is the chronic infusion of proteins directly to the basal ganglia. One recent program used a novel, chronically implanted delivery system consisting of a subcutaneous reservoir and multiple intracranial catheters to perform repeated CED infusions of glial cell line-derived neurotrophic factor as an alternative to intraventricular delivery [42, 43]. Although the study did not reach its primary endpoint, it was an important proof-of-principle study for successful chronic delivery of a protein to the parenchyma. There has not been a role thus far for chronic delivery of cells. Chronic delivery of viral vectors may be inadvisable due to antibody formation to the virus carrying the gene of interest over time. However, for other novel therapies, this may be an important delivery method moving forward.

The future of CNS delivery of cells, viral vectors and other biological agents for PD is bright. There is a movement towards less invasive and more efficient surgical approaches. New surgical tools, such as percutaneously mounted aiming devices and small twist drills, have transformed intraparenchymal delivery into an almost incision-less procedure. Posterior approaches through the occipital region to place laser fibers down the long axis of the hippocampus to treat seizures have been transformative in epilepsy surgery over the last decade. These procedures are now done routinely, and have a bleeding risk comparable to traditional frontal approaches for gene therapy or deep brain stimulation [44–47]. A posterior trajectory along the long axis of the putamen (analogous to the far anterior approach for cell transplantation utilized by Freed et. al. over 20 years ago) have made gene therapy procedures a single-pass affair, with greater efficiency and shorter procedure times [14].

There are newer, more cost effective iMRI-based delivery devices that are capable of performing multiple bilateral simultaneous infusions, and others are sure to follow [48–50]. New routes of administration for novel agents, such as intranasal delivery of antisense oligonucleotides, may provide a non-surgical option for delivery in the future [51]. Finally, recent studies have shown that focused ultrasound can be used to induce focal opening of the blood brain barrier, raising the possibility that an intravenous or systemically-administered agent could be delivered to a brain target without any direct parenchymal penetration [52]. It remains to be seen if adequate concentrations of the therapeutic could be achieved by this means of delivery, or what the implications might be for widespread systemic delivery of a given agent.

These explorations of less invasive options and alternative routes of delivery are encouraging, provided we strike an appropriate balance between safety, efficiency and invasiveness. It is natural to strive towards a non-surgical delivery option to make these potential treatments as safe as possible and attractive for patients. However, we must remember that the most important factor in delivering a

biologically-based treatment is getting enough of the therapeutic to the target to produce a clinically meaningful change.

CONFLICT OF INTEREST

Dr. Larson serves as a consultant for Aspen Neuroscience, Corlieve, Neurocrine Biosciences, and Sanofi; serves on the advisory board of Biogen and Sio (Axovant); and has grants from Brain Neurotherapy Bio, Neurocrine Biosciences, UniQure, and Voyager.

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