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REVIEW

Merging DBS with viral vector or stem cell implantation: "hybrid" stereotactic surgery as an evolution in the surgical treatment of Parkinson's disease

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Parkinson's disease (PD) is a complex neurodegenerative disorder that is currently managed using a broad array of symptom-based strategies. However, targeting its molecular origins represents the potential to discover disease-modifying therapies. Deep brain stimulation (DBS), a highly successful treatment modality for PD symptoms, addresses errant electrophysiological signaling pathways in the basal ganglia. In contrast, ongoing clinical trials testing gene and cell replacement therapies propose to protect or restore neuronal-based physiologic dopamine transmission in the striatum. Given promising new platforms to enhance target localization—such as interventional MRI-guided stereotaxy—the opportunity now exists to create hybrid therapies that combine DBS with gene therapy and/or cell implantation. In this mini-review, we discuss approaches used for central nervous system biologic delivery in PD patients in previous trials and propose a new set of strategies based on novel molecular targets. A multifaceted approach, if successful, may not only contribute to our understanding of PD pathology but could introduce a new era of disease modification.

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INTRODUCTION

The cardinal motor symptoms of Parkinson's disease (PD)—bradykinesia, rigidity, tremor, and postural instability—belie an extraordinarily complex disorder in which the etiopathogenesis is only partially understood.[1–5] Motor abnormalities in fact represent a relatively delayed feature of the disease. By the time a patient presents with motor symptoms, cyto- and histopathologic mechanisms have been advancing in both the central and peripheral nervous systems for up to a decade or more.[6] While current therapies are symptomatic and aimed at restoring neurochemical and electrical signaling within the basal ganglia (BG), efforts to address the genetic and molecular underpinnings of PD are still at relatively nascent stages.[7] In this mini-review, we will explore the landscape of current modalities of surgical PD treatments and discuss a novel therapeutic framework to be considered for evaluation in future human clinical trials.

FROM FETAL DOPAMINERGIC TRANSPLANTS TO STEM CELLS: AN HISTORICAL ACCOUNT OF DOPAMINE REPLACEMENT THROUGH CELL IMPLANTATION IN PD PATIENTS

The initial randomized, placebo-controlled trials to address the histopathologic basis of PD, *i.e.*, loss of nigrostriatal dopaminergic projections, utilized stereotactic injections of fetal mesencephalic tissue in PD patients versus a sham surgery.[8,9] These trials were designed utilizing data collected from over two decades of preclinical work establishing the efficacy of cell transplantation in Parkinsonian animal models and later humans.[10–18] Nevertheless, both trials resulted in a modest treatment effect—clinical benefit was shown only in a subpopulation of younger, less severely affected patients—and furthermore were associated with disabling dyskinesias in a subgroup of patients likely due to dopaminergic supersensitivity and graft-derived serotonergic hyperinnervation.[19]

Notwithstanding, several important themes emerged from these and prior open-label trials, including the successful demonstration of dopaminergic reinnervation in host tissue along with the ultimate amelioration of motor symptoms allowing a proportion of patients to achieve levodopa independence.[20–36] Additionally, Lindvall and Björklund[37] argue that insufficient dopaminergic cellular volume and production likely played a key role in the modest effect seen in the Freed and Olanow trials given the relatively low rate of fluorodopa uptake seen on subsequent positron emission tomography imaging. This, in addition to the lack of adequate immunosuppression and a patient cohort with severe disease burden, represented a significant divergence from previous trials and offers insight into

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some of the key factors that help determine graft survival. More recently, dopaminergic grafts implanted during these initial clinical trials have been recovered years later after the death of several patients and have demonstrated Lewy body and other pathologies characteristic of host tissue.[38,39] Motivated by these findings, Dehay *et al.*[40] argue that prevention of propagation of alpha-synuclein, the main component of Lewy bodies, may represent a novel therapeutic target for PD progression.

Building on the relative successes of these and other preclinical trials, Kim *et al.*[41] have proposed an approach utilizing stem cell technologies to address the shortcomings of fetal tissue transplants. First and foremost, use of stem cells solves the limitation posed by access and potential ethical considerations of fetal dopaminergic tissue. Moreover, stem cells substantially improve the substrate yield and consistency of transplants. This is likely to be a key factor in maximizing synaptic integration of dopaminergic (DA) neurons, since prior work has established the following principles: (i) in the natural course of PD, signs of motor deterioration follow a step function in that they appear after loss of ~70% of dopaminergic neurons in the substantia nigra pars compacta, a relatively consistent feature across symptomatic individuals[42] and (ii) following cell transplantation in the putamen, 50% uptake (~1/2 putaminal volume or ~100,000 tyrosine hydroxylase-positive neurons) must be achieved to improve motor symptoms.[43,44] A comprehensive strategy for re-attempting dopaminergic cellular implants may also include determining whether extrastriatal implantations along the mesolimbic dopaminergic pathway are warranted, since previous studies have suggested more effective transplant integration when this pathway is intact.[13,45] Finally, immunosuppressive therapy is being investigated as a possible therapeutic adjunct to enhance cellular integration and to answer the question of the role of inflammation in the native PD pathological mechanism.[46] Of course, an important underlying consideration with regard to efficacy will be to optimize patient selection, with an emphasis on younger individuals without advanced disease and severe dyskinesia. Already, several stem cell systems—including both embryonic stem cells and induced pluripotent stem cells—for repairing gene mutations exist, paving the way for the next round of human clinical trials using this promising methodology, especially among individuals with genetic forms of PD.[41,47–51]

The two approaches—fetal transplants and stem cells—continue development in parallel. In the European Union, the TRANSEURO trial and GForce-PD initiative continue to fund efforts to advance human fetal mesencephalic and stem cell transplants, respectively.[52] Thus, cell replacement as a strategy to treat PD remains an intensely active area of research with no consensus as of yet as to which represents the most promising path forward.

CAN GENE THERAPY CHANGE THE PARKINSONIAN BRAIN? STRATEGIES TO DIRECTLY CONTROL MOLECULAR BIOLOGY IN PD

The recent introduction of novel viral vector designs—such as adeno-associated viruses (AAV) and lentivirus—now make it possible to transduce neuronal populations within the central nervous system thus allowing for molecular modulation of key biological pathways. Accordingly, gene transfer technologies are being applied to PD in hopes of supporting dopaminergic neuron survival or modulating aberrant dopaminergic signaling within the BG. Viral vector-mediated gene therapies have so far largely targeted DA production pathways that originate in the substantia nigra pars compacta, namely, the enzymes responsible for DA synthesis such as tyrosine hydroxylase and aromatic amino acid decarboxylase, as well as neuroprotection through growth factor production including glial-derived neurotrophic factor and neurturin.[53–61] Another approach that has been employed is to attempt to mitigate excitatory glutamatergic outflow of the subthalamic nucleus (STN) by AAV-mediated delivery of glutamic acid decarboxylase (GAD) to the STN.[62] AAV-mediated delivery of GAD within STN projection neurons counterbalances excessive glutamatergic outflow to the globus pallidus interna (GPi) and substantia nigra reticulata. Using this approach, Lewitt and colleagues published initial results of a randomized, sham surgery–controlled, double-blinded phase 2 clinical trial involving 37 patients at seven centers in the United States. At the 6-month endpoint, the Unified Parkinson's Disease Rating Scale (UPDRS) total score for the AAV-GAD group (26.6, $n = 16$) was significantly lower than the sham-operated group (34.3, $n = 21$) ($P = 0.04$), demonstrating a short-term effect.[59–61] Nevertheless, a larger trial of AAV-GAD with longer follow-up is not planned, demonstrating a decline in optimism for gene therapy–based approaches that directly compete with deep brain stimulation (DBS). This is accompanied by several failed gene therapy trials using growth factor–based approaches.[63] As in the fetal cell studies, the lack of success in these trials is likely multifactorial including inadequate striatal coverage/tissue delivery of viral vectors, and the disease process itself mitigating growth factor signaling pathways.[64,65] Although the clinical trials have been unsuccessful, this important work has demonstrated the superior safety profiles of the use of viral vectors for delivery and expression of intraparenchymal biologicals in all trials to date. Much work remains to be done as a commercially viable product still does not exist.

HYBRID MODALITY STEREOTACTIC IMPLANTATION FOR PARKINSON'S DISEASE: RATIONALE FOR A NEW APPROACH

First approved by the Food and Drug Administration in 1997 for clinical use in PD patients, DBS represents the most successful symptomatic therapy to date for PD since the introduction of levodopa in the late 1960s.[66,67] Although the exact mechanism remains controversial, high-frequency stimulation of the STN or GPi modulates pathological BG circuits that ultimately result in improved motor control.[68,69] Part of the appeal of DBS technology is that it engenders a rapid improvement in symptoms and restores key quality-of-life measures for patients. But how long does this effect last? Long-term follow-up data show sustained improvement in certain UPDRS motor subscores—along with reduction in dyskinesias and levodopa equivalent dosages—up to 10 years post-implantation. However, Castrioto *et al*. reported that axial motor signs begin showing deterioration ~3 years following implantation likely due to progressive and unremitting pathology involving nondopaminergic pathways.[70,71] Derangement of verbal fluency, particularly following STN stimulation, and the emergence over time of other cognitive and nonmotor symptoms that do not respond to DBS have also been common findings in studies reporting long-term outcomes in DBS patients.[72] Overall, efficacy of hypokinesia reversal at 10 years is reduced to ~25% of preoperative baseline post-implantation.[70,73]

The potential benefit derived from combining DBS with stem cell or gene transfer technologies lies not only in the potential additive value of each of these modalities but it would also allow us to ask several fundamental questions about PD not possible with a monotherapy approach. The first such question is: What is the theoretical limit of effective motor control duration in PD patients? Moro *et al.*[74] suggest that patients with Parkin and PINK1 mutations may derive less benefit from bilateral STN stimulation in the first year

post-implantation than noncarriers (36 versus 56% motor UPDRS improvement). In their study, at 3–6 years, UPDRS scores normalized between the two groups (12 carriers versus 68 noncarriers) due to an increase in levodopa-induced dyskinesias and worsening axial symptoms in the noncarrier group. However, Piccini *et al.* followed a patient with unilateral putaminal embryonic implants and found using positron emission tomography that even after 10 years dopaminergic transmission remained at equivalent levels to the unimplanted side.[31,75–77] The upper limit of therapeutic efficacy using cell and gene therapy is unknown, with most trials achieving on average 2–5 years of follow-up. However, in a scheme wherein DBS is combined with these alternative modalities, effective motor control would be expected for at least 10 or more years from DBS alone, while the temporal dynamics of cell and gene therapy protocols can be evaluated on much longer timescales, for example, 15–20 years.

A second basic question concerning a hybrid stereotactic approach is: What would be the optimal sites for implantation in a combined DBS and biologic approach? We know from systematic analyses of DBS outcome data that while STN and GPi are ideal stimulation sites for dyskinesia and tremor control,[73] other targets that may be considered are the thalamic centromedian/parafascicular complex and caudal zona incerta.[78–80] Additionally, sites such as the pedunculopontine nucleus have shown some promise in selected cases in ameliorating gait and nonmotor symptoms.[81–87] Therefore, a variety of potential configurations for a hybrid stereotactic approach could be considered; for example, DBS lead implantation in GPi and GAD-based viral vector implantation into STN or stem cell delivery to the post-commissural putamen.[88] Alternatively, STN DBS could be considered in conjunction with STN cellular implants (see below) or striatal and nigral neurotrophic support mediated by viral vector platforms or modified cell therapies.

Perhaps the most important question highlighting the difference between this proposed approach and traditional single therapy approaches would be: Can we take advantage of possible synergism between DBS and molecular modulation of biological pathways? In stem cell cultures, several recent studies have documented enhanced neuronal proliferation, differentiation, and migration in response to an applied electric field.[89–99] Wang *et al.* demonstrated in olfactory bulb neural precursor cells that a biphasic electrical stimulation paradigm prevented apoptotic-induced cell death through activation of the PI3K/Akt (phosphatidylinositol 3′-kinase) pathway and brain-derived neurotrophic factor production.[100] Although not directly proven in dopaminergic neurons, the early conclusion is that electrical stimulation is an important mediator of stem cell transplantation survival through engagement of mechanisms promoting growth and differentiation and prevention of early death.

In animals, this question has been tested directly in a recent study in which rodents were implanted with DBS leads in the anterior nucleus of the thalamus, a site of stimulation for patients with medically intractable epilepsy. Neurons in the dentate gyrus, connected to the anterior nucleus of the thalamus through the fornix, showed a multi-fold increase in the number of new hippocampal neurons versus animals undergoing sham surgery. A separate arm showed that pharmacologic suppression of hippocampal neurogenesis could be rescued by anterior nucleus of the thalamus electrical stimulation.[101,102] Stimulation of another limbic target, the entorhinal cortex, also promotes neurogenesis within the dentate gyrus, and this stimulation-induced neurogenesis likely facilitates spatial memory as assessed in a water-maze test.[103] In many other animal models, including PD, a rapidly expanding literature has elaborated the modulation of expression of a variety of genes including transcription and trophic factors by DBS.[104–113] Thus, merging DBS with biologics has considerable potential and holds a distinct advantage over monotherapy approaches, of simultaneously addressing both immediate (pathological BG circuits) and long-term (pathological molecular pathways) PD mechanisms.

DBS CONTINUES TO EVOLVE ALONG WITH ADVANCED NEUROIMAGING PROCEDURES

Most DBS outcome studies show that implanting a single target on one side of the brain, *e.g.*, GPi or STN, is effective in controlling primarily contralateral body symptoms. However, in several published studies, some authors have used more than one target in a single side of the brain, *e.g.*, STN and pedunculopontine nucleus, to treat multiple and/or refractory symptoms, such as tremor and gait imbalance.[114,115] Thus, therapeutic interventions at multiple nodes within the BG can be additive. Furthermore, several developing technical platforms are aimed at more accurate and safe stereotactic targeting using either DBS or volume delivery of cells or gene vectors. For example, interventional MRI has been adapted for use with DBS.[116,117] With this technique, patients are placed under general anesthesia, and DBS leads are implanted while patients are in the bore of an MRI scanner using rapid MRI sequences that can be updated every few minutes. The advantage of this technique over traditional DBS surgery is that final placement of the lead is demonstrated in real time and can be adjusted if necessary prior to the end of the case (versus a separate operation to revise the lead). A second advantage of this technique is that the same targeting devices developed specifically for use with interventional MRI, for example, the ClearPoint, SmartFrame, and SmartFlow devices, can be used to safely infuse substrate containing stem cells or viral vectors intraparenchymally. This has already been demonstrated in both nonhuman primate models and humans.[118]

In summary, there is reasonable preliminary evidence that DBS itself can modulate not only neurophysiological aspects of pathologic circuits but also gene expression, neurogenesis and stem cell biology on a variety of time scales. Thus, a promising avenue of investigation will be further development of hybrid treatments that combine DBS with biological therapies. These hybrid treatments could be readily delivered using conventional or MRI-guided approaches and address issues identified with current approaches. As such, combined approaches would provide the following clinically and biologically relevant advances: (i) the ability to enhance the delivery of a therapeutic agent (spatially and temporally), (ii) the possibility of spatiotemporal control of a biological therapeutic, and (iii) the potential to develop novel therapies that would have immediate symptomatic benefit but may also mitigate neurodegeneration in the long term.

CONCLUSION

Although effective symptomatic treatments for PD exist, a disease-modifying approach is still lacking. Current cell transplantation and gene therapy trials have offered a glimpse of this prospect. However, future generations of these modalities must continue to evolve if they are to become viable treatment options for PD patients. Fortunately, alternate cellular and molecular strategies exist and suggest that we have not yet exhausted the possibilities for designing an effective cell- or gene-based therapy for PD. Hybrid approaches incorporating DBS lead implantation in conjunction with stem cell or viral vector therapeutics may capitalize on the additive contribution from each modality, given the complementary time frames on which each may achieve the optimal

effect. Additionally, it may be more cost effective to combine these approaches into a single treatment in which fundamental questions regarding the utility of hybrid stereotactic surgery can be addressed. These include extending effective motor control beyond what is currently possible with single modality therapy, determining optimal implantation sites, and possibly lowering the use of levodopa therapy and accompanying dyskinesias. The ultimate objective is to design a therapeutic approach that provides the crucial answers needed to advance PD treatment from control of symptoms to control of the disease.

CONFLICT OF INTEREST

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