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Recent advances in cell-based therapy for Parkinson disease

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✓In this review, the authors discuss recent advances in the field of cell therapy for Parkinson disease (PD). They compare and contrast recent clinical trials using fetal dopaminergic neurons. They attribute differences in cell preparation techniques, cell type specification, and immunosuppression as reasons for variable outcome and for some of the side effects observed in these clinical trials. To address ethical, practical, and technical issues related to the use of fetal cell sources, alternative sources of therapeutic dopaminergic neurons are being developed. The authors describe the progress in enrichment and purification strategies of stem cell–derived dopaminergic midbrain neurons. They conclude that recent advances in cell therapy for PD will create a viable long-term treatment option for synaptic repair for this debilitating disease. *(DOI: 10.3171/FOC/2008/24/3-4/E5)*

KEY WORDS • dopamine • Parkinson disease • stem cell • transplantation • ventral mesencephalon

PARKINSON disease is a debilitating neurodegenerative disorder characterized by the selective loss of nigrostriatal dopaminergic neurons and loss of DA in the striatum.[19] Different groups of DA neurons in the ventral midbrain exhibit differences in susceptibility to degeneration during PD. The most susceptible DA neurons are the A9 group found in the ventral tier of the substantia nigra pars compacta.[25,48,50,53] The A9 DA neurons project to the motor striatum and differ in gene expression from the less susceptible A10 DA neurons of the VTA, which project to the nucleus accumbens and limbic regions.[8,14,15,25,48,50,53] In particular, genes associated with energy-related metabolism, mitochondrial protein expression, and vesicle-mediated transport have been shown to vary between cell groups, thereby offering a clue to the selective vulnerability observed in the A9 neuronal subtype in PD.[8]

To combat the progressive loss of DA in patients with PD, current therapies focus on pharmacological substitution of DA using levodopa, which produces substantial clinical benefits for some years.[19] Pharmacological substitution, however, is eventually associated with reduced clinical benefits and troubling motor complications known as levodopa–induced dyskinesias.[19] Similarly, DBS of the pallidum or STN is a reversible and effective way to control the cardinal motor symptoms of PD,[44,45,72] but as disease progresses, a decline in treatment efficacy may be observed, and long-term benefits of pallidal or STN stimulation beyond 4–5 years remain to be established.[36,40,43,59,62,68,69,83]

Abbreviations used in this paper: DA = dopamine; DBS = deep brain stimulation; ESC = embryonic stem cell; FACS = fluorescence-activated cell sorting; NCAM = neural cell adhesion molecule; PD = Parkinson disease; PET = positron emission tomography; STN = subthalamic nucleus; SVZ = subventricular zone; VM = ventral mesencephalic;VTA = ventral tegmental area;.

What Is Cell Therapy and What Is Its Advantage Over Pharmacology?

As an alternative therapeutic strategy to pharmacological substitution or high-frequency deep brain electrical stimulation, cell-based therapies have been developed. Although obtaining and delivering the appropriate cell types to patients is challenging, the potential therapeutic benefit of physiological DA release over a sustained period of time is considerable. For patients with PD, initial studies focused on transplanting readily available cell sources, such as catecholaminergic adrenal medullary tissue into the striatum, but limited clinical benefits were observed, and survival of grafted cells was poor.[1,30,49,82] A major advancement in the field came with the use of fetal VM tissue as a cell source for transplantation. Fetal DA neurons transplanted into the striatum of patients with PD have survived, integrated, and provided motor benefits.[20,21,26,37,38,46,47,51,53,57,61,63,64,76,84] In this review, we discuss recent advances in the field of cell therapy for PD and describe technical considerations for improving clinical outcomes.

Influence of Cell Preparation on Patient Response

A variety of cell preparation methods have been used for fetal VM transplantation. The first fetal neural transplanta-

tion studies utilized dissociated fetal VM tissue transplanted into the striatum of patients in whom PD had developed after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine exposure.[84] These young patients exhibited marked motor improvement and an increase of fluorodopa uptake in the striatum on PET.[84] Indirect evidence of graft survival from fluorodopa uptake and postsynaptic DA receptor occupancy PET studies has indicated that fetal VM tissue transplanted into the striatum can survive for up to a decade and provide sustained motor benefits in PD.[63] However, few postmortem studies of the survival and integration of grafted DA neurons exist.

Our group has recently reported the first postmortem study of the survival and integration of DA neurons grafted as cell suspensions into the brains of 2 patients with advanced PD.[53] Human fetal VM tissue from aborted fetuses was dissected and incubated in hibernation medium supplemented with glial derived neurotrophic factor before dissociation for transplantation. Cells were bilaterally transplanted, using a custom-made transplantation cannula and microinjector system,[52] into the putamen of one patient and unilaterally into the striatum and ventral midbrain of another patient,[51] Both patients displayed marked clinical improvement without side effects, and fluorodopa uptake was significantly increased in the grafted regions. The patients died of unrelated causes 3.5 and 4.5 years, respectively, after transplantation. Postmortem analyses revealed robust graft survival and dense reinnervation in the grafted regions, with striatal grafts containing between 100,000 and 200,000 DA neurons and the nigral graft containing approximately 4000 DA neurons as demonstrated by immunoreactivity to tyrosine hydroxylase, the rate-limiting enzyme in DA synthesis. Of these transplanted DA neurons, approximately 70% exhibited an A9 phenotype as demonstrated by co-immunoreactivity with the G-protein coupled inward rectifying current potassium channel type 2 (GIRK-2). Furthermore, the transplanted A9 DA neurons were predominantly distributed in the periphery of the graft, where they were able to make connections with the host. In conclusion, it was shown that DA neuronal suspension grafts can survive, integrate and display the mature VM phenotypes, and provide functional benefits in the degenerating brain for years.[53] Importantly, these patients did not exhibit side effects such as dyskinesia.[53]

In some cases, transplantation of solid pieces of human fetal VM tissue has been beneficial and long-term survival of transplanted cells has been verified postmortem. One such patient survived for 18 months after transplantation of solid fetal VM tissue into the putamen.[38] Postmortem, numerous DA neurons were observed with partial reinnervation of the putamen.[38,39] Furthermore, electron microscopic examination revealed numerous synapses between graft and host neurons.[39] This histological data was associated with marked motor improvement and increased fluorodopa uptake in this patient.[38,39] In another postmortem analysis from the same group, a patient who survived for 19 months after transplantation had large grafts with extensive reinnervation of the putamen bilaterally, associated with marked motor improvement and increased striatal uptake of fluorodopa.[37]

The results from 2 US clinical trials of solid fetal VM tissue transplantation for PD have been reported. In the first trial, Freed et al.[21] utilized a fetal tissue preparation technique of solid tissue strands incubated in tissue culture medium for up to 4 weeks before transplantation. Forty patients either received bilateral putaminal transplants of fetal VM tissue from 2 embryos per side or received sham surgery. Robust graft survival was demonstrated by PET and confirmed postmortem. Although some objective clinical improvement was observed on standard rating scales for the younger but not the older patients, the study failed to meet its primary endpoint of clinical improvement on a patient self-report scale at 1 year after transplantation. Furthermore, troubling off-period dyskinesias were observed in 15% of the patients receiving neural transplantation.[21]

In the second trial, Olanow et al.[57] randomly assigned 34 patients to receive intraputaminal solid fetal VM tissue from 1 or 4 donors per side or undergo a placebo procedure. Fetal tissue was not cultured and was transplanted within 48 hours of harvest. Striatal fluorodopa uptake was significantly increased in both the 1- and 4-donor transplantation groups, and robust survival of DA neurons was found at postmortem examination of 4 patients. Nevertheless, the study failed to meet its primary endpoint. There was no significant improvement in the motor component of the Unified Parkinson's Disease Rating Scale, although a treatment effect was observed in patients with milder disease. In addition, off-medication dyskinesias were observed in as many as 56% of the patients who received transplants.

Although these trials of solid tissue transplantation failed to show significant clinical benefit, one of these studies was concluded after only 1 year, which is an insufficient period of time for the growth and integration of human fetal DA neurons and the development of functional effects. Indeed, some of these patients showed clinical improvement 2–3 years after transplantation surgery, consistent with observations from other clinical studies.[21,32,33]

In an attempt to overcome some of the limitations associated with obtaining human fetal neural tissue, a clinical trial of porcine VM tissue transplantation into the striatum of 12 patients with advanced PD was conducted.[71] This treatment was associated with some clinical benefits. Postmortem analysis in one of these patients 7 months after transplantation showed porcine neural cells that had extended axons and produced synapses in the host striatum. Although graft size was relatively small, this was the first study to demonstrate that neural xenografts could survive and provide appropriate growth of DA neurons in a patient's brain.

Immunosuppression in Clinical Trials

Although immunosuppression is required for xenotransplantation studies,[23,71] the need for immunosuppression in allografting trials remains controversial. Evidence indicates that differences in cell preparation techniques influence the host immune response to the grafted cells. For example, a recent study of parkinsonian non-human primates showed that solid fetal VM allografts elicited a stronger glial reaction than cell-suspension grafts.[67] Similarly, patients who received solid fetal VM grafts exhibited pronounced host immunological and microglial reactions postmortem.[21,57] Although immunosuppression was not used in one of these studies,[21] in the other study a 6-month course of immunosuppression was used, and clinical deterioration coincided

with the withdrawal of immunosuppression.[57] In contrast, cell-suspension grafts elicited a minor host immune response, and clinical benefits were sustained after 6 months of standard immune suppression.[53] The reduced host immune response observed for cell-suspension grafts compared with solid tissue grafts is most likely due to major histocompatibility complex Class I. Major histocompatibility complex Class I is expressed by donor blood vessels that predominate in solid tissue grafts. In contrast, host-derived angiogenic processes predominate in cell-suspension grafts.[24,42]

Dyskinesias in Clinical Trials

Prolonged levodopa treatment causes many patients to develop disabling motor complications known as dyskinesias.[19,55,58] In the 2 large clinical transplantation trials, off-period dyskinesias were observed in 15%[21] and 56%[57] of the patients who received transplants. In contrast, off-period dyskinesias were not observed in the 2 patients treated with fetal VM cell suspensions and reported on by our group, and one of these patients displayed improvement of dyskinesias after transplantation[53] consistent with observations from rodent transplantation studies.[41] It is possible that differences in cell-preparation techniques, grafting methods, and final cell composition play a major role in the differential development of off-period dyskinesias in some of the patients who received transplants. In the 2 trials in which dyskinesias were observed, solid—not dissociated—tissue pieces were transplanted, raising the possibility that cotransplantation of other cell types not relevant for ventral midbrain neural grafting contributed to the development of dyskinesias. Furthermore, one of these trials utilized a cell-culture step for up to 4 weeks before transplantation. Under such conditions it is conceivable that the less-vulnerable A10 DA neurons were selected at the expense of the more vulnerable but more appropriate A9 DA neurons. The A9 neurons have a better capacity to control the physiological release and synaptic availability of DA than the A10 neurons because of the higher presynaptic DA autoreceptor and DA transporter levels that occur in A9 neurons.[31,33]

Recently new light has been shed on the role of other cell types in the evolution of dyskinesias. Carta et al.[7] demonstrated that the serotonergic terminals in the DA-denervated striatum are responsible for levodopa-induced dyskinesias in rodents and that either lesioning the serotonergic afferents or dampening the serotonergic system with the serotonergic 5-HT1A and 5-HT1B autoreceptor agonists suppressed levodopa-induced dyskinesias. The same group subsequently demonstrated that serotonin neurons grafted into rodents cause a marked worsening in levodopa-induced dyskinesias when grafts contain little or no DA neurons.[6] In contrast, DA-rich grafts were able to markedly reduce levodopa-induced dyskinesias, regardless of the number of serotonin neurons grafted.[6] It is plausible that the interaction between serotonin and DA terminals may further explain the variable outcome with respect to levodopa-induced dyskinesias that has been observed in clinical trials, emphasizing the importance of controlling and optimizing the cell composition used for clinical neural transplantation.[6,31]

Alternative Sources of DA Neurons for Transplantation

Major obstacles have hindered the use of fetal tissue as a transplantable cell source. Primarily, the use of fetal tissue raises ethical concerns. Secondly, the routine use of aborted fetuses as a reliable cell source is not practical because of the low yield of DA neurons. Tissue from several fetuses is required for a single patient.[33] Alternatively, ESCs may provide an unlimited source of DA neurons for transplantation.[31,75] Embryonic stem cells are self-renewing, pluripotent cells from the inner cell mass of the preimplantation blastocyst.[16] These characteristics have established ESCs as an optimal source of transplantable immature DA neurons. Initial studies used mouse ESCs for transplantation into rodent models of PD. Whether transplanted into the brain or kidney capsule, mouse ESCs differentiated into all cell lineages, including DA neurons.[16] When transplanted at low density and dose into the striatum of hemiparkinsonian rats, mouse ESCs developed by default into large neuronal grafts that contained DA neurons.[4,74] The ESC-derived DA neurons matured and expressed markers typical for both A9 and A10 DA neuron subpopulations. Importantly, these transplanted DA neurons restored striatal DA storage capacity and cortical motor activation as measured by PET and functional magnetic resonance imaging, respectively. Furthermore, a gradual recovery from amphetamine-induced motor asymmetry mirrored the differentiation and maturation of the transplanted DA neurons.[4]

To increase the yield of ventral midbrain neurons in vitro, other studies of mouse ESCs have employed genetic strategies using factors important for the development of A9 DA neurons. For example, overexpression of the transcription factor Nurr1 significantly improved the differentiation of mouse ESCs toward midbrain DA neuron phenotypes.[9,11] Furthermore, overexpression of the homeodomain transcription factor Pitx3 preferentially enhanced differentiation into the A9 DA neuron phenotype.[9]

More recently, human ESCs have become the focus of attention. When human neural precursor cells derived from human ESCs were grafted into hemiparkinsonian rats, a small fraction of the grafted cells developed into DA neurons, causing partial behavioral recovery.[3] Subsequently, enrichment for a DA neuronal population prior to transplantation has been accomplished by several groups.[60,70,77,86,87] For example, Perrier et al.[60] demonstrated that coculture of human ESCs on mouse stromal feeder cells yielded neuroepithelial cells that could be patterned toward ventral midbrain and hindbrain fate and terminally differentiated into midbrain DA neurons.[60] The addition of the bone morphogenic protein antagonist Noggin further markedly enhances the development of neuroepithelial precursors from human ESCs and favors DA neurogenesis.[73]

A major concern in ESC-derived neural transplantation is the potential risk of tumor formation. Transplantation of human ESC-derived cells into parkinsonian rats has resulted in teratoma formation in some cases.[5,70,73] As a step toward using ESC-derived DA neurons in the clinic, a selection technique is required to eliminate the risk of tumor formation by immature cells and to enrich for DA neurons.[31,33,65]

Fluorescence Activated Cell Sorting

Fluorescence activated cell sorting, initially developed for hematological research and analysis,[28] has recently been applied to select the progeny of human ESCs at different stages of neuronal differentiation based on the expression of specific cell-surface markers. During neuronal differentiation, the surface molecules CD24 and neural cell adhesion molecule (NCAM) are upregulated. Therefore, these neuronal markers can be used for positive selection strategies prior to transplantation.[66] In addition, genetic engineering of ESCs has been combined with FACS sorting. For instance, mouse ESCs overexpressing the proneural gene *Sox1* with a fluorescent marker were selected using FACS. This resulted in the isolation of neural progenitor cells and the elimination of tumorigenic pluripotent stem cells in vitro and after transplantation.[10] Furthermore, FACS of differentiated mouse ESCs expressing a fluorescent protein under the genetic control of the A9 DA neuron transcription factor Pitx3 yielded a highly enriched neuronal population expressing markers of the A9 DA neuron phenotype. When transplanted into hemiparkinsonian rats, these isolated DA neurons survived and restored motor symmetry.[27]

Non-ESC Sources for Cell Transplantation

An alternative source of donor cells may be obtained through manipulating easily accessible fibroblasts. These non-neural cells would require considerable manipulation to overcome previous differentiation cues. Although cells exhibiting some characteristics of dopaminergic neurons have been derived from mesenchymal sources, the functional significance of these cells when transplanted into models of PD remains to be examined.[79] A more promising approach may involve reprogramming adult cells to become ESC-like.[78] A proof of principle hereof was recently offered by some unexpected results observed in mice. Induced pluripotent stem cells were created by introducing the transcription factors Oct-3/4 and Sox2 and the genes *c-Myc* and *Klf4* into mouse embryonic or adult fibroblasts.[78] The transcription factor Nanog conferred a similar response.[56] The function and safety of DA neurons derived from induced pluripotent stem cells remains to be tested in animal models of PD, and translation of this technique to use in human cells will also be of clinical significance.

Using Endogenous Cells for Cell Therapy

An intriguing alternative approach to cell therapy for PD is to instruct DA neurogenesis using endogenous stem cells. In the substantia nigra of patients and PD models, there is no compelling evidence for endogenous progenitor cells replacing A9 DA neurons,[12,22] but endogenous stem cells generate new neurons in other regions of the adult brain, namely the olfactory bulb and the dentate gyrus of the hippocampus.[34,54] Transplantation studies of fetal DA neurons in the patient's putamen and PD models have provided a proof of principle that new DA neurons in the putamen/striatum can improve motor symptoms. The SVZ is medial to these striatal targets; thus instructive cues to the SVZ may expand the neurogenic niche to include the DA-depleted striatum.[12]

In the adult rodent and human brain, stem cells in the SVZ generate neuroblasts that migrate and integrate into the olfactory bulb.[13] Preliminary studies in rodent models of PD have examined the induction of proliferation and migration by exogenous factors.[12,18] Intraventricular infusions of epidermal growth factor–receptor agonists such as epidermal growth factor and transforming growth factor-α increase proliferation and cell migration from the SVZ.[12,18] Studies in rodent models and PD patients have shown that DA contributes to the proliferation of endogenous progenitor cells in the SVZ.[2,29,85] Dopamine D2 and D3 receptors regulate cell proliferation in the adult SVZ.[35,80] The results of 2 controversial studies have suggested, however, that intraventricular infusions of the DA D3 receptor agonist, 7-hydroxy-N,N-di-n-propyl-2-aminotetralin (7-OH-DPAT) can generate new dopaminergic neurons in the substantia nigra pars compacta.[80,81] Our interpretation of those studies is is that the results are due to a reactivation of the DA phenotype in damaged neurons rather than the generation of new DA neurons. Further studies of DA receptor modulation in the non-neurogenic substantia nigra are required to understand the potential therapeutic benefits from administration of this and other drugs.

Future Directions, Toward Clinical Applications

Cell therapy for PD has significantly advanced in recent years. Open-label clinical trials have provided proof of principle that transplantation of fetal DA neurons can improve patients' neurological symptoms.[20,26,37,38,46,47,51,53,61,63,64,76,84] Also, 2 heralded "double-blind" clinical trials have shown benefits in subgroups of patients.[21,57] We propose that technical improvements in cell preparation and delivery will standardize this potential clinical outcome and reduce the risk of side effects. To address ethical, practical, and technical issues regarding the use of fetal tissue for transplantation, alternative sources of therapeutic DA neurons are being developed. Improvements in functional and safety considerations with respect to these alternative cell sources are now being rigorously studied. The neurosurgical community needs to stay aware of advances in the field of restorative neurological surgery as a viable long-term alternative to pharmacological substitution or DBS. The pace of innovation will continue to drive cell therapy toward exciting clinical applications for PD.

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