Stem Cell Therapy for Parkinson’s Disease: A Road Map for a Successful Future

Román Vidaltamayo,1,2 José Bargas,1,4 Luis Covarrubias,1,3 Arturo Hernández,1,4 Elvira Galarraga,1,4 Gabriel Gutiérrez-Ospina,1,5 and René Drucker-Colín1,2

Cell transplant therapy for Parkinson’s disease (PD) has been in use for over 2 decades as an experimental treatment. Different cell types have been proposed as better therapeutic alternatives. However, source availability and therapeutic value of the transplants as compared to current pharmacological options have precluded the use of this kind of surgery in the majority of PD patients. In this article, we discuss the suitability of different types of stem cells for PD therapy, the requirements that the donor cells should fulfill in order to improve upon current methods, and propose alternatives for evaluating the efficacy of PD cell therapy.

Introduction

Cell therapy for Parkinson’s disease (PD) has been in use for over 2 decades, primarily as an experimental therapeutic alternative. Limited availability of donor tissue and modest clinical outcomes has precluded widespread recommendation for every PD patient. The question arises as to why transplantable cells have had limited success.

In this work, we review the current state of cell therapy for PD and we discuss the perspectives of using stem cell technology to generate better sources of dopaminergic tissue and the feasibility of testing the functionality of grafted tissue using corticostriatal slices of affected brains.

Partial Success of Current Cell Therapy for PD Could be Related to Limitations in Standardized Donor Material

Several longitudinal studies have analyzed over 300 PD patients transplanted with various dopaminergic cell types, including fetal and cadaveric donor adrenal gland tissue (reviewed in [1]) and fetal mesencephalic tissue (reviewed in [2]), the latter having become the preferred source of dopaminergic cells for this experimental therapy. Most of the open label studies were designed to serve as proof of principle that dopamine (DA)-producing cells can secrete sufficient levels of neurotransmitter to control motor symptoms in the patients. The primary end point was, in most of the experimental designs, improvement of the motor symptoms in the off-phase of pharmacological therapy [3–6].

Most of the grafted patients that showed positive clinical outcomes during the off-period of drug therapy improved their motor symptoms by 30%–60%, as measured by the United Parkinson’s Disease Rating Scale (UPDRS), regardless of the source of dopaminergic tissue used, with similar improvements observed with fetal adrenal and mesencephalic tissue. However, there is limited availability of fetal tissue due to either ethical or moral issues [3–9].

Using mesencephalic fetal tissue grafts, clinical benefits can be observed 1 year after transplantation, but recently it has been suggested that the maximal motor improvement occurs 2–3 years after surgery [5,10,11]. In this regard, long-term survival and activity (as measured by dopamine recapture imaging) of the grafted tissue, correlating with motor symptoms relief, has been observed up to 14 years after transplantation [12].

However, recent double-blind studies have shown that there is no significant improvement in the clinical outcome of transplanted patients when compared to placebo surgery, especially in patients with more severe motor deficits [3,13].

Moreover, some patients receiving dopaminergic tissue grafts tend to develop severe dyskinesias, which require further surgery (such as pallidotomy or deep brain stimulation) for their clinical management [2,3].
Similarly, in animal PD models (rodents and nonhuman primates), cell therapy shows limited success. Nearly all transplanted animals show improvements in motor symptoms, decreasing after transplantation by roughly 50% or better, as demonstrated by different behavioral assessments [1,14–23] (see Table 1).

Thus, the clinical observations and the animal experimental data suggest that current cell therapy strategies have probably reached its higher limit in clinical benefit for PD and that alternative strategies should be designed to overcome the hurdles imposed by the lack of standardized dopaminergic tissue preparations.

It is well accepted that the adverse effects, and the lack of clear clinical advantage of cell therapy for PD, are related to the nature of donor tissue used [2]. Dyskinesias are thought to be caused by the presence of serotoninergic cells in the mesencephalic tissue or the formation of “hot spots” of high dopamine secretion by the grafted cells among silent areas of the striatum [24–26]. In order to overcome these limitations, it is necessary to have access to a more homogeneous source of dopaminergic cells, which express the correct phenotype of nigrostriatal neurons, since some of the dopaminergic neurons of fetal mesencephalic tissue belong to the ventral tegmental (A10) area as opposed to the nigrostriatal (A9) area [27–31]. Preparations currently in use vary from 1% to 10% of total cells belonging to dopaminergic lineage [4,6,11]. These cells should show correct regulation of dopamine secretion, such as feedback by dopamine transporter [27–31]. Furthermore, a homogeneous source of nigrostriatal, dopamine-secreting neurons will allow for standardization of the surgical procedure in order to avoid the formation of hot spots and to ensure long-term survival and decrease rejection of the graft. Stem cell technologies represent our best option to achieve the goal of implementing cell therapy as a viable option for PD patients.

### Table 1. Motor Symptoms of Rodent Models of PD Decrease Roughly 50% After Transplantation of Various Kinds of Dopaminergic Tissue

<table>
<thead>
<tr>
<th>Cell source</th>
<th># Publications</th>
<th>Motor benefit (% decrease in rotational behavior)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal substantia nigra</td>
<td>38</td>
<td>45%–75%</td>
</tr>
<tr>
<td>Adrenal medulla</td>
<td>13</td>
<td>30%–45%</td>
</tr>
<tr>
<td>Fetal ventral mesencephalon</td>
<td>49</td>
<td>45%–77%</td>
</tr>
<tr>
<td>Human ventral mesencephalon</td>
<td>3</td>
<td>40%–65%</td>
</tr>
<tr>
<td>Superior cervical ganglion</td>
<td>3</td>
<td>30%–40%</td>
</tr>
<tr>
<td>Genetically engineered cells</td>
<td>6</td>
<td>35%–45%</td>
</tr>
<tr>
<td>Syngeneic bone marrow</td>
<td>1</td>
<td>30%–40%</td>
</tr>
<tr>
<td>Xenogeneic dopaminergic</td>
<td>3</td>
<td>35%–55%</td>
</tr>
<tr>
<td>Neonatal SVZ</td>
<td>3</td>
<td>35%–55%</td>
</tr>
<tr>
<td>Adrenal cell lines</td>
<td>3</td>
<td>35%–40%</td>
</tr>
<tr>
<td>Encapsulated cell lines</td>
<td>2</td>
<td>35%–40%</td>
</tr>
<tr>
<td>Neuronal cell lines</td>
<td>2</td>
<td>35%–65%</td>
</tr>
<tr>
<td>Neural precursor cells</td>
<td>7</td>
<td>45%–80%</td>
</tr>
<tr>
<td>ES and iPS cells</td>
<td>10</td>
<td>50%–70%</td>
</tr>
<tr>
<td>Total</td>
<td>143</td>
<td></td>
</tr>
</tbody>
</table>

A non-exhaustive, albeit characteristic, list of published articles show that current cell therapy approaches in rodent models of Parkinson’s disease (PD) have moderate benefits on motor symptoms. It is clear that rotational behavior improvements are slightly better than 50%, although almost every transplanted animal responds to treatment.

Abbreviations: ES, embryonic stem; iPS, induced pluripotent stem; PD, Parkinson’s disease; SVZ, subventricular zone.

**What Kind of Stem Cell Is Best Suited to Provide Nigrostriatal Dopaminergic Neurons?**

Different cell types have been proposed as viable candidates to generate mature nigrostriatal neurons suitable for transplant. Adult neural stem cells, obtained from the subventricular zone (SVZ), appeared to be the best candidates, because: (a) their natural developmental fate is to become dopaminergic neurons in the olfactory bulb, (b) cell proliferation in the SVZ is readily increased in response to different kind of injuries to the brain (see Fig. 1), and (c) SVZ cells can be cultured and expanded as neural precursor cells (NPCs) in vitro, which could lead to a steady production of source material for transplants [32,33].

The capability of SVZ neuroblasts to differentiate into dopaminergic neurons in the olfactory bulb could be the major reason to use them to regenerate the dopaminergic input to the Parkinson’s affected striatum. However, the particular dopaminergic phenotype of the bulb interneurons could be inappropriate for this task. These interneurons secrete GABA on top of dopamine and their dopamine output appears to be constant, and these 2 features could actually be detrimental to a regulatory role within the striatum [34–36]. Moreover, it has been suggested that the expression of the paired-like transcription factor Pitx3 is necessary to achieve the correct phenotype of substantia nigra (SN/A9) dopaminergic neurons [37–40]. In Pitx3 null mutants SN/A9 dopaminergic neurons fail to develop [40] and it is clear that periglomerular and granule cells in the olfactory bulb, which represent the two major dopaminergic neuron types in this tissue, do not express Pitx3 (http://www.gensat.org) [41].

In this respect, data from one of us (GGO) show that there is a population of neural stem cells residing in the RMS, which generate preferentially periglomerular interneurons...
of the bulb [42]. This population could be activated in response to insult to the brain, the same way proliferation in the SVZ increases after different types of noxious stimuli to the central nervous system, such as hypoxia, and exposure to neurotoxins, such as 6-hydroxydopamine (6-OH-DA), the widely used drug to induce parkinsonism in animals [32,43,44].

This increased proliferation in the SVZ and within the RMS could correlate with the increase in number of periglomerular cells observed in animal models of PD [45] and could also explain the increases observed in the bulb of Parkinson’s patients postmortem [46], which have been related to the olfactory deficit observed in these patients [44,46–50].

Taken together, all these observations suggest that the differentiation potential of cells arising from the SVZ is limited to the particular subset of interneurons in the olfactory bulb and even if we could generate enough NPCs in vitro, these adult neural stem cells would not fulfill all the requirements to become the source of choice for PD cell therapy.

The next potential candidate for cell replacement in the nigrostriatal system was embryonic stem cells (ESC). The “default” pathway of differentiation of these cells is to become neural cells, and it has been shown that ESCs in culture easily differentiate into NPCs when exposed to inducing signals, such as FGF [51–55]. When these “primed” ESCs are transplanted into the striatum of 6-OH-DA-injured animals, they can improve the rotational behavior and they appear to innervate the host striatum and fire action potentials [17]. However, one major disadvantage of using ESCs for transplantation is the tendency of the grafted tissue to form tumors, primarily teratocarcinomas derived from undifferentiated ESCs still present in the transplanted population [56–58]. Tumor growth can be prevented by separation of pluripotent cells from the ones that have initiated differentiation, either by fluorescence or magnetic-activated cell sorting (FACS or MACS), or by inducing that all of the transplanted cells to have committed to the dopaminergic SN/A9 differentiation pathway [58].

In order to induce this correct fate, it is required that we identify all the cell mechanisms involved in defining the SN/A9 phenotype, especially those signals present in the developing midbrain that lead to the correct program of gene expression to be activated, so we can reproduce this permissive environment in vitro. Data from one of us (LC) shows that such a permissive environment exists in the developing midline of the ventral midbrain, which can induce differentiation of ESCs into dopaminergic midbrain neurons [59]. For ESCs to be responsive to the environmental cues of the ventral embryonic midbrain, the cells should be committed to an ectodermal fate, which is achieved by culturing them as embryoid bodies (EB).

When dissociated cells from these EBs are seeded on top of a flap derived from the midbrain region of E10.5 embryos, a large fraction, nearly 40%, of the inoculated cells differentiate into Lmx1a+/Pitx3+/Th+ neurons, which corresponds
to the expression profile of SN/A9 dopaminergic cells, only in the region (ventral midline) where naturally occurring DA neurons arise. If transplanted EB cells fall in a midbrain region outside the ventral midline, the cells instead acquire the phenotype of the neurons surrounding them (within the Nkx and Pitx7 domains).

Noteworthy, when ESCs are induced to differentiate into committed NPCs, by exposure to the FGF and Sonic hedgehog (Shh), and then transplanted onto the midbrain flaps, the number of TH+ cells in the ventral midline is dramatically reduced to nearly 5%, and these cells are not restricted to the natural SN/A9 domain and can be observed outside the ventral midline at roughly the same numbers [59].

Taken together, these results show that current protocols designed to improve the yield of SN/A9 cells from ESC cultures could actually be detrimental to achieve this goal and that further work is required to identify the correct signaling network to induce the generation of these types of neurons. However, they also serve as proof of principle that the identification of these signals, and the correct timing of their presentation in order to induce stem cell differentiation into SN/A9 dopaminergic neurons, is an attainable goal.

Recently, adult stem cells have arisen as a viable source of transplant material for PD. The use of adult stem cells derived directly from affected patients would allow for allogenic transplant approaches, which potentially would obviate the need for immunosuppressive therapy in PD. Rodent and human adult stem cells derived from the bone marrow (mesenchymal stem cells, MSCs) can be induced to differentiate into TH+, DA producing cells, which appear to be immature neuroblasts of DA lineage that show weak electrical activity related to low expression levels of voltage-gated ion channels [60,61]. Functional maturity of these induced MSCs improves when expression of the RE-1 (REST) silencing factor is knocked down by siRNA methods [62].

Differentiation of MSCs can be induced either by exposure to morphogenic proteins (FGFs and Shh) [23,63] or by lentiviral transduction of the transcription factor Limx1a [60]. When induced human MSCs are transplanted into the striatum of 6-OH-DA-treated rats, there is a 50% reduction in apomorphine-induced rotational behavior. This effect is not observed when rats are transplanted with “naïve” non-induced hMSCs [63]. In contrast, in vitro differentiated rat MSCs show similar (roughly 50%) decreases in amphetamine-induced rotational behavior, but it must be noted that even the transplantation of non-induced rMSCs reduces rotations by nearly the same factor in 6-OH-DA-treated rats [23]. This observation has been interpreted as a result of a trophic action of rMSCs on the remaining DA neurons of the injured side [23,64–66]. Correlating with this observation, it has been shown that co-culturing ventral mesencephalic tissue with rMSCs increases expression of the Th gene in the mesencephalic cells [67]. Whether these differences between human and rat MSCs derive from intrinsic distinct capabilities of the cells or to differential response to the accepting niche (ie, allogeneic vs. xenogeneic transplants) remains to be determined.

Lately a second source of adult stem cells for PD therapy has been identified. These cells are derived by reprogramming skin fibroblasts to become induced pluripotent stem (iPSC) cells. Because the originating fibroblasts are harvested from adult individuals, iPSC cells are actually tailor made to accommodate the needs of a specific individual, preventing rejection of the potential transplants [68–71]. Although the brain is thought to be an immunologically privileged site, it has been well documented that survival of the grafts used for PD therapy is increased by the use of continuous immunosuppression, and the use of iPSC cells could overcome this particular limitation [13]. Another advantage to advocate the use of iPSC cells is to avoid the ethical issues presented by the use of precursors reprogrammed by somatic cell nuclear transfer (SCNT) into oocytes.

When transplanted to the striatum of 6-OH-DA-injured rats, mouse fibroblast-derived iPS cells can improve the rotational behavior induced by amphetamine [72]. As observed with other dopaminergic tissues, ipsilateral turns decrease by 50%, which seem to be the current limit of benefits achieved by cell therapy, as discussed earlier.

It is now clear that for cell therapy to become a viable option of treatment of PD patients, we need more homogeneous sources of dopaminergic neurons. The donor cells should ideally consist in pure preparations of dopamine-secreting SN/A9 cells that could allow the determination of the effective “dose” of cells that should be transplanted. Having access to such population of dopaminergic cells will also allow the determination of the best surgical technique (number of sites, dispersion within the tissue, etc.), which in turn will translate in better control of double-blind studies. Nevertheless, as with all comparisons between treatments, it is necessary to compare the efficacy of cell therapy to current pharmacological schemes for PD treatment, and determine if transplants could eventually replace L-DOPA treatment, or if cell therapy will only be an option for the most severe cases refractory to currently available therapies.

Which Is the Best Way to Evaluate the Functional Efficiency of Stem Cell Therapy for PD?

In order for cell therapy to emerge as a viable option for PD patients, grafted tissue must not only ameliorate motor symptoms, but also avoid development of incapacitating adverse effects, such as dyskinesias.

Ideally, grafted dopaminergic neurons should be able to reconstruct the nigrostriatal pathway, in such way that DA modulation of the basal ganglia circuits is reestablished, as well as the regulatory inputs to the DA-secreting neurons, that allow compensatory mechanisms, such as feedback, to occur. Even with the best stem cell-derived dopaminergic cell, the adult brain likely lacks the correct environment permissive to neuronal regeneration from the substantia nigra into the caudate-putamen (striatum).

Nevertheless, it is possible that the transplanted cells could form local neural circuits within the striatum that allow compensation for the loss of modulatory inputs from the SN [73–75]. These local circuits should not only modulate activity of the basal ganglia pathways, both through the D2 and the D1 dopamine receptors, but should also be subject to feedback regulation; in such way that dopamine availability does not sharply fluctuate [2].

For us to be able to evaluate the formation and function of these neural circuits, we need better functional tests, to complement the information obtained with the currently available behavioral ones, such as feeding and rotational behavior. The use of positron emission tomography (PET) coupled to magnetic resonance imaging (MRI) techniques has reduced the breach between behavioral and functional data.
The availability of radioactive markers of dopamine neuron activity, such as 6-[[18]F]-fluoro-L-DOPA ([18]F-DOPA), allows the evaluation of PD progression in vivo [76,77] and graft survival in transplanted PD patients [4,78]. However, it has been shown that [18]F-DOPA is not well suited for evaluation of dopamine re-uptake in small animal PET scanning and other radiotracers of DAT binding, such as [11]C-[(+)-α-dihydrotetrabenazine ([11]C-DTBZ), have better specificity and spatial resolution when evaluating animal models of PD [79].

DAT binding and l-DOPA decarboxylation markers probe the survival of dopaminergic tissue, but in order to evaluate the dopamine-release capabilities of the tissues, it is necessary to use radioactively labeled dopamine receptor antagonists, such as [11]C-raclopride, which can be displaced from the receptors by dopamine liberated from nerve terminals

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**FIG. 2.** Imaging cell ensembles in corticostriatal slice preparations by calcium-sensitive dyes. (A) Schematic drawing representing active neurons (circles) in corticostriatal slices, under different imaging conditions. An electrode stimulating the cortex induces the coactivation of a set of neurons, which fire with similar patterns (state 1, green circles in left panel). When the glutamatergic agonist NMDA is applied to the recording bath, a different set of neurons fires with a new pattern (state 2, red circles in middle panel). When a cortical stimulus is simultaneously applied when NMDA is in the bath, a third pattern of firing is observed in a third set of neurons (state 3, cyan circles in right panel). (B) Transitions from one state to another can be controlled by applying the correct stimulus. However, at any given state, not all the cells belonging to an ensemble fire simultaneously, and this generates different trajectories (distinct colored circles) that the ensembles can follow to transit from one state to the next one. (C) When the trajectories of NMDA-elicited activity, with no cortical input, are further analyzed over time, it can be shown that they represent new functional states, in which distinct neuron assemblies participate. This suggests that network assembly activity is an intrinsic property of the striatum. (D) Transition between different states of NMDA-stimulated cell assemblies follows a predetermined pathway, and neural activity transits from state 1 to state 2 and then to state 3 in strict order, and the probability for each of these transitions to occur. Once a given state is reached, there is also the probability of the cell assemblies to remain engaged in the same firing pattern, depicted by the recurring arrows and their respective probabilities. This pathway could represent a “motor program” in which different cell assemblies are engaged sequentially. (E) Interestingly, fast GABA transmission is required for establishing the sequential transitions of the “motor program” induced by NMDA. Under simultaneous application of NMDA and the GABA antagonist bicuculline in the recording bath, a higher number of active neurons is observed, and instead of sequential transitions among 3 states, most of the cells remain “locked” in a single pattern of activity (state 1, red circles), with minor transitions to a more silent state (2, cyan circles) with fewer active cells (F). This suggests that modulation of GABAergic transmission is required for a motor program to be activated correctly and it has been proposed that activity of GABAergic spiny interneurons within the striatum is deregulated in Parkinson’s disease (PD) [82]. Arrows in A and C depict cells that remain active in every distinct state, which could represent the “core” generators of the motor programs in the striatum.
located in situ and exposed to amphetamine [5]. Using these approaches, it has been shown in transplanted human brains that the grafted cells remain active and it has been suggested that they can form connections with neurons residing in the host striatum [5,12]. However, the spatial resolution achieved with the current available PET scanners, roughly 2 mm ($2 \times 10^{-2}$ m), is insufficient because single cell events can only be resolved with 100-fold higher resolutions ($10–20$ μm).

One of us (JB) has recently designed a brain slice preparation in which individual cell activity can be observed and evaluated, using calcium-imaging techniques [80]. When corticostriatal slices are loaded with a calcium-sensitive fluorescent dye, it is possible to analyze single-cell activity in a widespread area of the striatum. Furthermore, it was possible to analyze the activity of neural ensembles, that is, groups of cells for which there is a clear correlation of firing patterns and that could represent functional microcircuits within the striatum (Fig. 2) [80,81].

Different cells within the striatum belong to different ensembles. However, there are some cells that belong to most of the ensembles present in a given slice. These cells serve as central pattern generators (CPGs), which are thought to represent memory traces in the nervous system [80,81,83,84]. It is possible that CPG activity within the striatum is related to the encoding of motor programs that are brought into action by cortical stimulation. In this regard, it is worth noting that unstimulated corticostriatal slices show mostly silent cells, with some uncoordinated activity. Ensemble activity is triggered by stimulation of the cortical fibers or exposition to the glutamatergic agonist NMDA.

These results suggest the existence of some intrinsic microcircuits in the striatum, which could be the actual modulators of motor activity provided by this area of the brain. Thus, it would be necessary to determine if the segregated pathways represented by neurons expressing either the D1 or the D2 dopaminergic receptors, belong to distinct activity ensembles, since deregulation of the D1 pathway has been proposed to be responsible for the dyskinesias observed in PD patients after prolonged exposure to l-DOPA [85–87]. It also remains to be demonstrated if there is a significant difference between the ensemble activities present in intact brain compared to the PD affected striatum.

Nonetheless, the use of this brain slice preparation will allow us to study if dopaminergic cells transplanted in vivo can integrate into local microcircuits within the striatum and if the formation of new modulatory connections could compensate for the loss of the naturally occurring dopaminergic input from the SN and reestablish control of the motor functions.

**Concluding Remarks**

More than 20 years have elapsed since cell therapy was first used as an option for PD treatment [7,88] and different groups have generated enough data to prove that replacement of lost dopaminergic neurons in the striatum can improve the motor symptoms of this disease. However, cell therapy for Parkinson's disease has not been able to fulfill its initial promises and remains a merely experimental approach, due to its modest clinical benefits and its major adverse effects, such as dyskinesias.

These limitations could be due to the lack of a perfect source of dopaminergic donor cells able to generate a homogeneous population of dopamine-secreting SN/A9 neurons, which can integrate into the circuits of the affected striatum.

Stem cell technology appears to be the best way to generate these donor cells, by amplifying a precursor population and inducing its differentiation such that each transplantable cell is committed to the dopaminergic SN/A9 phenotype and still remains capable of making functional connections to the intrinsic neurons of the striatum in order to form regulatory circuits. The use of iPSCs might also obviate administration of immunodepressants.

Nevertheless, we are still far from understanding the signaling network that directs differentiation of pluripotent cells into fully functional dopaminergic SN/A9 neurons, and a concerted effort from research laboratories of different expertise would be required to tackle this problem.

We have also reached a point where we can address basic questions about microcircuitry in the brain. Understanding how the motor circuits in the basal ganglia work will also allow us to design better therapeutic strategies.

**Acknowledgments**

We would like to thank Ruben Garcia-Montes and Pablo Valle-Leija for generating the images included in Figure 1. This work was supported by IMPULSA of the Universidad Nacional Autónoma de México (UNAM). R. Vidal-tamayo is supported by Proyectos Apoyo a la Investigación e Innovación Tecnológica (PAPIIT), UNAM Grant no. IN211307 and Consejo Nacional de Ciencia y Tecnología (CONACYT) Grant no. J1-56295.

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Address correspondence to:
Dr. René Drucker-Colin
Depto. de Neurociencias
Instituto de Fisiología Celular, UNAM
Apdo. Postal 70–600
D.F. 04510
México

E-mail: drucker@servidor.unam.mx

Received for publication June 11, 2009
Accepted after revision August 28, 2009
Prepublished on Liebert Instant Online August 28, 2009