Bilateral Fetal Nigral Transplantation into the Postcommissural Putamen in Parkinson's Disease

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We performed fetal nigral transplantations in 4 Parkinson's disease (PD) patients. Solid grafts were bilaterally implanted into the postcommissural putamen using 3 to 4 donors per side aged 6% to 9 weeks postconception. Transplant deposits were separated by no more than 5 mm in three dimensions. Cyclosporine was employed for a total of 6 months. Patients were evaluated at baseline and at 1, 3, and 6 months postoperatively. Striatal 18-fluorodopa uptake was assessed by positron emission tomography at baseline and at 6 months postoperatively. The procedure was well tolerated in all patients. One patient had a clinically asymptomatic superficial cortical hemorrhage along the needle tract and a second had transient postoperative confusion and hallucinations. All patients experienced clinically meaningful benefit. Significant improvement ($p < 0.05$) was detected in total UPDRS score during the “off” state, Schwab-England disability score during the “off” state, percent “off” time, and percent “on” time with dyskinesia. Increased striatal fluorodopa uptake was observed bilaterally in each patient, with mean increases of 53% on the right ($p = 0.01$) and 33% on the left ($p = 0.08$). Our study demonstrated clear and consistent improvement in clinical features and striatal fluorodopa uptake following fetal tissue transplantation in patients with advanced PD whose condition was not improved preoperatively by drug manipulation. These preliminary results are encouraging and support further studies to evaluate grafting strategies as a therapy for PD.


Parkinson's disease (PD) is a progressive neurodegenerative disorder that is associated with loss of neurons in the substantia nigra pars compacta and a decline in striatal dopamine. Current therapy is based primarily on a dopamine replacement strategy using the dopamine precursor levodopa. However, chronic levodopa therapy is associated with adverse effects and increasing disability. Therefore, there has been a search for alternative therapies that can reverse functional disability in patients with advanced PD who cannot be satisfactorily controlled with existing medications. Neural grafting is a rational consideration as a therapy for PD because (i) PD is associated with a relatively well-characterized and specific dopamine neuronal degeneration, (ii) dopamine replacement therapy provides dramatic clinical benefit, (iii) dopamine neurons subserve a modulatory function and under physiological conditions provide tonic stimulation of striatal dopamine receptors, and (iv) there is a well-defined target area for transplantation. Several studies demonstrated the capacity of intrastriatal grafts of embryonic dopaminergic neurons to survive, produce dopamine, form connections with host neurons, and provide long-lasting amelioration of motor dysfunction in animal models of Parkinsonism (reviewed in [1]). These studies led to the initial clinical trials of fetal nigral transplantation as a treatment for PD [2–7]. However, the observed benefits have been inconsistent and for the most part modest. This may relate to the specific transplant variables employed such as target site, number of donors, donor age, and distribution of implanted tissue within the target region. We performed fetal nigral transplantation...
in PD patients using a protocol designed to maximize the likelihood of graft survival and dopamine reinnervation of the target site based on existing information. Solid fetal nigral grafts were bilaterally implanted into the posterior (postcommissural) putamen using 3 to 4 donors per side aged 6½ to 9 weeks postconception (PC). Fetal deposits were separated by no more than 5 mm in three dimensions. We present the clinical and positron emission tomography (PET) results at 6 months in the first 4 patients who have undergone fetal nigral transplantation according to this protocol.

Materials and Methods

Patient Selection

Patients with PD were selected from the Movement Disorder Center at the University of South Florida. PD was diagnosed according to the core assessment program for intracerebral transplantation (CAPIT) protocol [60] and included resting tremor, rigidity, bradykinesia, and a good response to levodopa therapy. Entry criteria included stable dose of levodopa/carbidopa for a minimum of 3 months prior to entry into the study, Hoehn-Yahr stage of III or less during the "on" state, clinically meaningful disability during the "off" state, and predictable motor fluctuations. Exclusion criteria included dementia; a previous intracranial procedure; a clinically significant medical, neoplastic, or infectious disease; and a clinically significant laboratory abnormality. Patients were screened serologically prior to entry into the study for the following: human immunodeficiency virus type 1 (HIV-1; antibody [Ab], antigen [Ag]), HIV-2 (Ab), human T-cell lymphotropic virus type 1 (HTLV-I; Ab), hepatitis A virus (HAV; IgM), hepatitis B (HB surface Ag, HB core Ab), hepatitis C virus (HCV; Ab), cytomegalovirus (CMV; IgM, IgG), toxoplasma (IgG, IgM), syphilis (rapid plasma reagent [RPR]), and herpes simplex virus (HSV, IgG). Patients were not included in the study if there was serological evidence of infection with syphilis, HAV, HBV, or hepatitis. Patients who were CMV or toxoplasma negative were also excluded to eliminate the risk of transplanting these common contagious agents to a naive recipient.

Study Design

Patients who met entry criteria and signed informed consent were entered into the study. Solid grafts of fetal human mesencephalon derived from donor embryos aged 6½ to 9 weeks PC were implanted bilaterally into the postcommissural putamen in staged procedures separated by approximately 4 weeks. Implanted tissue was derived from 3 to 4 embryos per side and placed so that deposits were distributed at approximately 5-mm intervals throughout the three-dimensional configuration of the postcommissural putamen. Cyclosporine (CSA) was employed for approximately 6 months. Patients were evaluated at baseline and at 1, 3, and 6 months postoperatively. Each evaluation included UPDRS, Hoehn-Yahr, and Schwab-England assessments during both "on" and "off" states in accordance with the CAPIT protocol. Timed motor tests (supination-pronation, stand-walk-sit, step seconds, Purdue Peg-Board, finger tapping, and hand-arm movements between two points) were also performed during "on" and "off" states. Evaluations during "off" states were performed in the early morning, approximately 12 hours after the previous dose of medication ("practically defined off"). "On" scores were determined at the time of peak response to the morning dose of levodopa. Percent "on" time with and without dyskinesia was calculated based on a self-assessment calendar. All evaluations were performed by the same investigator and a standardized video examination was obtained at the time of each evaluation. Striatal 18-fluorodopa (FD) uptake on PET was assessed preoperatively and 6 months postoperatively.

Donor Tissue

Tissue was obtained from women undergoing elective abortion in accordance with federal, state, and local laws; National Institutes of Health (NIH) guidelines; and the Uniform Anatomical Gift Act as adapted by the State of Florida. Informed consent for the use of the cadaver fetus was sought only after the patient had signed surgical consent for abortion. No monetary or other inducement was provided to the patient, abortionist, or abortion clinic. Donation of embryonic tissue was made without restriction as to individual recipient or whether the tissue would be employed in human trials. A low-pressure aspiration abortion technique was employed using sterile technique [8]. There was no alteration in the indication, timing, or methodology of the abortion procedure. Each donor was screened using maternal blood for HIV-1 (Ab, Ag), HIV-2 (Ab), HTLV-1 (Ab), HAV (IgM), hepatitis B virus, (HB surface Ag, HB core Ab), HCV (Ab), CMV (IgM), toxoplasma (IgM), syphilis (RPR), and HSV (IgM). In addition, fetal tissue immediately adjacent to the mesencephalon was cultured for aerobic and anaerobic bacteria, yeast, HSV, and CMV. Donors were excluded if there was evidence of infection with HIV-1 or -2, HTLV-1, syphilis, or hepatitis B or C. In addition fetal tissue was not employed if the mother had a history of prostitution, injection drug abuse, more than 10 transfusions in the preceding year, evidence of active vaginal herpes, a temperature higher than 100.5°F, or a white blood cell (WBC) count higher than 15,000/mm³.

Donor age was staged according to the atlas of O'Rahilly and Muller [9] for donors younger than PC week 8 and for older donors by a combination of foot length, heel length, and greatest length [10, 11]. The mesencephalon was dissected and stored in "hibernation medium" at 8°C for up to 2 days. Immediately before transplantation, the mesencephalon was further dissected into ½-mm³ pieces. The final tissue dissection was performed in chilled Hank's balanced salt solution (HBSS, Gibco).

Localization of Target

Patients were placed in a standard CRW magnetic resonance imaging (MRI)–compatible stereotactic frame using local anesthesia. The putamen was visualized on a high-field-strength MRI (1.5 T) using a fast spin-echo sequence (TR 3200/TE 17/matrix 256 × 256/field of view 3 cm). Axial images were obtained using contiguous 3-mm sections extending from below the putamen to above the caudate in a plane such that a perpendicular line passing through the midputamen would intersect the coronal suture. Coronal sections were obtained in 3-mm intervals in a plane perpendicular to the axial cuts beginning 3 cm anterior to the coronal suture and progressing caudally through the putamen. Target sites for im-
plantation were based on a determination of the "zero point" in the putamen defined as the point midway between its rostral and caudal aspects on the lowest axial section. This point was the initial target site for transplantation and permitted identification of all other target sites based on a single stereotactic measurement.

Surgical Procedure

After target sites had been determined, patients were transferred to the operating theater and sedated with fentanyl and Propofol. The airway was protected with a laryngeal mask airway. A grid array in the shape of the putamen, with holes at 5-mm intervals, was placed onto the stereotactic frame. The grid array was aligned so that its axial plane was parallel to the plane of the axial MRI and its longitudinal axis parallel to the axis of the midline of the brain. While the patient was under local anesthesia, a burr hole was placed to accommodate the site of entry of the stereotactic transplant needle. Initially, a cortical entry point 3 cm lateral to midline was employed. After the first operation on the third patient (the fifth procedure), a cortical entry point 1.5 cm from the midline was utilized so that the superficial needle tract remained entirely within the superior frontal gyrus. The transplant needle consisted of an outer cannula with a diameter of 1.3 mm and an inner cannula with a diameter of 1.2 mm tapering to 0.9 mm (the size of a 20 gauge needle). Tissue was microdissected into 7-mm³ pieces and aspirated into the stereotactic needle using a 100-µl Hamilton syringe in a volume of approximately 16 to 20 µl of HBSS. The transplant needle was then placed into the "zero point" on the grid array and directed to the "zero point" of the putamen. Each needle tract contained tissue from one half of a mesencephalon (= one substantia nigra). Within each needle tract, four deposits in a volume of approximately 4 to 5 µl were implanted at approximately 5-mm intervals. Injections were made at a rate of 2 µl/30 sec with an interval of 1 minute between deposits. Following the last deposit in each tract, 3 µl of HBSS was injected, and the needle was left in place for 2 minutes to avoid graft withdrawal. Subsequent needle trajectories utilized the same burr hole and cortical entry point by angling the grid array in the sagittal and coronal planes. A total of six to eight needle tracts per side were made.

Perioperative Management

Immunosuppression with CsA, 6 mg/kg/day, was initiated 2 weeks before the first transplant procedure, reduced to 2 mg/kg/day 2 weeks after the second procedure, and discontinued after 6 months. Renal function was monitored on a routine basis. Antibiotic treatment with piperacillin (3 g intravenously every 6 hours), cefazoline (Fortaz) (2 mg intravenously every 8 hours and fluconazole (200 mg intravenously/orally every morning) was initiated immediately before surgery and continued for 5 days or for a full course of appropriate treatment if cultures were positive. Vancomycin (1 gm intravenously every 12 hours) was used in place of piperacillin if patients were allergic to penicillin. MRI was performed on the first postoperative day. Following surgery, antiparkinsonian medications were reinstituted at their preoperative dose and efforts were made to maintain this dose throughout the study except in the case of toxicity or an inadequate clinical response.

Positron Emission Tomography

PET was performed at the University of British Columbia on an ECAT 953-31B camera according to a standard protocol [12]. Briefly, medication was withheld overnight and the patient was given 200 mg of carbidopa orally 1 hour before scanning. The patient was positioned in the scanner using gantry-mounted lasers to align the orbitomeatal line with the detector rings. Head movement was restrained by an individually molded thermoplastic face mask that was used for subsequent scans. A transmission scan was performed to correct for attenuation of radioactivity. FD (3–5 mCi) was injected intravenously and 12 sequential scans were made at 10-minute intervals. Serial arterial blood samples were obtained to define peak level and elimination time course of blood radioactivity. The peripheral metabolism of FD to 3-O-methylfluorodopa was determined using an alumina extraction method [13]. Regions of interest (ROIs) were placed on an integral image generated from the last 60 minutes of emission data. Four circular ROIs measuring 8.8 mm in diameter were positioned manually along the long axis of each striatum so that one covered the head of the caudate and three covered the putamen. Three background circular ROIs were placed on the occipitoparietal cortex on each side, taking care to avoid the ventricles. Similar sets of ROIs were placed on the five contiguous slices where the striatum was best seen. The scans were analyzed to determine the striatal FD uptake rate constant (K) using the method of Patlak and colleagues [14]. The K for each ROI in each slice was averaged to determine the mean K for the right and left caudate and putamen. To determine the significance of changes between scans in individual subjects, we used the data generated in separate studies of the reproducibility of FD PET in normal and PD patients scanned with a similar protocol [15, 16].

Statistical Analysis

For each variable, a repeated-measures analysis of variance was performed using PROC GLM in the SAS statistical software package. To test for the presence of an overall time effect on each variable, a univariate adjusted F test for within-subject effects was used. This test was followed up by testing each of the means at 1, 3, and 6 months postoperatively for a difference from the mean at the preoperative baseline visit by using an F test derived from the repeated-measures model.

Results

Four patients underwent bilateral transplant procedures according to the above-described protocol and have been followed for 6 months. Patient data at the time of entry are summarized in Table 1. Surgery was well tolerated and patients were discharged from the hospital within 48 to 72 hours. All cultures of fetal tissue (n = 29) were negative and antibiotics were discontinued after 5 days. MRI was performed the day following surgery. No evidence of blood was detected within any of the transplant sites. MRI studies with gadolinium were performed after the first four procedures and did not demonstrate areas of enhancement.
suggestive of a breakdown in the blood-brain barrier (BBB).

A comparison of clinical scores at baseline and at 6 months following the transplant procedure is shown in Table 2. Significant improvement ($p < 0.05$) was detected in total UPDRS score during the "off" state, Schwab-England disability score during the "off" state, percent "off" time, and percent "on" time with dyskinesia. Percent "off" time was reduced from a mean of 34.1% at baseline to 12.1% at the time of the final visit. Dyskinesia was markedly reduced in all patients and disappeared in 2. These benefits were observed in each patient and generally began between postoperative months 1 and 3 (Fig. 1). Painful "off" period dystonia, present at baseline in Patients 1, 2, and 4, virtually disappeared by the +month postoperative visit. Gait status improved following introduction of carbamazepine and reduction of levodopa dose. Patient 3 had an asymptomatic superficial cortical hemorrhage in measures of renal function.

The results of FD PET in individual patients at baseline and at 6 months are shown in Table 3 and illustrated in Figure 2. Bilateral increases in putaminal $K_i$ were observed in each patient. Mean putaminal $K_i$ increased by 53% on the right ($p = 0.012$, t test, two-tailed) and by 33% on the left ($p = 0.08$). There were smaller but nonsignificant increases in the caudate $K_i$'s.

Patient 1 experienced confusion and hallucinations beginning 3 weeks after the second transplant procedure, possibly due to subclinical seizure activity. Men-

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Duration (yr)</th>
<th>UPDRS (on/off)</th>
<th>% &quot;off&quot; time</th>
<th>% &quot;on&quot; time w/dyskinesia</th>
<th>Schwab-England score (on/off)</th>
<th>Hoehn-Yahr score (on/off)</th>
<th>Levodopa/Perigolide (mg/day)</th>
<th>Selegiline Hydrochloride (mg/day)</th>
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<tr>
<td>1</td>
<td>M</td>
<td>59</td>
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<td>17/78</td>
<td>48</td>
<td>19.5</td>
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<td>M</td>
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<td>12</td>
<td>13/70</td>
<td>30</td>
<td>30</td>
<td>90/65</td>
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<tr>
<td>3</td>
<td>F</td>
<td>61</td>
<td>22</td>
<td>27.5/68.5</td>
<td>30.5</td>
<td>69.5</td>
<td>90/40</td>
<td>2/4</td>
<td>700</td>
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<td>4</td>
<td>M</td>
<td>50</td>
<td>15</td>
<td>22/104.5</td>
<td>28</td>
<td>75</td>
<td>85/50</td>
<td>2/3</td>
<td>800</td>
<td>1.5</td>
</tr>
</tbody>
</table>

UPDRS = unified Parkinson's disease rating scale.

<table>
<thead>
<tr>
<th>Table 2. Comparison of Mean ($\pm$ standard error of mean) Baseline and 6-Month Scores Following Fetal Nigral Transplantation</th>
</tr>
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<tbody>
<tr>
<td>UPDRS &quot;on&quot;</td>
</tr>
<tr>
<td>ADL</td>
</tr>
<tr>
<td>Motor</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>UPDRS &quot;off&quot;</td>
</tr>
<tr>
<td>ADL</td>
</tr>
<tr>
<td>Motor</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>% &quot;Off&quot; time</td>
</tr>
<tr>
<td>% &quot;On&quot; time with dyskinesia</td>
</tr>
<tr>
<td>Hoehn-Yahr score &quot;on&quot;</td>
</tr>
<tr>
<td>Hoehn-Yahr score &quot;off&quot;</td>
</tr>
<tr>
<td>Schwab-England score &quot;on&quot;</td>
</tr>
<tr>
<td>Schwab-England score &quot;off&quot;</td>
</tr>
</tbody>
</table>

UPDRS = unified Parkinson’s disease rating scale; ADL = activities of daily living; NS = not significant.

Discussion

We report significant improvement in clinical function and striatal FD uptake 6 months following fetal nigral transplantation in 4 patients with advanced PD. Significant improvement was observed in total UPDRS score during "off" periods, percent "on" time, percent "on" time without dyskinesia, and functional disability during "off" state. Gait was improved in 3, painful "off period" dystonia resolved in 3, and freezing episodes disappeared in 2. Increased striatal FD uptake was de-
It is difficult to compare our results with other results reported in the literature because of differences in patient selection, transplantation technique, and method of evaluation. Nonetheless, our results appear to compare favorably to those reported 6 months following transplantation in the peer-reviewed literature [2, 4, 6, 7] including patients with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism [17]. All of our patients experienced clinically relevant improvement that persisted throughout the 6-month observation period. This time course of recovery is similar to that observed in other studies [4]. Increased striatal FD uptake on PET at this time point, as we detected in each patient, has only been reported for 2 other patients [7, 18]. Interestingly, each underwent transplantation with multiple donors.

Benefits in our patients were obtained using a protocol that was designed to optimize donor age, number of donors, distribution of cells, and target site. We used cells derived from donors aged 6½ to 9 weeks PC. The optimal donor age for graft survival following transplantation is thought to be from the time dopaminergic cells first appear in the ventricular zone to when they differentiate and extend neuritic processes [19]. Once neuritic processes are formed, cells are less likely to survive transplantation, possibly because they may be axotomized during dissection and preparation. Dopamine neurons first appear in the ventricular zone at 5½ to 6½ weeks PC [20, 21]. Neuritic process are first identified at PC week 8 and reach the striatum at PC week 9. These observations suggest that the ideal donor age for grafting human embryonic mesencephalic dopamine cells is between 5½ and 9 weeks PC. Our previous study on human to rodent nigral xenografts confirmed that optimal survival is obtained with embryos aged 5½ to 8 weeks PC for suspension grafts and 6½ to 9 weeks PC for solid grafts [22]. We employed solid grafts and hence used fetal donors aged 6½ to 9 weeks PC. Only three other groups [2, 5, 7] have consistently utilized donor tissue from this donor age "window."

We employed fetal nigral tissue derived from 3 to 4 donors per side. The precise number of donors required to provide functional benefit in patients with PD is unknown and can only be estimated from animal experiments. The smallest number of transplanted dopaminergic neurons demonstrated to provide significant behavioral improvement is 120 in the rodent [23] and 2,000 in the marmoset [24]. The human striatum is substantially larger and it is likely that a greater number of neurons will be required to achieve meaningful clinical benefit. It has been estimated that in humans 60,000 dopamine neurons project to the putamen and that up to 20,000 dopamine neurons from a single human fetus survive transplantation into immunosuppressed rodents [25]. It may thus be necessary to transplant mesencephalic tissue from 3 donors into each putamen to restore complete dopaminergic activity. However, full dopaminergic innervation may not be required to reverse the symptoms associated with striatal dopamine deficiency as clinical features of PD are not seen prior to a 60 to 80% reduction in nigral neu-
<table>
<thead>
<tr>
<th>Patient No.</th>
<th>R Pregraft</th>
<th>R Postgraft</th>
<th>L Pregraft</th>
<th>L Postgraft</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0071</td>
<td>0.0105&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>0.0111&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>0.0109&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>0.0126&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>0.0122</td>
</tr>
<tr>
<td>4</td>
<td>0.0075</td>
<td>0.0104&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0076</td>
<td>0.0089</td>
</tr>
<tr>
<td>Mean (± SEM)</td>
<td>0.0071 ± 0.0002</td>
<td>0.0108 ± 0.0006&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0081 ± 0.0012</td>
<td>0.0108 ± 0.0007&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

| Caudate nucleus | |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| 1               | 0.0130          | 0.0143          | 0.0144          | 0.0143          |
| 2               | 0.0143          | 0.0135          | 0.0107          | 0.0136          |
| 3               | 0.0153          | 0.0177          | 0.0198          | 0.0223          |
| 4               | 0.0144          | 0.0121          | 0.0129          | 0.0129          |
| Mean (± SEM)    | 0.0143 ± 0.0005 | 0.0144 ± 0.0012 (NS) | 0.0145 ± 0.0019 | 0.0158 ± 0.0022 (NS) |

<sup>a</sup>p < 0.05.  
<sup>b</sup>p < 0.01.  
<sup>c</sup>p = 0.08.  

NS = not significant compared to baseline; SEM = standard error of mean.

Fig. 2. Preoperative (top row) and postoperative (bottom row) fluorodopa positron emission tomography scans in the 4 Parkinson's disease patients who underwent bilateral transplantation. Images have been normalized to background activity in each individual and scaled to each other. Note that striatal fluorodopa uptake in the putamen is increased bilaterally in each patient.
rons and striatal dopamine [26]. On the other hand, it is not known what percent of embryonic cells survive transplantation into the human striatum. To maximize the likelihood of obtaining a detectable clinical benefit, we employed a minimum of 3 donors per side in our protocol. Only one other group has bilaterally implanted 3 or more donors per side [17]. Interestingly, while these patients had MPTP-induced parkinsonism and not PD, the pattern and magnitude of benefit in this group most closely resemble those observed in our patients.

We distributed tissue within the target region so that deposits were separated by no more than 5 mm in three dimensions. Even if the correct number of donor cells are transplanted, improper distribution may result in a suboptimal clinical response. We estimate, based on our experimental observations in rodents, that human fetal nigral neurons implanted into the striatum extend processes for approximately 2.5 mm. Further, dopamine diffusion from implanted dopaminergic neurons is highly limited [27, 28]. This implies that donor cells should be distributed throughout the putamen at intervals no greater than 5 mm in all three dimensions. Only two other groups addressed the issue of tissue distribution. Freed and coworkers [2] separated needle tracks by 4 mm in the anteroposterior but not the mediolateral plane of the putamen. Spencer and colleagues [6] separated deposits by 4 mm but only transplanted tissue into the caudate nucleus.

We implanted fetal tissue into the postcommissural putamen. Based on our desire to optimize the concentration of implanted nigral cells, we chose to limit placement of grafts into one somatotopically defined region. In rodent and primate experiments, functional recovery following dopamine implantation is site-specific [24, 29, 30]. Embryologically, differentiation of the postcommissural putamen differs from that of the anterior putamen, which is more closely linked spatially and temporally to the caudate nucleus [31]. In PD there are several reasons for selecting the postcommissural putamen as the primary site for neural grafting. Both autopsy and PET studies in PD demonstrated greater dopamine depletion within the posterior putamen than the anterior putamen/caudate nucleus [26, 32], and degeneration of the substantia nigra preferentially occurs in regions that project to the posterior putamen [33]. In primates, the postcommissural putamen receives input from the precentral motor fields [34, 35] and microstimulation studies within the posterior putamen evoke discrete movements of contralateral body parts [36]. In contrast, the caudate nucleus and anterior putamen are less related to primary motor circuitry and receive input primarily from the prefrontal cortex and frontal eyefields [35, 37]. Thus the postcommissural putamen appears to be distinct from the anterior putamen/caudate and a rational target site for fetal nigral grafting in PD. No other group has exclusively targeted the postcommissural putamen or implanted such a high concentration of fetal nigral tissue into this specific region. It is possible that implantation into the caudate will provide additional benefits and that best results will be obtained with transplantation into both the putamen and caudate.

We transplanted tissue bilaterally into the postcommissural putamen. Following unilateral transplantation into the putamen, improvement primarily occurred on the contralateral side in both rodents [30] and PD patients [2, 3]. Further, PET demonstrated that following unilateral transplantation, FD uptake is increased on the transplanted side but declines on the unoperated side [5, 18]. These observations suggest that better results may be obtained with bilateral grafts. Only two other groups have transplanted bilaterally [2, 17] and only the former involved PD patients.

We employed immunosuppression with CsA. The central nervous system (CNS) is a relatively immunologically privileged site and the need for immunosuppression in neural transplantation is not established. Fetal allografts in rodents and nonhuman primates have been observed to survive for extended periods of time without immunosuppression [38, 39]. Transplantation of fetal tissues into the CNS of nonhuman primates does not induce a detectable immunological response or donor-specific sensitization [40] and clinical improvement has been reported in some patients who received fetal grafts without immunosuppression [2]. However, there is concern that the surgical trauma or the graft itself could disrupt the BBB and permit the immune system access to graft antigens within the brain [41]. CsA has been shown to improve survival of xenografts in rodent models [23, 42] and there are examples of allogenic neural graft rejection in immunologically disparate rodents [43]. This is particularly relevant in our protocol where tissue from 6 to 8 immunologically unrelated donors is transplanted into a single individual. Further, lack of graft rejection in rodents and primates does not ensure similar results in humans. CsA was not employed by the group reporting the highest proportion of clinical failures [44] and there are as yet no autopsy-proved examples of robust graft survival following fetal nigral transplantation in nonimmunosuppressed patients. As failure to employ immunosuppression might preclude optimal graft survival and clinical response, we elected to use immunosuppression.

The mechanism responsible for the clinical benefit observed in our patients cannot be established based on this open clinical trial. In animal models, behavioral improvement following fetal nigral transplantation is thought to relate primarily to survival of grafted neurons, neuritic outgrowth with synaptic connectivity, and graft-derived dopamine production [45]. How-
ever, several additional factors could contribute to a beneficial clinical response following nigral transplantation. These include a placebo effect, increased dopamine availability due to a breakdown in the BBB, immunosuppressants, and host-derived sprouting due to surgical trauma or a trophic effect.

It is unlikely that benefits observed following nigral grafting in PD are solely due to a placebo effect as benefits are not detected immediately, a similar pattern of improvement has been observed in other nigral transplant studies [2, 4, 17], and benefits are associated with an increase in striatal FD uptake, which increases over time [5]. Lindvall and coauthors [5] also argued that a placebo response is unlikely because improvement primarily occurs on the contralateral side following a unilateral transplant procedure. There is no evidence to suggest a long-lasting breakdown of the BBB. Experimental data suggest that the BBB closes rapidly following neural transplantation [46]. Contrast-enhanced MRI in our patients as well as in other studies [5] showed no evidence of BBB breakdown following transplantation. Carbipoda was not detected in the ventricular cerebrospinal fluid of adrenal transplant patients following levodopa/carbipoda administration [47]. Finally, clinical benefit following transplantation was most pronounced during “off” episodes, when circulating levels of levodopa are presumably at their lowest. The possibility that CsA might influence the signs and symptoms of PD and confound interpretation of the transplant effect is also considered. CsA increases spontaneous and amphetamine-induced locomotor behavior in Sprague-Dawley rats [48]. There is also some evidence suggesting that inflammation or autoimmune response may contribute to the pathogenesis of PD [49, 50]. Controlled studies to more definitively determine the effect of CsA in PD are required.

It is possible that the graft or the lesion associated with the transplant procedure could induce sprouting of resident host fibers. Implants of adrenal medulla into mice [51], monkeys [52], and PD patients [53] can induce a tyrosine hydroxylase–immunoreactive (TH-IR) sprouting response from residual host ventral mesencephalic dopaminergic neurons despite failure of adrenal cells to survive. Nonetheless, it is unlikely that sprouting due to the surgical trauma or a trophic effect is responsible for the clinical improvement observed in the present study. All published observations of TH-IR sprouting occurred in the caudate nucleus. There have been no reports of sprouting in the posterior putamen, the target of transplantation in this project. No behavioral improvements have been noted in primate models of parkinsonism following sham surgery with injection of saline solution [54] or implantation of nonnigral tissue [55]. Increased striatal FD uptake observed on PET could be due to host-derived sprouting rather than survival of grafted neurons. However, striatal FD uptake is not increased following adrenal transplantation in monkeys [56] or in PD patients [57] despite greater striatal trauma. Finally, we now have autopsies confirmation of robust survival of implanted fetal nigral neurons without evidence of host-derived sprouting in a PD patient 18 months following a fetal nigral transplantation procedure [58]. Based on the above, it is likely that the functional recovery observed in our patients is due, at least in part, to survival of grafted nigral neurons.

The mechanism responsible for the dramatic reduction in percent “off” time and dyskinesia in our patients is unknown. Change in levodopa dose does not readily account for these benefits as improvement was observed in 2 patients in whom the dose of levodopa was not changed and prior to change of dose in the other 2. Further, preoperative dose manipulation did not provide simultaneous benefit in these parameters in any patient. A graft-related increase in dopamine neurons and terminals could permit more physiological storage of dopamine and stimulation of dopamine receptors, thereby accounting for an increase in “on” time coupled with a reduction in levodopa-induced dyskinesia [59].

In summary, we demonstrated clinical benefit and increased striatal FD uptake on PET 6 months following fetal nigral transplantation in patients with advanced PD. As progressive improvement beyond 6 months has been reported [5], it is possible our patients will experience further benefit and long-term follow-up is essential. The quality of improvement we observed may relate to the specific transplant variables employed, including restricted donor age window, multiple donors, bilateral transplantation, and wide dispersion of grafts throughout the three-dimensional configuration of the postcommissural putamen. Further studies to define the transplant variables associated with the optimal clinical response are required. In addition, the need for immunosuppression in neural transplantation and any effect CsA may have on the signs and symptoms of PD must be determined. The results of our uncontrolled clinical trial are encouraging. However, fetal transplantation must still be considered an experimental procedure and ultimately, studies that more precisely define the contribution of the lesion and placebo effect will be necessary before its value as a treatment for PD can be established.

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