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Simultaneous intrastriatal and intranigral fetal dopaminergic grafts in patients with Parkinson disease: a pilot study

Report of three cases

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The main neural transplantation strategy in Parkinson disease (PD) has been focused on reinnervating the striatum. The clinical results reported in patients who receive transplants have been limited and do not justify the use of neural transplantation as a routine therapeutic procedure for PD. Identifying the optimal target for transplantation may be one of the critical factors for optimizing clinical outcomes. Evidence from preclinical studies indicates that simultaneous intrastriatal and intranigral grafts (double grafts) may produce a more complete functional recovery. The authors report the clinical and positron emission tomography (PET) scanning results in three patients enrolled in a safety and feasibility pilot study who received double grafts and who have been followed for up to 13 months posttransplantation.

Patients included in the study had idiopathic PD. All patients underwent detailed assessments before and after surgery, in accordance with the Core Assessment Program for Intracerebral Transplantation. The patients received implants of fetal mesencephalic cell suspensions in the putamen and substantia nigra (SN) bilaterally. There were no intraoperative or perioperative complications. Follow-up PET scans demonstrated an increase in the mean fluorodopa uptake constant values in the putamen and SN 12 months postsurgery. Improvements were also noted in the total Unified Parkinson's Disease Rating Scale, Hoehn and Yahr, Schwab and England, and pronation/supination scores after transplantation. The authors demonstrate the feasibility of reinnervating the SN and striatum by using a double transplant strategy in humans.

KEY WORDS • Parkinson disease • neural transplantation • stereotactic neurosurgery

To date, the main transplantation strategy in PD has been focused on reinnervating the striatum by the ectopic placement of dopaminergic grafts.[3,10,13,15,16,20,24,25,37,44] Patients receiving intrastriatal grafts have shown robust reinnervation of the striatum, as documented by PET and postmortem studies.[19,20] There is also recent evidence from [^{11}C]-raclopride PET studies that grafted dopaminergic cells release synaptic dopamine 10 years after transplantation.[38] Although the results reported in patients who receive transplants have demonstrated some clinical benefit, improvement has been limited and has not reached a level high enough to justify the use of neural transplantation as a routine therapeutic procedure for PD. Furthermore, in a recent double-blind, sham-surgery, controlled trial of fetal tissue transplantation in the putamen of patients with severe PD limited clinical benefits were shown only in a subset of younger patients.[11] Several variables may influence the clinical efficacy of neural transplantation for PD.[12,23,27,34] Choosing the optimal target for transplantation may be one of the critical factors involved in optimizing clinical outcomes. The failure to restore dopaminergic reinnervation to the SN by ectopically placed intrastriatal grafts is likely a contributing factor in the limited clinical efficacy of neural transplantation in patients with PD.

We have previously demonstrated that simultaneous intrastriatal and intranigral grafting (double grafts) may promote reconstruction of the nigrostriatal pathway and pro-

Abbreviations used in this paper: DMEM = Dulbecco's modified Eagle medium; GABA = γ-aminobutyric acid; GDNF = glial cell line–derived neurotropic factor; K_i = uptake constant; MR = magnetic resonance; PD = Parkinson disease; PET = positron emission tomography; SN = substantia nigra; UPDRS = Unified PD Rating Scale; VM = ventral mesencephalic.

duce a more complete behavioral recovery in the rodent model of 6-hydroxydopamine-induced PD.[28,30,32] Furthermore, the intranigral graft appears to be necessary for the restoration of complex sensorimotor behaviors such as the adjusting step performance in the hemiparkinsonian rat.[2] This observation is important because these sensorimotor behaviors are more relevant to the human condition. It is well known that dopamine is released within the SN pars reticulata by dendrites of pars compacta neurons.[4,5] There is also evidence that levodopa-induced rotational behavior is dependent on both striatal and nigral mechanisms.[39] Reinnervation of the striatum and SN may be essential to optimize graft-derived functional improvement. This notion is also supported by a recent study in which enhanced functional recovery was observed in hemiparkinsonian rats that received simultaneous intrastriatal dopaminergic and intranigral GABAergic grafts.[45] Intranigral transplantation of fetal SN allografts in the hemiparkinsonian rhesus monkey has also been shown to produce behavioral recovery.[42] All of these animal studies indicate that the SN may be an important transplantation target for PD.

Evidence from these preclinical studies has prompted a safety and feasibility pilot study of double grafts in patients with PD, which is currently ongoing in our center. We report here the clinical and PET scan results in three patients enrolled in this study who have been followed for up to 13 months posttransplantation. Clinical improvement correlated with an increase in fluorodopa uptake in the striatum and SN posttransplantation. This report provides, for the first time, evidence of the feasibility of grafting the SN and striatum in patients with PD.

Methods

Patient Selection

The inclusion criteria for selecting these patients consisted of a diagnosis of idiopathic PD made independently by two neurologists and preoperative PET scanning results consistent with PD. Also, the patients must have responded well to levodopa from the onset of the disease, but maximum tolerated medication must no longer provide adequate relief of symptoms, or there must be unacceptable side effects. The patients underwent a detailed assessment for at least 6 months before surgery, in accordance with the Core Assessment Program for Intracerebral Transplantation.[21] The patients were screened for serological evidence of infection with syphilis, hepatitis B and C, human immunodeficiency virus, cytomegalovirus, and human T-cell lymphotropic virus.

Patient Assessment

Preoperative and postoperative clinical assessments were performed on an outpatient basis at regular intervals by using the UPDRS.[9] A timed motor task of hand pronation/supination was also used. All tests were conducted at maximum "on/off" periods as defined by the Core Assessment Program for Intracerebral Transplantation protocol. Video recordings were assessed by a neurologist in a blinded fashion. Throughout the trial, patients and caregivers maintained diaries that included a registry of medication. The exact permutation test for paired samples to test the statistical significance between mean values pre- and postoperatively was performed using a commercially available software package.[7]

Cell Suspension Preparation

Fetal VM tissue was obtained from women undergoing elective abortions in the pregnancy termination unit of our center under strict guidelines of a protocol approved by the University and Hospital ethics review boards. No alteration in the indication, timing, and methodology of the abortion procedure was permitted. Fetal tissue was collected with maternal consent from women who tested negative for human immunodeficiency virus, human T-cell lymphocyte virus, hepatitis B, hepatitis C, and syphilis, and who were undergoing elective abortion by suction curettage between the 6th and 9th week of gestation for reasons unrelated to this transplantation procedure. Tissue obtained in women who had fever, an elevated white blood cell count, or cultures positive for gonorrhea, chlamydia, detectable antibodies to cytomegalovirus or herpes simplex virus was not used.

Fetal ventral mesencephalus were dissected under sterile conditions and tissue samples of each specimen were cultured for aerobic and anaerobic organisms. The ventral mesencephalus were then placed in storage for 6 days of hibernation at 4˚C in 2 ml of a low-sodium, phosphate-buffered, calcium-free hibernation medium consisting of (in mM): 30 KCl, 5 glucose, 0.24 $MgCl_2$, 10.95 NaH_2PO_4, 5 Na_2HPO_4, 20 lactic acid, 32.18 KOH, and 164.7 sorbitol (pH 7.4). Hibernated tissue treated with GDNF received recombinant human GDNF[1,26,29,31] (1 μg/ml hibernation media).

Cell suspensions of VM tissue were incubated in 0.1% trypsin/0.05% DNAse/DMEM at 37˚C for 20 minutes and then rinsed four times in 0.05% DNAse/DMEM. The tissue was then mechanically dissociated using successively smaller sterilized micropipettes until a "chunky" cell suspension was achieved. This suspension was not homogeneous and contained small aggregates of cells. A final concentration of approximately 100,000 cells/μl was used, with viabilities of 98 to 99% as determined by the trypan blue dye exclusion method. Three fetal donors were used for each transplanted side (putamen and SN), for a total of six donors per patient.

Surgical Procedure

The surgical transplantation procedures were performed in two stages 4 weeks apart. On the day of surgery, the patient was fitted with a Leksell stereotactic headframe after application of local anesthetic agents. The stereotactic coordinates for targets in the postcommissural putamen and SN were calculated using T_1- and T_2-weighted MR images and a computerized stereotactic planning workstation.

Transplantation was performed with the patient receiving a local anesthetic and sedation was induced with a combination of midazolam (0.25–1-mg bolus doses) and propofol (10–20 mg bolus followed by infusion of 15–40 μg/kg/min). A burr hole was placed at the level of the coronal suture and a customized transplantation cannula (outer diameter 0.8 mm) was inserted into four different targets approximately 3 mm apart in the postcommissural putamen and one target in the ipsilateral SN. A 50-μl Hamilton sy-

ringe, which was fitted with a custom-made microinjector, was used to load the cell suspension into the transplantation cannula.[31] The cell suspension was deposited along each of four putaminal trajectories that had been previously calculated on the patient's MR study. Four injections of approximately 2.5 μl (250,000 cells) were made in each trajectory, for a total of 10 μl per trajectory. Approximately 4,000,000 cells were deposited in each postcommissural putamen. For the SN injections, two 2.5-μl deposits were made for a total of 5 μl (500,000 cells). The patients received 1 g of Ancef intravenously before the skin incision was made, and three more doses of 1 g of Ancef were administered intravenously every 8 hours postoperatively. Each patient underwent MR imaging with a 1-tesla magnet T_1- and T_2-weighted axial, coronal, and sagittal images were obtained 24 hours postsurgery to check for target accuracy, and all were discharged from the hospital 48 hours after surgery.

Perioperative Management

The patients received immunosuppressive medication (5–8 mg/kg/day cyclosporin A) beginning 2 weeks before their admission. Doses were then tapered to 2 mg/kg/day and continued for 6 months. Immunosuppressive doses were adjusted on the basis of drug levels in serum, and renal function was monitored closely to detect immunosuppressive toxicity. Postoperatively, every effort was made to keep antiparkinsonian medications at their preoperative level, and modifications were made only for clinical reasons.

Neuroimaging Studies

The PET scans were performed preoperatively and at 6 and 12 months postsurgery in all three patients at the McConnell Brain Imaging Centre (Montreal Neurological Institute, McGill University). Scans were performed on the Siemens ECAT HR+ Positron Emission Tomograph in three-dimensional mode, with a resolution of approximately 5 mm full width at half maximum in all directions at the center of the field of view. Patients received a bolus injection of 3 to 5 mCi of fluorodopa into the antecubital vein over 2 minutes. Their heads were immobilized within the aperture of the PET scanner by a form-fitting vacuum device. One hour before the scan, patients received 150 mg carbidopa orally to prevent the peripheral breakdown of fluorodopa. On the day of the study, patients did not take their antiparkinsonian medications, and they did not eat breakfast before the scan. After the injection of fluorodopa, PET data were acquired for 90 minutes in 27 time frames of varying durations. In addition, all patients underwent high-resolution MR imaging with a 1.5-tesla magnet; T_1-weighted $1 \times 1 \times 1$–mm images were obtained for anatomical coregistration.

The PET scans were automatically realigned to MR images for each patient.[8] The MR images were transformed into standardized stereotactic space.[6] Regions of interest were then drawn onto the MR image in stereotactic space on the basal ganglia (caudate nucleus and putamen), midbrain, and cerebellum. The cerebellum was used as a reference region to calculate the fluorodopa by using the graphic method of Patlak and Blasberg.[36] In addition, K_i maps were generated by calculating this value at each voxel. Fluorodopa K_is were taken of the putamen, caudate, and midbrain areas.

TABLE 1

Demographic factors and baseline scores in three patients who underwent VM tissue transplantation for PD

Case No.	Age (yrs), Sex	Duration of PD (yrs)	Score (on/off)		
			UPDRS	Schwab–England Scale	Hoehn–Yahr Scale
1	59, F	14	90.5/139.0	60/20	2.5/5
2	52, F	11	47.5/78.0	90/60	2.5/3
3	48, M	10	65.0/75.0	80/70	2.5/2.5

Follow-up MR images were obtained with gadolinium enhancement 6 and 12 months postoperatively to check for disruption of the blood–brain barrier.

Sources of Supplies and Equipment

The exact permutation tests were performed using StatExact software, version 4, which was acquired from StatSoft, Tulsa, OK. The GDNF was purchased from Prepro Tech, Inc., Rocky Hill, NJ. The DNAse and DMEM were obtained from Sigma Chemical Co., Chicago, IL, and the trypsin from Worthington, Freehold, NJ. The Leksell stereotactic head frame and the stereotactic planning workstation (Surgiplan) were acquired from Elekta AB, Stockholm, Sweden. The MR imager (Signa-Horizon) was purchased from General Electric Medical Systems, Milwaukee, WI, and the high-resolution MR imager (Gyroscan) was acquired from Philips Medical Systems, Eindhoven, The Netherlands. The PET scanner (model ECAT HR+) was obtained from Siemens, Erlangen, Germany.

Results

Surgical Procedure

Three patients in whom PD was diagnosed (Table 1) underwent bilateral transplantation of fetal VM tissue into the putamen and SN (Fig. 1). Each patient underwent two surgical procedures 4 weeks apart. In each session, the putamen and SN on one side of the brain received transplanted tissue. All patients tolerated the surgical procedures well and there were no intra- or perioperative complications. One patient (Case 3) who had a history of hypertension developed a hemorrhage in his right frontal lobe 3 months after his first transplant, which required surgical evacuation. The origin of the hemorrhage was considered to be hypertensive in nature and unlikely to be related to the transplant procedure. The hemorrhage was confined to the subcortical white matter and did not reach the putamen. The patient experienced residual arm weakness after the event.

Brain MR images obtained 24 hours postsurgery demonstrated that the deposits of transplanted tissue were made in the desired targets (Fig. 2). The MR images obtained with gadolinium enhancement at 6 and 12 months posttransplantation demonstrated no areas of enhancement, which indicated no blood–brain barrier breakdown or any other abnormality in the transplanted areas.

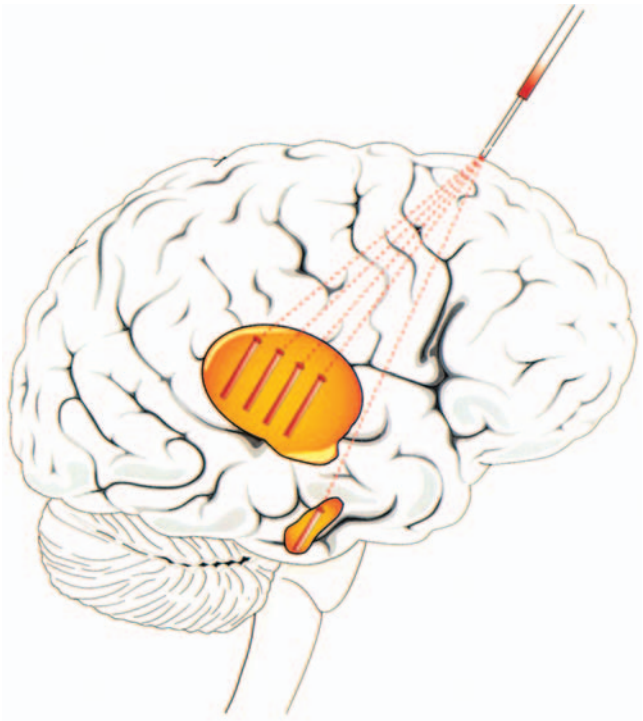

FIG. 1. Diagram illustrating the double transplant strategy. Four graft deposits are implanted into the putamen and one is implanted into the SN.

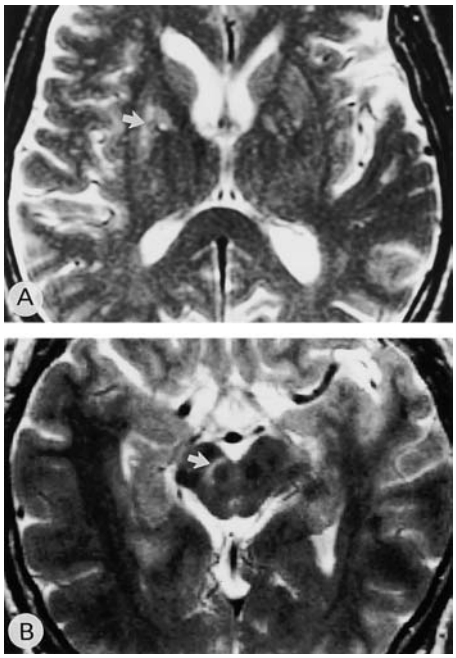

FIG. 2. Axial T_2-weighted MR images obtained 24 hours postoperatively in a patient with PD who received simultaneous intrastriatal and intranigral fetal VM grafts. A: Axial MR image depicting four tracts of graft deposits into the right putamen *(arrow)*. B: Axial MR image depicting a graft deposit into the right SN *(arrow)*.

Clinical Outcome

The patients in Cases 1 and 2 were followed for 13 months, whereas the patient in Case 3 was followed for only 3 months because his subsequent clinical assessment was disrupted by his hypertensive hemorrhage. Therefore preoperative and the last three postoperative scores were analyzed for the three patients (Fig. 3). The mean total UPDRS scores in the on state improved from 67.66 preoperatively to 45.66 postoperatively ($p = 0.07$), and in the off state the mean total UPDRS scores improved from 97.33 to 61.33 postoperatively ($p = 0.04$). The mean Hoehn and Yahr[17] scores also improved, from 2.5 before surgery to 2.16 at last assessment in the on state ($p = 0.04$), and from 3.5 before surgery to 2.66 at last assessment in the off state ($p = 0.04$). The mean Schwab and England scores improved from 50 preoperatively to 66.67 at last assessment in the off state ($p = 0.04$); no significant mean change was observed in the Schwab and England[41] scores in the on state. Improvement was also observed in the timed motor task of hand pronation/supination bilaterally. This change was marginally significant in the off state only. For the right hand, the mean pronation/supination improved from 7.51 turns before the surgery to 13.67 turns at last assessment ($p = 0.06$), and for the left hand, the mean scores improved from 8.65 turns before surgery to 16.5 turns at last assessment ($p = 0.07$).

The patient in Case 1 had a decrease in levodopa requirements from 600 mg/day at baseline to 325 mg/day postsurgery, which represents a 46% decrease. In the patient in Case 3, levodopa requirements decreased 65%, from 1150 mg/day at baseline to 400 mg/day postsurgery. No change in the levodopa dose requirement occurred in the patient in Case 2.

TABLE 2

*Preoperative and 1-year postoperative [^{18}F]fluorodopa K_i values and percentage change in three patients who underwent VM tissue transplantation for PD**

Case No.	Region	K_i Value		Percentage Change
		Preop	Postop	
1	lt put	0.004202	0.002629	−37.4
	rt put	0.002427	0.004864	+100.4
	lt mid	0.002467	0.003025	+22.6
	rt mid	0.002127	0.003613	+69.9
2	lt put	0.002744	0.003886	+41.6
	rt put	0.002181	0.001458	−33.2
	lt mid	0.002181	0.004048	+40.4
	rt mid	0.002450	0.002813	+14.8
3	lt put	0.002575	0.003908	+51.8
	rt put	0.002427	0.005654	+133.0
	lt mid	0.003481	0.004140	+18.9
	rt mid	0.002745	0.005700	+107.7

* Mid = midbrain; put = putaminal.

Fluorodopa-Enhanced PET Scans

Preoperative PET scans confirmed that the patients had a marked decrease in putaminal fluorodopa uptake consistent with the diagnosis of idiopathic PD.[22,43] Twelve months postoperatively, there was an increase in fluorodopa K_i values in the midbrain when compared with preoperative values in all three patients (Table 2). Putaminal fluorodopa K_i

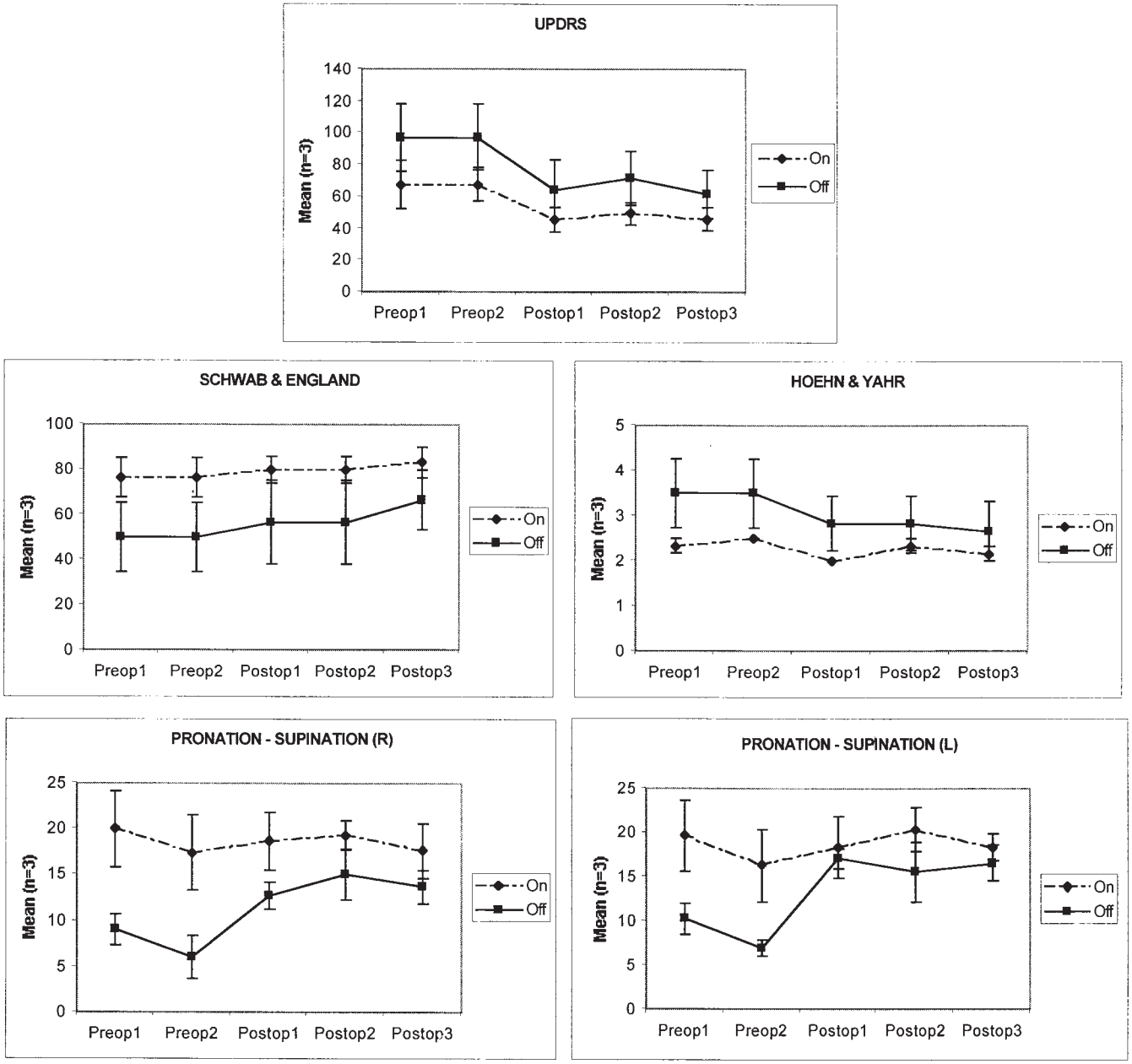

FIG. 3. Graphs showing the mean performance scores before and after surgery in the on and off state for total UPDRS, Hoehn and Yahr, and Schwab and England scores, and the timed motor task of pronation/supination for the right (R) and left hand (L). Error bars are marked with 95% confidence intervals. Overlapping error bars postoperatively indicate nonsignificant differences between the on and off states. n = number of patients; postop1, -2, and -3 = last three postoperative scores; preop1 and -2 = first and second preoperative scores.

values also increased from the baseline in all three patients, with the exception of the left putamen in the patient in Case 1 and the right putamen in the patient in Case 2, in which K_i values decreased, indicating that the graft did not survive in these locations.

Discussion

In this study we have provided evidence that transplantation of tissue in the SN as well as in the putamen is feasible in humans. This early clinical experience indicates that double grafting is relatively safe in humans, because no intraoperative or perioperative complications or adverse effects related to the transplant procedure were observed in these patients.

Although there is evidence that intranigral grafts can survive in animal models of PD and induce functional improvements,[2,28,30,32,33,35,42,46] this is the first evidence that intra-

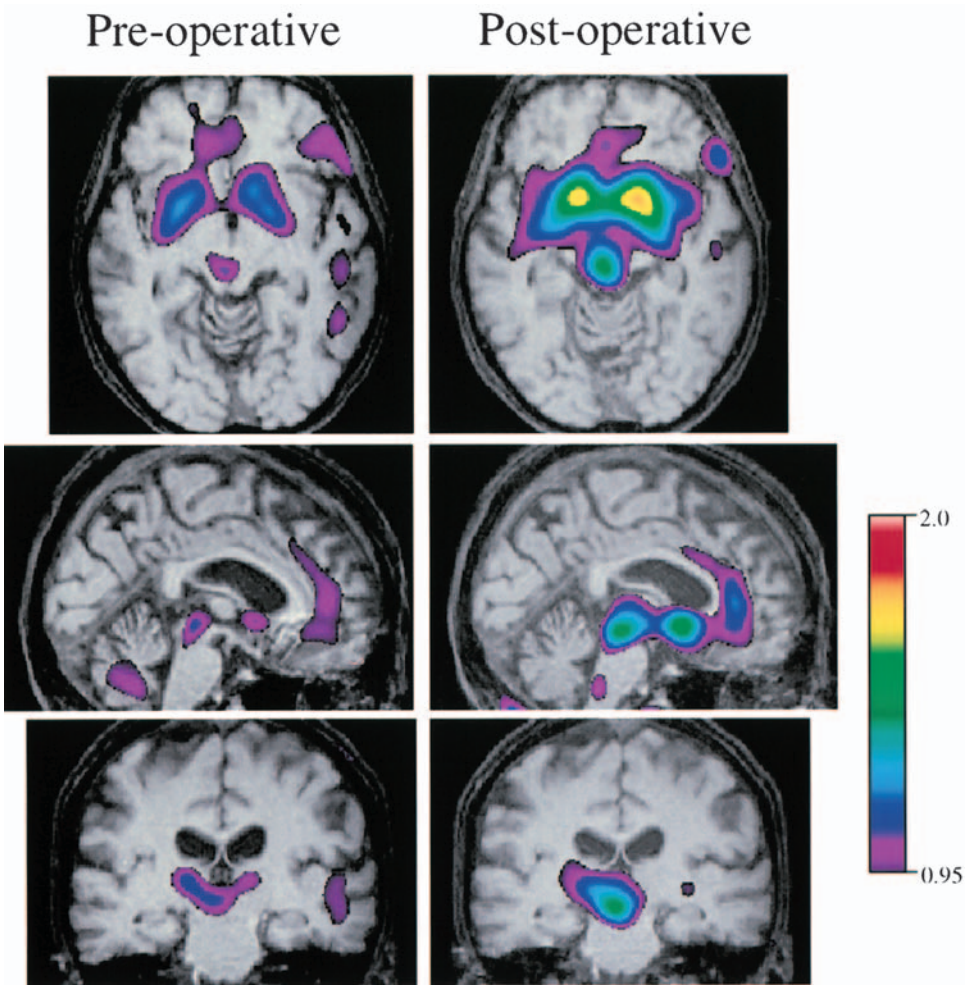

FIG. 4. Preoperative and postoperative fluorodopa PET scans obtained in a patient with PD (Case 3) who received simultaneous intrastriatal and intranigral fetal VM grafts. These images consist of parametric maps of fluorodopa K_i transformed into stereotactic space and overlaid on the patient's MR image (also in stereotactic space). The parametric maps were smoothed using an 8-mm gaussian filter. The axial, coronal, and sagittal sections demonstrate an increase in K_i in the midbrain and putamen bilaterally, likely resulting from surviving grafted dopamine neurons.

nigral grafts can survive in humans (Fig. 4). Postoperative PET scans demonstrated a significant increase in fluorodopa uptake in the midbrain bilaterally in these patients 12 months after transplantation. Assessment of fluorodopa uptake by using PET scanning is the only valid method of assessing graft survival in vivo.[11,13,24,29,38,44] Furthermore, correlation of graft survival with fluorodopa uptake on PET scans has been made by postmortem examination in a patient who received these transplants.[19,20] A recent PET study in which [^{11}C]-raclopride contrast was used also indicates that grafted dopaminergic cells can release dopamine 10 years after transplantation.[38]

We have previously demonstrated in a rodent model of PD that grafts in the striatum and SN induce a better functional recovery compared with intrastriatal grafts alone.[2,28,30,32] This beneficial effect may result from restoration of dopaminergic reinnervation of the host SN. It is well known that dopamine is released in the SN pars reticulata by dendrites of pars compacta neurons.[4,5] Nigral dopamine is believed to enhance GABA release from striatonigral efferents through presynaptic (D1) dopamine receptors,[40] reducing GABA transmission in the ventromedial thalamus[14] and increasing locomotor activity.[18] Furthermore, there is evidence that levodopa-induced rotational behavior is dependent on both striatal and nigral mechanisms.[39] The main reason for the ectopic placement of dopaminergic tissue is the apparent inability of grafts placed in the ontogenic location (SN) to grow axons over long distances to reach their target (striatum). This strategy has failed, however, to restore dopaminergic innervation to the SN or to reconstruct the nigrostriatal pathway. The inability of intrastriatal grafts to restore the dopaminergic innervation to the SN may be an important factor in limiting the clinical efficacy of fetal tissue transplantation. It is clear that the current neural transplantation strategy for PD, in which the striatum has been targeted as the optimal site for dopaminergic graft placement, has not achieved the degree of clinical benefit necessary to consider this procedure to be an effective surgical option for PD. This notion is supported by results of a recent double-blinded, sham-surgery, controlled trial of

fetal tissue transplantation into the putamen that showed limited clinical benefits in patients who received the transplants.[11] Determining the appropriate target for transplantation is a crucial step in optimizing neural transplantation as a restorative therapy for PD.

In this pilot study we demonstrate the feasibility of reinnervating the SN and striatum by using a double transplant strategy in humans. Reinnervation of the SN in addition to the striatum may be crucial to improve clinical outcomes in patients with PD, and it deserves further investigation.

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