TRANSPLANTATION OF EMBRYONIC DOPAMINE NEURONS FOR SEVERE PARKINSON'S DISEASE

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ABSTRACT

Background Transplantation of human embryonic dopamine neurons into the brains of patients with Parkinson's disease has proved beneficial in open clinical trials. However, whether this intervention would be more effective than sham surgery in a controlled trial is not known.

Methods We randomly assigned 40 patients who were 34 to 75 years of age and had severe Parkinson's disease (mean duration, 14 years) to receive a transplant of nerve cells or undergo sham surgery; all were to be followed in a double-blind manner for one year. In the transplant recipients, cultured mesencephalic tissue from four embryos was implanted into the putamen bilaterally. In the patients who underwent sham surgery, holes were drilled in the skull but the dura was not penetrated. The primary outcome was a subjective global rating of the change in the severity of disease, scored on a scale of −3.0 to 3.0 at one year, with negative scores indicating a worsening of symptoms and positive scores an improvement.

Results The mean (±SD) scores on the global rating scale for improvement or deterioration at one year were 0.0±2.1 in the transplantation group and −0.4± 1.7 in the sham-surgery group. Among younger patients (60 years old or younger), standardized tests of Parkinson's disease revealed significant improvement in the transplantation group as compared with the sham-surgery group when patients were tested in the morning before receiving medication (P=0.01 for scores on the Unified Parkinson's Disease Rating Scale; P=0.006 for the Schwab and England score). There was no significant improvement in older patients in the transplantation group. Fiber outgrowth from the transplanted neurons was detected in 17 of the 20 patients in the transplantation group, as indicated by an increase in ^{18}F-fluorodopa uptake on positron-emission tomography or postmortem examination. After improvement in the first year, dystonia and dyskinesias recurred in 15 percent of the patients who received transplants, even after reduction or discontinuation of the dose of levodopa.

Conclusions Human embryonic dopamine-neuron transplants survive in patients with severe Parkinson's disease and result in some clinical benefit in younger but not in older patients. (N Engl J Med 2001;344: 710-9.)

A FTER several years of treatment with levodopa and other drugs,[1] motor fluctuations ranging from bradykinesia to hyperkinesia develop in many patients with Parkinson's disease. No drug therapy has eliminated these fluctuations. However, the implantation of embryonic dopamine neurons into the brain may improve motor control. We and others have reported that transplanted dopamine neurons survive and that patients may have progressive clinical improvement over a period of three to four years.[2-21]

All these studies were unblinded, and the number of patients in each was small. Even with standardized scoring with the use of the Unified Parkinson's Disease Rating Scale (UPDRS),[22] the Schwab and England scale,[23] and the scale of the Core Assessment Program for Intracerebral Transplantations,[24] the variability in surgical methods within and between centers has made it difficult to compare the results of dopamine-neuron implantation in different groups of patients.

We conducted a double-blind, sham-surgery–controlled trial of the implantation of embryonic dopamine neurons in patients with severe Parkinson's disease. Our goals were to determine whether the implanted neurons survived and led to improvement in the symptoms and signs of Parkinson's disease and to examine the effect of age on the efficacy of implantation.[25-28]

METHODS

Patients

All patients considered for the study had had Parkinson's disease for more than seven years with at least two of the three main signs: bradykinesia, rigidity, and tremor at rest. All patients had improvement in response to levodopa, with improvement of at least 33 percent in the total UPDRS score after a first morning dose of levodopa,[22] and had base-line ^{18}F-fluorodopa positron-emission tomographic (PET) scans compatible with the presence of Parkinson's disease, with a diminution of ^{18}F-fluorodopa uptake that was more severe in the putamen than in the caudate nuclei.[29] Before patients were enrolled in the study, their clinical response to drug therapy was optimized. Criteria for exclusion from the study included a score on the Mini–Mental State Examination of less than 24, hallucina-

tions or delusions during levodopa therapy, epilepsy, previous brain surgery, severe depression, cerebrovascular disease, evidence on magnetic resonance imaging of another neurologic disorder, and a medical contraindication to surgery.

The protocol and a consent form describing the risks and potential benefits of the study were approved by the institutional review boards of the University of Colorado, Columbia University, North Shore University Hospital, and a performance and safety monitoring board appointed by the National Institutes of Health. A separate consent form for the donation of fetal tissue from elective abortions was also approved by these groups. Written informed consent was requested from the women who donated tissue only after the women had consented to the abortion procedure, as required by federal law. All donors and all patients with Parkinson's disease had negative serologic tests for hepatitis B and C virus, human immunodeficiency virus types 1 and 2, and syphilis. Cultures of embryonic tissue performed before transplantation were negative for human herpesvirus, cytomegalovirus, fungi, and bacteria.

Evaluation of the Patients

All of the patients were admitted to the Irving Center for Clinical Research at Columbia University for three to four days on five occasions during the study: twice before surgery for base-line assessments and 4, 8, and 12 months after surgery. The patients kept diaries of their symptoms for one week before each inpatient evaluation. The patients also submitted global ratings of their symptoms by mail one week before each postoperative admission.

Inpatient testing included assessments that used the UPDRS and the Schwab and England scale. The UPDRS is a comprehensive inventory of symptoms and signs of Parkinson's disease, including mentation and mood; activities of daily living and motor performance; and muscle rigidity, speech, and gait.[22] The scores for the three parts of the UPDRS range from 0 (normal) to 176 (worst possible). The Schwab and England scale measures performance in the activities of daily living, with 100 percent denoting normal and 0 denoting completely disabled.[23] For both tests, the state of the patient was defined as "off medication" when testing was conducted before the patient had a first morning dose of levodopa and at least 12 hours after the administration of levodopa the previous day.[24] The "on medication" scores refer to the best test scores recorded during the day while the patient was taking medication.

Because the doses of levodopa and other antiparkinsonian drugs are individualized for each patient, change in drug therapy can confound the interpretation of surgical outcomes. We sought to refrain from changing patients' preoperative drug schedules if possible. However, dyskinesias develop in most patients after transplantation,[2,5] making adjustments of the doses necessary. In all cases, the decisions about whether to change doses of drugs were made by physicians who were not aware of the patients' treatment-group assignments.

Randomization

Patients were randomly assigned in groups of 10 to undergo sham surgery or transplantation, with adjustments to balance the groups according to age, sex, and duration of disease. The patients, the examining physicians and nurses at Columbia–Presbyterian Medical Center, the nurse coordinator at the University of Colorado, and the PET imaging staff at North Shore University Hospital were unaware of treatment-group assignments throughout the study; only the statistician and the surgical team at the University of Colorado Hospital were aware of the assignments.

Method of Transplantation

All surgical procedures were performed at the University of Colorado Hospital. On admission, patients received oral phenytoin at a dose of 15 mg per kilogram of body weight. After a stereotactic ring was affixed to the skull, magnetic resonance imaging was used to establish coordinates for four needle passes in the axial plane of the putamen, extending from its full anterior-to-posterior dimension (about 35 to 40 mm). Two needle tracks were created in each

side of the brain above the frontal sinus, one about 7 mm higher than the other.

Human embryonic mesencephalic tissue containing dopamine neurons was recovered from fragments of embryos aborted seven to eight weeks after conception. Tissue was extruded through a sterile glass extruding device as strands 200 μm in diameter and was cultured in F12 medium containing 5 percent human placental serum.[4,5] Production of dopamine by the tissue during culture was monitored by measurement of homovanillic acid concentrations in the culture medium. Tissue was transplanted up to four weeks after it had been obtained.

Only in the operating room did the neurosurgeon learn whether a sham operation or transplantation would be performed. Surgery was performed with the patient awake, with local anesthesia administered to the skin of the forehead. Four twist-drill holes through the frontal bone were made along the planned axis of the tracks. The tissue implants were placed with the use of a stainless-steel guide cannula with a graduated outer diameter of 1.5 to 0.6 mm. A rounded stylet was contained in the bore of the cannula during its passage to the posterior tip of the putamen. The stylet was then replaced with a needle containing embryonic tissue, usually in a total volume of 20 μl, which was deposited continuously as the needle was withdrawn through the putamen, a distance of 35 to 40 mm. After a two-minute wait for the stabilization of pressure, the cannula was removed from the brain. Each implant consisted of tissue from a single embryo. The patients' ability to speak and to move all of their extremities was tested after each injection. The patients in the sham-surgery group underwent an identical procedure except that the dura mater was not penetrated after the twist-drill holes had been made in the frontal bone. No patient received immunosuppressive drugs.

Imaging Studies

At North Shore University Hospital, ^{18}F-fluorodopa scanning was performed before and 12 months after surgery. PET images were quantified by a rater who was unaware of treatment-group assignments, as described previously.[30,31] We estimated the striatal uptake of ^{18}F-fluorodopa by subtracting the occipital background signal from the striatal activity and dividing the result by the occipital activity. The difference between uptake by the putamen at base line and 12 months after surgery was the measure of the growth of the transplant.

Outcome Variables

A subjective global rating of clinical improvement or deterioration, scored by the patients, was the primary outcome variable. Patients chose phrases with corresponding point values ranging from "parkinsonism markedly worse" (−3) through "no change" (0) to "parkinsonism markedly improved as compared with before surgery" (+3). Patients chose a phrase, rather than a number, to characterize their condition. The only global rating score used for analysis of the primary outcome was the value mailed in by patients 12 months after surgery. Secondary outcomes were the growth of transplants as estimated by means of ^{18}F-fluorodopa PET scans and the clinical outcomes, assessed in terms of UPDRS and Schwab and England scores while patients were off medication and as reported in the diaries kept by patients. Changes in drug doses and the results of neuropsychological assessments were tertiary outcomes.

Safety

Serious adverse events were defined as illnesses and incidents that necessitated hospitalization or caused death. These events were reported to the monitoring board immediately. Asymptomatic hemorrhage along a needle track during surgery was considered to be an adverse event but not a serious adverse event.

Transplantation in Patients with Previous Sham Surgery

Patients randomly assigned to sham surgery had the option of receiving an implant of dopamine neurons after they had complet-

ed the double-blind phase of the study, which lasted for one year after the original surgery. Fourteen of the 20 patients in the sham-surgery group received transplants in subsequent operations.

Statistical Analysis

Student's t-test was used to compare the two groups with respect to the primary outcome variable. The statistician performed an interim analysis of the primary outcome variable after 20 patients had completed the blinded phase of the study. Only the members of the performance and safety monitoring board were informed of the outcome, and they allowed the study to continue. The t-test was also used to compare the results of the ^{18}F-fluorodopa measurements. The generalized-estimating-equation method[32-34] was used to analyze the other, secondary outcome variables (UPDRS scores, Schwab and England scores, and data from the diaries). This method assumes that the characteristics of a single patient are likely to be correlated over time. Repeated measures for each patient were treated as a cluster. All reported P values are two-sided. No formal adjustments were made for multiple comparisons of secondary end points.

RESULTS

The characteristics of the 19 women and 21 men who enrolled in the study are shown in Table 1. The patients who were 60 years old or younger had better responses to drugs, as indicated by the differences between the UPDRS scores recorded when patients were off medication and those recorded when they were on medication. Enrollment took place from April 1994 to April 1997. The operations began in May 1995 and were completed in January 1998.

A total of 39 patients completed the study. One patient in the transplantation group died in an automobile accident seven months after surgery. There were no serious perioperative complications. Magnetic resonance images of the brain were obtained in all patients within 24 hours after surgery. Except for an asymptomatic hemorrhage noted in one patient at the time of surgery and confirmed on magnetic resonance imaging, there were no abnormal findings.

Scores on the Global Rating Scale

Among the subjective global rating scores reported by patients from home 12 months after surgery, only those of the younger patients in the transplantation group (60 years old or younger) were positive, indicating improvement; all other patients had negative scores, indicating worsening disease. There were no statistically significant differences between the treatment groups. The mean ($\pm$SD) global rating score was 0.0 ± 2.1 among the 19 patients in the transplantation group, and -0.4 ± 1.7 among the 20 patients in the sham-surgery group (P=0.62). For the younger patients, the scores were 0.5 ± 2.1 in the transplantation group and -0.3 ± 1.7 in the sham-surgery group (P= 0.36). For older patients (more than 60 years old), the scores in the respective groups were -0.7 ± 2.0 and -0.4 ± 1.7 (P=0.80). The week after these ratings were made, at hospital admission 12 months after surgery, with patients and examining staff still unaware of treatment-group assignment, the patients viewed a preoperative video of themselves and gave themselves new global-rating scores. Patients in both age groups and both treatment groups changed their scores to positive values. For younger patients, the new scores in the transplantation and sham-surgery groups were 1.0 ± 1.8 and 0.3 ± 1.6, respectively (P=0.35). For older patients, the new scores were 0.2 ± 2.0 and 0.3 ± 1.7, respectively (P=0.90).

UPDRS Scores

The total UPDRS scores recorded when patients were off medication at one year were similar in the two treatment groups (P=0.11) (Fig. 1). Among the younger patients, those who received transplants had significantly greater improvement in UPDRS scores than those in the sham-surgery group (P=0.01). The

TABLE 1. Base-Line Characteristics of Patients with Parkinson's Disease.*

Characteristic	All Patients (N=40)	Sham-Surgery Group ≤60 yr (N=11)	Sham-Surgery Group >60 yr (N=9)	Transplantation Group ≤60 yr (N=10)	Transplantation Group >60 yr (N=10)
Sex — no. (%)					
Female	19 (48)	6 (55)	3 (33)	5 (50)	5 (50)
Male	21 (52)	5 (45)	6 (67)	5 (50)	5 (50)
Age — yr	57±10	49±6	66±5	50±8	65±4
Duration of disease — yr	14±6	13±6	15±7	13±3	15±6
UPDRS score while on medication	22±14	16±6	30±15	12±6	27±11
UPDRS score while off medication	63±21	61±21	71±20	58±19	59±21
Improvement from "off" to "on" — %	65	74	58	79	54
Diary score	3.0±1.8	2.5±1.9	4.0±1.5	2.5±1.5	3.3±1.9

*Plus–minus values are means ±SD. Higher scores on the Unified Parkinson's Disease Rating Scale (UPDRS) and in the diary reflect more severe symptoms of Parkinson's disease. The worst possible UPDRS score is 176, and the best score is 0. The worst possible diary score is 5, and the best score is 0.

score on this scale (on which higher values indicate more severe symptoms) decreased by 15 percent from base-line values in the transplantation group as a whole and 28 percent among the younger patients in the transplantation group. When only the motor components of the UPDRS were analyzed, the scores when patients were off medication decreased 18 percent for the transplantation group as a whole (P=0.04) and 34 percent for the younger patients in this group (P=0.005). The signs in which improvement occurred were rigidity and, in the younger patients, bradykinesia. Tremor did not improve in either age group. Transplantation resulted in a greater improvement in the UPDRS scores recorded for men while off medication than for women while off medication (P=0.04). For

each age group and overall, there were no significant differences between the transplantation and sham-surgery groups with respect to the best UPDRS scores recorded during a day of testing while patients were on medication.

Schwab and England Scores

There was significantly greater improvement from base line in Schwab and England scores recorded when patients were off medication in the transplantation group than in the sham-surgery group (P=0.008) (Fig. 1). Only the younger patients who received transplants had improvements (P=0.006 for the comparison with the sham-surgery group). The best Schwab and England scores recorded when patients were on
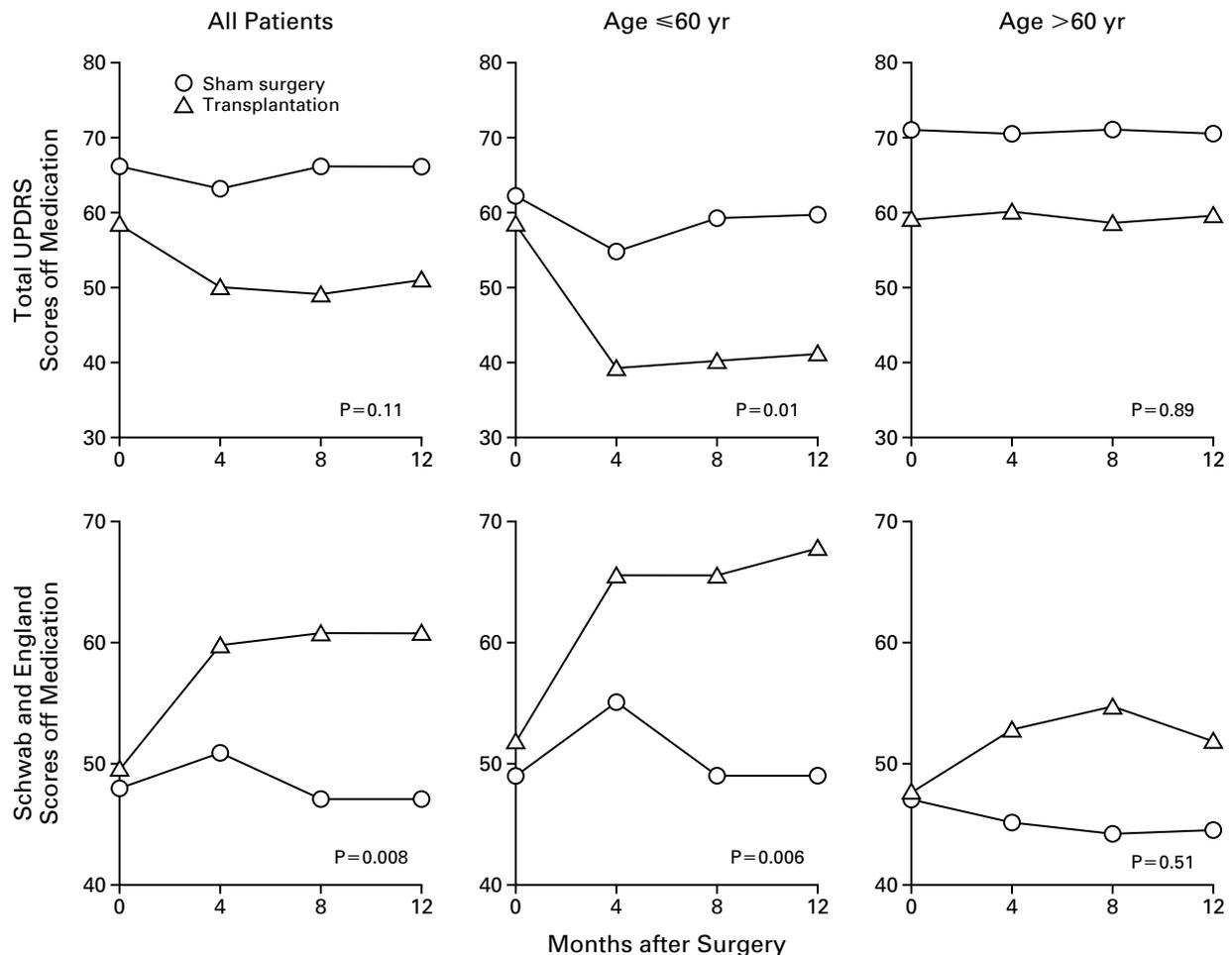

Figure 1. Unified Parkinson's Disease Rating Scale (UPDRS) Scores and Schwab and England Scores for Patients in the Sham-Surgery and Transplantation Groups while off Medication.

For the UPDRS scores, the higher the score, the worse the parkinsonism (worst possible score, 176; best possible score, 0). For the Schwab and England scores, the higher the score, the better the performance in the activities of daily living (worst possible score, 0; best possible score, 100). The scores at 0 months are the average of the scores on two base-line tests. The P values are for the comparisons between the scores in the two groups at 12 months.

medication did not differ significantly between the transplantation and sham-surgery groups.

Patients' Diaries and Drug Doses

There were no significant differences between the treatment groups in terms of the patients' diary scores or drug doses one year after surgery (data not shown).

Adverse Events

Serious adverse events that necessitated hospitalization or caused death during the one-year follow-up period are listed in Table 2. One serious adverse event, a subdural hematoma first detected about six weeks after surgery, was judged to be "possibly" related to the surgery, since the magnetic resonance image had been normal on the day after surgery. The subdural hematoma resolved without intervention. More serious adverse events occurred in the transplantation group than in the sham-surgery group (eight and one, respectively). A total of 313 nonserious adverse events of various degrees of severity were reported by the 40 patients; the 275 events of types that were reported more than once are listed in Table 3. There were no significant differences in the severity of adverse events between the transplantation and sham-surgery groups.

Growth of Transplants

Typical ^{18}F-fluorodopa PET scans from patients in the transplantation and sham-surgery groups are shown in Figure 2. The PET scans of 16 of the 19 patients in the transplantation group (84 percent) were correctly identified by a blinded rater as positive for transplant growth, and in only 1 of the 20 patients in the sham-surgery group (5 percent) was the scan incorrectly judged to show transplant growth. Quantitative analysis of the scans at base line and at one year revealed a significant increase in radionuclide uptake in the putamen among patients in the transplantation group (percent change from base line, 40 ± 42; P<0.001) but no significant change in uptake in the sham-surgery group (-2 ± 17 percent, P=0.40), yielding a significant difference (P<0.001) between the transplantation and sham-surgery groups. The increases in ^{18}F-fluorodopa uptake in the putamen were similar in the younger and older patients in the transplantation group.

Postmortem Analysis of the Brains of Two Patients with Transplants

A 66-year-old woman died in an automobile accident seven months after transplantation surgery when a tree fell across the highway during a storm. Examination of the brain revealed a small right anterior subarachnoid hemorrhage. Histologic examination of the substantia nigra showed degenerating pigmented dopamine neurons with Lewy bodies — findings compatible with the presence of idiopathic Parkinson's

TABLE 2. THE NINE SERIOUS ADVERSE EVENTS THAT OCCURRED DURING 12 MONTHS OF FOLLOW-UP.

EVENT	MONTHS AFTER SURGERY	TREATMENT GROUP	RELATION TO SURGERY
Wrist fracture	1	Transplantation	Unlikely
Subdural hematoma	<2	Transplantation	Possible
Cerebral infarction	4	Transplantation	Unlikely
Elective shoulder surgery	5	Transplantation	Unlikely
Fatal motor vehicle accident	7	Transplantation	Unlikely
Suicide attempt with anti- parkinsonian drugs	9	Transplantation	Unlikely
Myocardial infarction	12	Transplantation	Unlikely
Myocardial infarction	12	Transplantation	Unlikely
Hysterectomy	14*	Sham surgery	Unlikely

*This event occurred after 12 months but before treatment-group assignment was known.

TABLE 3. NONSERIOUS ADVERSE EVENTS.*

EVENT	SHAM-SURGERY GROUP	TRANSPLANTATION GROUP
	number	
Increased "off" time or severity	67	53
Increase in episodes of "freezing"	14	16
"On" states not as good or delayed	7	11
Increase in dyskinesia	25	20
Increase in depression	8	8
Confusion, hallucination, or increase in psychosis	1	8
Headache	9	7
Increase in constipation	7	2
Falling	3	9
Total	141	134

*"Freezing" is the sudden loss of the ability to move, particularly while walking. "Off" time refers to periods in which movement is slow, often when levodopa and other drugs are at low blood levels. "On" describes the state in which movement is more rapid and sometimes complicated by abnormal dyskinetic motion, usually while the patient is taking levodopa.

disease (Fig. 3). The putamens containing the transplants were sectioned at 40-μm intervals along the axial plane, and the sections were tested for immunoreactivity to tyrosine hydroxylase as previously described.[35] All four transplant tracks contained large numbers of dopamine neurons with fiber outgrowth extending 2 to 3 mm from the cell bodies. The two tracks on the left side contained 18,204 and 20,188
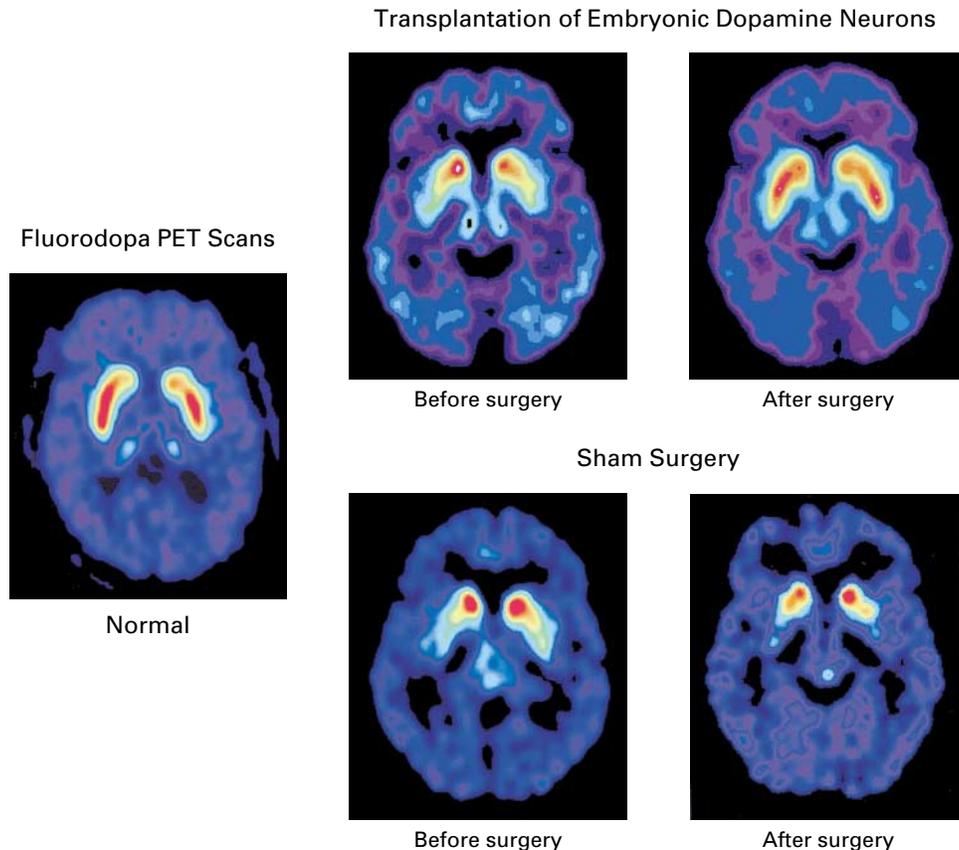
Transplantation of Embryonic Dopamine Neurons

Fluorodopa PET Scans

Normal

Before surgery

After surgery

Sham Surgery

Before surgery

After surgery

Figure 2. Change in ^{18}F-Fluorodopa Uptake in the Brains of Patients with Parkinson's Disease after Transplantation, as Shown in Fluorodopa PET Scans.

In the panel on the far left, an axial section through the caudate and putamen of a normal subject shows intense uptake of ^{18}F-fluorodopa (red). On the right side, the upper panels show preoperative and 12-month postoperative scans in a patient in the transplantation group. Before surgery, the uptake of ^{18}F-fluorodopa was restricted to the region of the caudate. After transplantation, there was increased uptake of ^{18}F-fluorodopa in the putamen bilaterally. The lower panels show ^{18}F-fluorodopa scans in a patient in the sham-surgery group. There was no postoperative change in ^{18}F-fluorodopa uptake.

cells, and the two tracks on the right side contained 12,523 and 11,592 cells. Neuromelanin and Lewy bodies were not detected in the transplanted dopamine neurons.

A 68-year-old man underwent transplantation and completed the one-year follow-up. When he was examined three years after transplantation, his total UPDRS score while off medication had decreased (improved) by 33 percent from base line. Shortly thereafter, at the age of 71, the patient died of an acute myocardial infarction. Histologic examination of his brain revealed Lewy bodies in pigmented dopamine neurons in the substantia nigra. Surviving dopamine neurons were seen in all four transplant tracks (right side, 22,760 and 14,036 cells; left side, 4780 and 2060 cells). Dopamine neurons in all transplant tracks con-

tained neuromelanin granules. Each transplant site had dopamine-neuron outgrowth that extended the full width of the putamen, demonstrating that a three-year period is sufficient for nearly complete reinnervation of the putamen. An ^{18}F-fluorodopa PET scan obtained two years after transplantation showed a 100 percent increase in uptake over base line. The PET signal was not lateralized as might have been predicted on the basis of the differences in dopamine-neuron counts in the two sides of the brain.

Immunostaining with antibodies to the lymphocyte marker CD3 and HLA class II antigen in these two patients revealed some inflammatory cells in the transplant tracks and perivascular areas. The degree of inflammatory response did not appear to be correlated with the number of surviving dopamine neurons.

Subsequent Follow-up

Since the completion of the double-blind protocol, follow-up of the patients has continued. Evaluation at up to three years in the 19 patients in the original transplantation group showed a 28 percent improvement over base line in total UPDRS scores while off medication (38 percent improvement among the younger patients and 14 percent among the older patients; P=0.004 and P<0.001, as determined with the general-estimating-equation method, for the total group and the younger patients in the group, respectively).

Of the 33 patients who ultimately received transplants and who have now survived for as long as three years after surgery, dystonia and dyskinesia developed in 5 (15 percent) and persisted after a substantial reduction in or elimination of therapy with dopamine-agonist drugs.[36] The five patients were all 60 years old or younger at the time of surgery, and all had had severe fluctuations in symptoms of Parkinson's disease before surgery. Three received transplants during the initial double-blind phase, and the other two were originally in the sham-surgery group and received transplants after the one-year blinded portion of the study had ended. Symptoms in all five patients had improved during the first year after transplantation. Because of the lack of efficacy of the transplants in older patients and the late appearance of dyskinesia in some younger patients, the six remaining patients in the sham-surgery group (four who were older than 60 years, and two who were 60 years old or younger) were advised against undergoing transplantation by means of the current method.

DISCUSSION

The goals of this trial were to determine whether transplanted embryonic dopamine neurons survived and improved symptoms and signs of Parkinson's disease and to define the effect of age on the outcome of transplantation. ^{18}F-fluorodopa PET scanning in 19 transplant recipients and postmortem examination of 2 patients revealed that transplanted cells survived in 17 of 20 patients in the transplantation group, regardless of age and without immunosuppression. Autopsy results in two older patients (66 and 71 years old) confirmed the growth of the transplants in the putamen. These results demonstrate that the cellular and chemical signals that support the development of embryonic dopamine neurons are present in patients with Parkinson's disease. Neuromelanin granules typically found in mature human dopamine neurons were first seen in neurons three years after transplantation, an interval consistent with the normal development of these neurons.

The clinical outcome of transplantation was more variable and was in part affected by the age of the patient. A subjective, patient-scored global rating was the primary outcome variable. Although younger patients in the transplantation group scored highest on this scale, the change in the scores of these patients was not significantly different from the change in the scores in the sham-surgery group. Standardized tests of Parkinson's disease conducted before the first morning dose of levodopa (off medication) revealed greater improvement among younger patients in the transplantation group than among those in the sham-surgery group.[22-24] When patients were off medication, the

Figure 3 (facing page). Surviving Dopamine Neurons in the Putamen in Two Patients with Parkinson's Disease Treated with Transplantation.

Immunocytochemical analysis for tyrosine hydroxylase, a protein present in dopamine neurons, was performed as previously described.[35] Panels A, B, and C show the left superior putamen of a 66-year-old woman who died in an automobile accident seven months after transplantation. Panel A shows a section of the brain superimposed on the preoperative magnetic resonance imaging scan. The red lines are projections of the needle tracks on the scan at the time of surgery. Dopamine neurons and fibers in the transplant tracks are aligned along this trajectory and are marked by the dark brown immunoperoxidase staining where there was a reaction. Cell bodies are confined to the central 1 mm of the track. The intense staining in the surrounding area represents dopamine nerve fibers growing out 2 to 3 mm from the cell bodies. The section also demonstrates substantial residual innervation of the caudate nucleus, located anterior and medial to the putamen. Panel B shows an enlargement of the same image. The bar in Panels A and B represents 1 cm. At seven months, fiber outgrowth from the transplant has only partially filled the putamen. Panel C presents a more highly magnified view of the transplant track (bar, 1 mm) with cell bodies in the central track and abundant fiber outgrowth from the cells. The inset shows a hematoxylin-and-eosin–stained section of the patient's substantia nigra, demonstrating a Lewy body in a pigmented dopamine neuron (arrowhead; bar, 15 μm). Panels D, E, and F illustrate the histologic features and tyrosine hydroxylase immunoreactivity of the transplant in the putamen of a 71-year-old man who died of an acute myocardial infarction three years after transplantation. Panel D shows the unstained track of the transplant (arrows) in the putamen. Panel E shows tyrosine hydroxylase immunostaining of an adjacent section of the brain (scale bar in both panels, 1 cm). Although the track of the transplant can be seen, most obvious is the relatively homogeneous and rich reinnervation of the surrounding putamen. Panel F is a more highly magnified view of cell bodies and fibers in the track of the transplant (bar, 0.1 mm). About 22,000 dopamine neurons survived in this track. The reinnervation of the putamen with dopamine-neuron fibers is more complete in this patient than in the woman who died seven months after transplantation. The upper inset shows a transplanted dopamine neuron with neuromelanin granules (arrowhead; bar, 25 μm; hematoxylin and eosin). The lower inset shows hematoxylin-and-eosin staining of the patient's substantia nigra, with a Lewy body in the perikaryon of a degenerating dopamine neuron (arrowhead; bar, 25 μm).
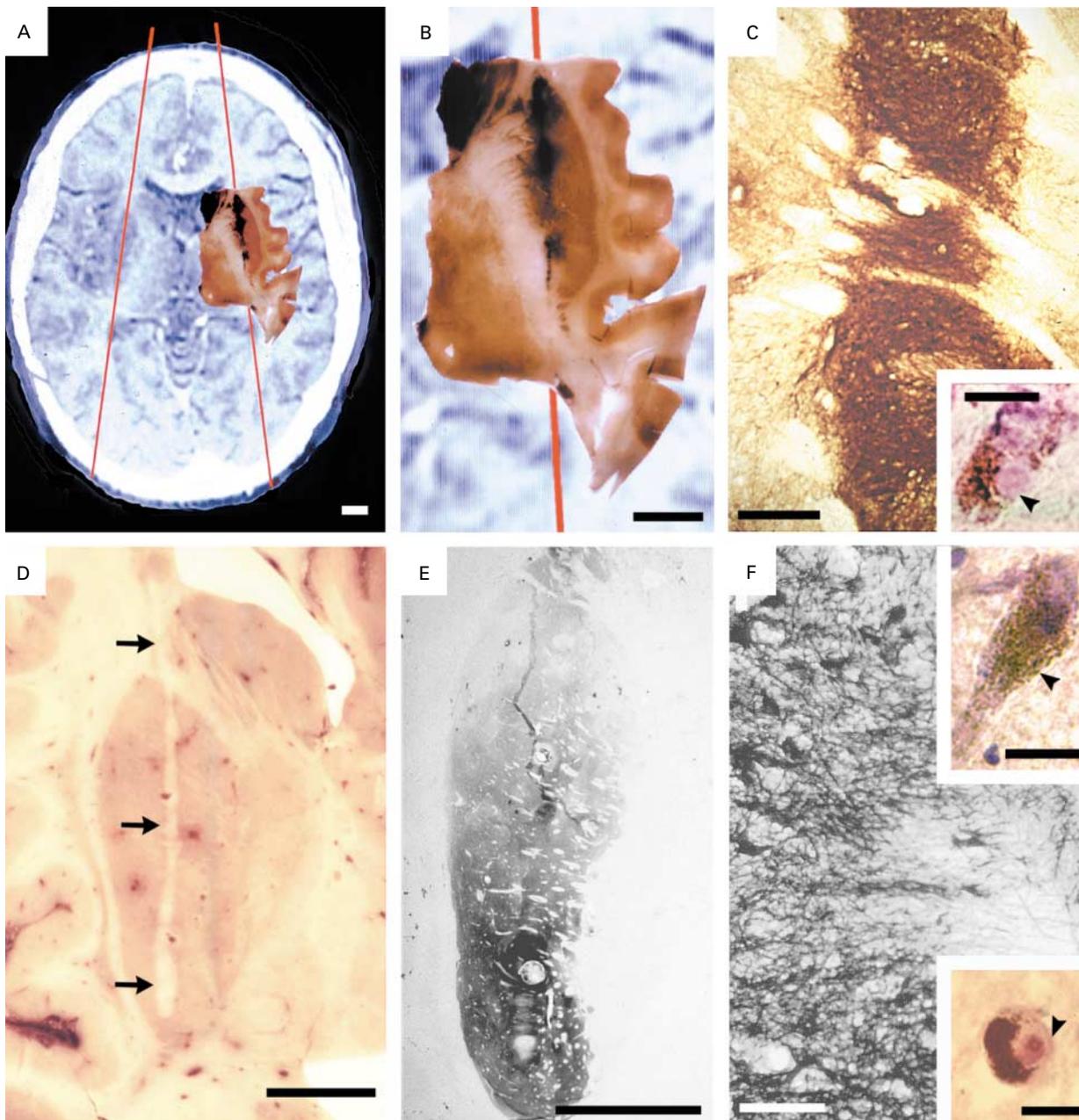
Schwab and England scores, which rate performance in the activities of daily living, improved significantly both in the transplantation group as a whole and among the younger patients in the group. The changes after transplantation were equivalent to about half the effect of levodopa, and this reduced the severity of the signs and symptoms that had previously been associated with being off medication. The time course and magnitude of clinical changes in our double-blind study are similar to those described in open studies.[2-21] Although analysis according to sex was not specified as an outcome variable, men who received transplants scored significantly better than women on the UPDRS and Schwab and England tests while off medication.

There were no significant differences between the best scores reported for patients in the transplantation group while on medication and those in the sham-surgery group. These scores reflect both the effects of transplantation and the effects of the drugs, and this increases variability. The study design maintained preoperative doses of drugs insofar as this was possible. We and others have reported that dyskinesia oc-

curs after transplantation and is usually improved by a reduction in the drug doses.[2,4,5,15]

The late development of dystonia and dyskinesia, more than one year after surgery, in five patients who had received transplants deserves comment. Parkinsonism in these patients improved during the first year after transplantation, even with substantial reductions in dosage or the discontinuation of levodopa. The subsequent appearance of dystonia and dyskinesia implies that the continued fiber outgrowth from the transplant has led to a relative excess of dopamine. The simplest response to this outcome would be to transplant less tissue in the future. The distribution of the tissue is also likely to be important. Because the depletion of dopamine in patients with Parkinson's disease is more severe in the dorsal and caudal putamen,[37] and since the most bothersome dyskinetic movements have been of the head and upper extremities, which are controlled by the more ventral portions of the putamen,[38] transplanting tissue dorsally and not ventrally in the putamen may be a prudent course for the future.

The fact that parkinsonism did not improve in the older patients during the first year after transplantation, despite the growth and development of dopamine neurons, may reflect a lower degree of plasticity of the brain or more diffuse brain disease in the older group. The fact that responses to drug therapy before surgery were less good in the older patients supports the contention that there are physiologic differences between younger and older patients.

Because we examined four secondary outcomes, multiple comparisons may have exaggerated the statistical significance of the results. Conservative analysis would suggest that the standard cutoff of $P=0.05$ to indicate significance should be lowered to $P=0.0125$. The small P values for the ^{18}F-fluorodopa PET results ($P<0.001$), the UPDRS scores for younger patients while off medication ($P=0.01$), and the Schwab and England scores for younger patients while off medication ($P=0.006$) suggest that these are unlikely to be false positive results.

We chose to conduct this study without the use of immunosuppressant drugs because of the success of transplantation of allogeneic fetal brain tissue in animals without immunosuppression, and because of our previous observations that allogeneic nerve-cell transplants do not induce humoral or cellular immunity in humans or in nonhuman primates.[11,12] Some researchers have used continuous immunosuppression with cyclosporine and other drugs,[3] and others have tried short-term immunosuppression.[13] Only a controlled clinical trial can establish whether immunosuppression will lead to a better or a worse outcome.

In summary, transplants of embryonic dopamine neurons survive in the putamen of patients with Parkinson's disease, regardless of age. Transplantation had some benefits in patients 60 years old and younger, but not in older patients. The occurrence of late dys-

tonia and dyskinesia in five of the patients with transplants indicates that the surgical technique may need further refinement.

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