Ventura Arjona, M.D.
Servicios de Neurocirugía and Neurología, Hospital Universitario Virgen de las Nieves, Granada, Spain

Adolfo Mínguez-Castellanos, M.D.
Servicios de Neurocirugía and Neurología, Hospital Universitario Virgen de las Nieves, Granada, Spain

Rafael J. Montoro, M.D., Ph.D.
Laboratorio de Investigaciones Biomédicas Hospital Universitario Virgen del Rocío, Universidad de Sevilla, Sevilla, Spain

Angel Ortega, M.D.
Servicios de Neurocirugía and Neurología, Hospital Universitario Virgen de las Nieves, Granada, Spain

Francisco Escamilla, M.D.
Servicios de Neurocirugía and Neurología, Hospital Universitario Virgen de las Nieves, Granada, Spain

Juan José Toledo-Aral, M.D., Ph.D.
Laboratorio de Investigaciones Biomédicas Hospital Universitario Virgen del Rocío, Universidad de Sevilla, Sevilla, Spain

Ricardo Pardal, Ph.D.
Laboratorio de Investigaciones Biomédicas Hospital Universitario Virgen del Rocío, Universidad de Sevilla, Sevilla, Spain

Simón Méndez-Ferrer, M.S.
Laboratorio de Investigaciones Biomédicas Hospital Universitario Virgen del Rocío, Universidad de Sevilla, Sevilla, Spain

José M. Martín, M.D., Ph.D.
Servicios de Neurocirugía and Neurología, Hospital Universitario Virgen de las Nieves, Granada, Spain

Miguel Pérez, Ph.D.
Servicios de Neurocirugía and Neurología, Hospital Universitario Virgen de las Nieves, Granada, Spain

Majed J. Katati, M.D., Ph.D.
Servicios de Neurocirugía and Neurología, Hospital Universitario Virgen de las Nieves, Granada, Spain

Eduardo Valencia, M.D.
Servicios de Neurocirugía and Neurología, Hospital Universitario Virgen de las Nieves, Granada, Spain

Teresa García, M.D.
Servicios de Neurocirugía and Neurología, Hospital Universitario Virgen de las Nieves, Granada, Spain

José López-Barneo, M.D., Ph.D.
Laboratorio de Investigaciones Biomédicas Hospital Universitario Virgen del Rocío, Universidad de Sevilla, Sevilla, Spain

Reprint requests:
José López-Barneo, M.D., Ph.D., Laboratorio de Investigaciones Biomédicas, Edificio de Laboratorios, 2ª planta, Hospital Universitario Virgen del Rocío, Avenida Manuel Siurot s/n, E-41013 Sevilla, Spain.
Email: Jose.L.Barneo.sspa@juntadeandalucia.es

Received, October 23, 2002.
Accepted, March 27, 2003.

AUTOTRANSPLANTATION OF HUMAN CAROTID BODY CELL AGGREGATES FOR TREATMENT OF PARKINSON'S DISEASE

OBJECTIVE: In this study, we assessed the feasibility of autotransplantation of carotid body (CB) cell aggregates into the striatum for the treatment of patients with Parkinson's disease (PD).

METHODS: Six patients with advanced PD underwent bilateral autotransplantation of CB cell aggregates into the striatum. They were evaluated clinically preoperatively and for 18 months after surgery according to the recommendations of the Core Assessment Program for Intracerebral Transplantation.

RESULTS: No major complications or adverse events resulted from the cell implantation or surgical procedures. During the course of the study, there was no significant aggravation of dyskinesia or decline in cognitive function in any of the patients. Five of the six patients who underwent transplantation manifested a measurable degree of clinical improvement evidenced by standardized clinical rating scales for PD. A decrease in the blinded Unified Parkinson's Disease Rating Scale Part III in the "off" state, the main measure of transplant efficacy in our study, was found to be maximal (between 26 and 74%) at 6 months after surgery. At 1 year, clear reductions in the blinded Unified Parkinson's Disease Rating Scale Part III were maintained in three patients (24, 38, and 52%, respectively). Modest improvement was seen in two patients (13 and 17%), and the sole patient who showed no improvement had the most fibrosis in the CB. The age of the patient and the state of the CB tissue were adversely correlated with clinical improvement after CB autotransplantation.

CONCLUSION: This pilot study indicates that CB autograft transplantation is a relatively simple, safe, and viable therapeutical approach for the treatment of patients with advanced PD. More studies are needed to optimize the procedure and to assess its general applicability for the treatment of patients with PD.

KEY WORDS: Autografts, Carotid body, Clinical outcome, Parkinson's disease, Transplantation

Neurosurgery 53:321-330, 2003 DOI: 10.1227/01.NEU.0000073315.88827.72 www.neurosurgery-online.com

Parkinson's disease (PD) is caused by the progressive loss of midbrain dopaminergic neurons. Cellular replacement therapies have been used as a complementary treatment when patients become less responsive to L-dopa (4, 8, 14, 15). The most broadly used approach to replace these midbrain dopaminergic neurons in patients with PD is the intrastriatal implantation of human embryonic ventral mesencephalic neurons, a technique that has been used in numerous patients with PD, with sustained clinical improvement observed in some cases (6–8, 13, 17, 18, 25, 26, 32, 35). Intrastriatal grafting of porcine embryonic mesencephalic neurons also has been performed in patients with PD (31). The transplantation of fetal mesencephalic neurons remains an experimental therapy with restricted applicability, however. Although it increases dopamine concentration locally, it has little restorative action on the nigrostriatal pathway and does not always produce the expected beneficial effects (3, 8). Moreover, the use of human fetal cells raises numerous ethical, legal, and practical issues (e.g., the scarcity of human embryonic tissue), whereas the use of xenografts is hampered by the need to use immunosuppression and the risk of

intraspecies viral infection. Intracerebral administration of trophic factors for dopaminergic neurons (2, 9, 12, 29, 38) and autotransplantation of dopaminergic carotid body (CB) cell aggregates (5, 20, 28, 33) are two approaches that recently have been developed in animal models to complement fetal cell grafts.

The CBs contain neural crest-derived dopaminergic glomus cells, which function as arterial oxygen sensors and release large amounts of dopamine in response to hypoxia (19). CBs are particularly well suited for intracerebral grafting because, unlike most other organs, low oxygen tension—a condition present in the cell-depleted striatum—stimulates CB cell growth (21). In addition, CB transplants have the additional clinical advantage that their unilateral surgical resection causes no significant side effects (11, 36). Because intrastriatal transplants of CB cell aggregates are able to effect notable histological and functional recovery in parkinsonian rats (5, 33, 34) and in monkeys treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (20), we undertook this pilot study to assess the feasibility, safety, and efficacy of CB autotransplantation in patients with PD. We report the outcomes in six patients during the course of 18 months after transplantation. A preliminary account of this work was published previously in abstract form (22).

PATIENTS AND METHODS

Patient Selection and Clinical Evaluation

Our pilot study included six patients with PD with motor complications. Their clinical characteristics are listed in *Tables 1* and *2*. The main criteria for inclusion into this study were 1) diagnosis of PD according to the London Brain Bank criteria (10), 2) age younger than 65 years, 3) history of PD for more than 5 years, 4) presence of motor fluctuations, 5) Hoehn and Yahr Stage 3 or above in the "off" state, 6) major functional disability after optimal pharmacological treatment, and 7) the ability to provide freely informed consent and follow the study protocol. The main exclusion criteria were 1) the presence of other diseases that might carry greater surgical risk or might interfere with study outcomes, 2) previous cranial surgery, 3) quality of life limited for reasons other than PD, and 4) cognitive deficit or psychiatric comorbidities. Patient consent was obtained according to the Declaration of Helsinki, and the procedures were approved by the Ethical Review Committee of the University Hospital Virgen de las Nieves, Granada, Spain, where patient selection and surgery were performed.

Clinical evaluations were performed according to the guidelines described in the Core Assessment Program for Intracerebral Transplantation (16). All of the patients were evaluated preoperatively and at 3, 6, 12, and 18 months postoperatively. Patient 3 missed the evaluation at 18 months. During every clinical visit, each patient was assessed according to protocol with regard to the different motor phases (i.e., predefined baseline "off" state, best "on" and worst "off" states) on the basis of the Unified Parkinson's Disease Rating Scale (UPDRS), Hoehn and Yahr staging, the Schwab and England scale, the dyskinesia rating scale, timed motor tests, a diary of fluctuations for the preceding week, and videotape recording. The UPDRS III subscale in the "off" state, which was evaluated by an independent neurologist in a blinded fashion (with the exception of rigidity) on the basis of masked and randomly presented videotape sequences, provided the most objective estimation of each patient's state. UPDRS II and total UPDRS in the "off" state also were used as major scores in patient evaluation (*Table 2*). Additional scores were the time spent in the "off" state, which was evaluated as from 1 (1–25% of the waking time) to 4 (76–100% of waking time), and the scale of dyskinesias, which was calculated by the arithmetic mean of intensity (0–5) and duration (0–5) (*Table 2*). The clinical evaluations conducted at 6 and 12 months were completed routinely on the basis of a comprehensive neuropsychological evaluation of each patient to assess visuomotor coordination, perception, attention, verbal and visual memory, language, and executive functions. The clinical assessment in the predefined baseline "off" state was performed at least 12 hours after patients' antiparkinsonian medication was withheld. All patients were L-dopa-responsive preoperatively. A stable medication regimen was administered during the first 18

TABLE 1. Clinical characteristics of the six patients who underwent autograft transplantation[a]

Characteristics	Patients					
	1	2	3	4	5	6
Sex/age (yr)	M/55	F/46	M/61	F/53	M/58	F/47
Duration of disease (yr)	16	7	13	8	18	7
Hoehn and Yahr score (off)	4	3	4	3	4	3
Carotid body histology	1+	4+	−	3+	2+	2+

[a] −, almost complete absence of tyrosine hydroxylase (TH)-positive cells in glomeruli; 1+, minimal distribution of TH-positive cells in glomeruli; 2+, moderate distribution of TH-positive cells in glomeruli; 3+, predominantly uniform distribution of TH-positive cells in glomeruli; 4+, uniform distribution of glomeruli with TH-positive cells.

TABLE 2. Major clinical scores (in "off" state) for the six patients who underwent autograft transplantation[a]

Evaluation	Patients (% change)					
	1	2	3	4	5	6
Before transplantation						
Total UPDRS	130	46	58	59	98	51
UPDRS II	35	13	17	17	29	12
UPDRS III (blinded)	78	27	35	31	60	29
Time in "off" state	2	2	3	1	1	2
Dyskinesia rating scale	2.5	0	1	3.5	1.5	2
L-dopa-equivalent dose (mg)	914	750	850	671	1818	500
6 mo after transplantation						
Total UPDRS	102 (−22%)	16 (−65%)	66 (+14%)	39 (−34%)	65 (−34%)	36 (−29%)
UPDRS II	28 (−20%)	7 (−46%)	17 (0%)	12 (−29%)	22 (−24%)	9 (−25%)
UPDRS III (blinded)	58 (−26%)	7 (−74%)	40 (+14%)	20 (−35%)	36 (−40%)	21 (−28%)
Time in "off" state	1	1	3	2	1	3
Dyskinesia rating scale	2.5	0	1	2.5	2	1
Change in L-dopa-equivalent dose (mg)	(0%)	(−20%)	(0%)	(−30%)	(0%)	(0%)
12 mo after transplantation						
Total UPDRS	105 (−19%)	23 (−50%)	55 (−5%)	50 (−15%)	83 (−15%)	30 (−41%)
UPDRS II	32 (−9%)	7 (−46%)	14 (−18%)	12 (−29%)	22 (−24%)	8 (−33%)
UPDRS III (blinded)	59 (−24%)	13 (−52%)	35 (0%)	27 (−13%)	50 (−17%)	18 (−38%)
Time in "off" state	2	1	3	1	2	1
Dyskinesia rating scale	2	0	0	3.5	1.5	1.5
Change in L-dopa-equivalent dose (mg)	(0%)	(−30%)	(0%)	(−30%)	(0%)	(+40%)
18 mo after transplantation						
Total UPDRS	115 (−12%)	25 (−46%)	—	51 (−14%)	93 (−5%)	35 (−31%)
UPDRS II	33 (−6%)	7 (−46%)	—	14 (−18%)	25 (−14%)	11 (−8%)
UPDRS III (blinded)	68 (−13%)	15 (−44%)	—	28 (−10%)	60 (0%)	20 (−31%)
Time in "off" state	1	1	—	1	2	1
Dyskinesia rating scale	2	0	—	3.5	1.5	1
Change in L-dopa-equivalent dose (mg)	(0%)	(−30%)	—	(−45%)	(0%)	(+40%)

[a] L-dopa-equivalent dose (in mg) was calculated on the basis of the following equivalences: 100 mg standard L-dopa = 140 controlled-release L-dopa = 1 mg pergolide = 1 mg pramipexole = 5 mg ropinirole = 10 mg bromocriptine = 10 mg selegiline. UPDRS, Unified Parkinson's Disease Rating Scale; —, no data.

months after surgery; modifications were allowed only when major clinical changes occurred.

Surgical Techniques

A single operation was performed with the patient under general anesthesia. During the first part of the surgery, the right CB, firmly attached to the adventitia of the internal carotid artery near the bifurcation, was removed with microscopic guidance. The piece of tissue, which was resected with the CB inside it (*Fig. 1A*), was taken to a laboratory near the operating room and placed on a Petri dish with aseptic Tyrode's solution, where it was cleaned of surrounding fat and connective tissue. The CB appeared as a well-individualized ovoid organ measuring 3 to 4 mm in the larger diameter (*Fig. 1B*). After isolation, the CB was trimmed into pieces of 200 to 300 μm with fine scissors (*Fig. 1C*). Approximately 100 pieces, or cell aggregates, per CB were obtained. A CB piece was fixed and kept for subsequent histological analysis. Following the protocols used previously in parkinsonian animals (5, 20, 33), CB cell aggregates from Patients 1 to 5 were subjected to mild enzymatic treatment (1 mg/ml trypsin, 1 mg/ml collagenase, and 0.2 mg/ml deoxyribonuclease) for 20 minutes before transplantation. To shorten the operation, enzymatic treatment was not used in Patient 6, because we had determined that in parallel studies on animals, it did not seem to improve graft survival. After washing the enzymes, CB cell aggregates were resuspended in fresh Tyrode's solution, placed on a dish, and transferred to the operating room. Cleaning and cutting

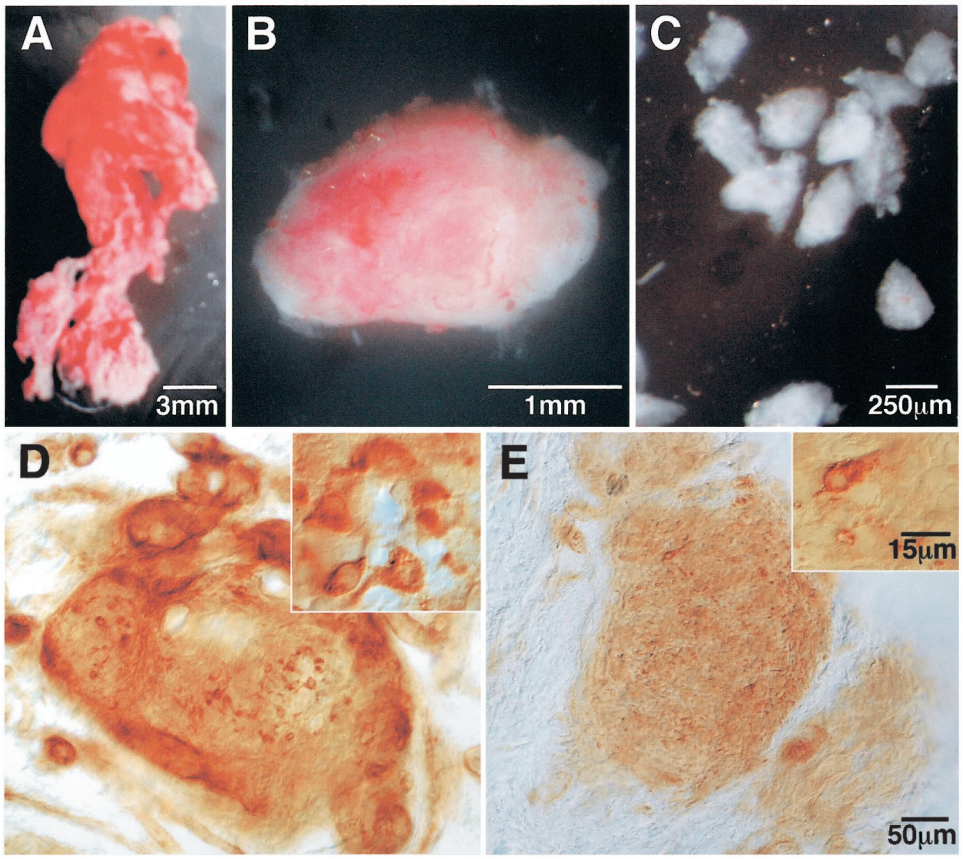


FIGURE 1. *Morphological analysis of the CBs.* A, *tissue resected from the carotid bifurcation in Patient 5.* B, *appearance of the CB after removal of surrounding connective and fat tissue.* C, *CB cell aggregates used for transplantation.* D, E, *immunostaining of CB sections obtained from Patients 2 and 6 with the use of antibodies against TH, illustrating the presence of clusters or glomeruli of dopaminergic glomus cells.* D *and* E insets, *examples of intensely stained glomus cells.*

into pieces of the CB was performed in approximately 90 minutes while the stereotactic localization procedure was initiated. A Cosman-Roberts-Wells stereotactic frame was affixed to the patient's cranium, and a computed tomographic scan was obtained to determine the coordinates of the anterior and posterior commissures. Striatal target coordinates were chosen according to the Schaltenbrand and Wahren atlas (30) and calculated with the aid of a software package (manufactured at the Departments of Neurosurgery and Medical Physics, University Hospital Virgen de las Nieves, Granada, Spain). We selected three targets in each putamen (anterior, middle, and posterior) for Patients 1 through 4. To test whether it produced better clinical outcome, two additional targets in the head of each caudate nucleus were included for Patients 5 and 6.

The second step of the operation was implantation. Two burr holes of 14-mm diameter were made (one on each side of the cranium) just frontal to the coronal suture and 4 cm to each side of the midline. A stainless steel cannula of 1.2-mm outer diameter was used to make the tracks into the predetermined targets in the brain. A piece of plastic tube connected to a Hamilton syringe was placed inside the cannula and introduced into the brain. In each track, 10 to 15 CB cell aggregates suspended in 10 μl Tyrode's solution, which had been aspirated into the tip of the plastic tube, were deposited in two steps as the cannula was withdrawn form the target. After each injection, a 1-minute pause was allowed for pressure stabilization before the cannula was withdrawn from the brain. Next, to ascertain that all CB cell aggregates had been injected into the predetermined brain target, the cannula with the attached plastic tube was flushed with fresh solution. For Patient 5, however, the requisite CB cell aggregates were deposited successfully only in the right hemisphere. In the left hemisphere, the four needle tracks were made, but because of several technical problems that arose during surgery, the amount of CB tissue deposited was less than 30% of that on the contralateral side.

Carotid Body Histology

The pieces of CB tissue were fixed overnight with 4% paraformaldehyde in phosphate-buffered saline at 4°C and then sectioned into 30- to 100-μm-thick slices with a vibratome. Immunohistochemical studies were performed with polyclonal anti-tyrosine hydroxylase (TH) antibody (1:1000) followed by a secondary biotinylated antirabbit antibody (1:200). These histological studies were performed to confirm the identity of the transplanted tissue and to determine the state of the CB organ, because the CB is known to become more fibrous and the number of glomus cells decreases with age. Moreover, the possible involvement of the CB in PD is still under discussion (23, 27). On the basis of the relative abundance of TH-positive cells and glomeruli characteristic of the CB (24) (*Fig. 1, D* and *E*), we established a qualitative classification of the CB histology ranging from − (indicating almost complete absence of TH-positive cells organized in glomeruli) to 4+ (indicating uniform distribution of glomeruli with TH-positive cells) (*Table 1*).

RESULTS

Safety and General State of the Patients

Intrastriatal CB autotransplantation seems to have been tolerated well by all patients, who were discharged from the

hospital within the first week after surgery. Postoperative magnetic resonance imaging performed before each patient discharge showed cannula tracks within the putamen and caudate, without signs of edema or hemorrhage. During patient hospitalization, two adverse events were documented: Patient 3 had nosocomial pneumonia that was treated satisfactorily with antibiotics, and Patient 6 experienced mild edema of cervical soft tissues that resolved without complications within a few days. During the 18-month follow-up period, the only adverse events observed were in Patient 3, who had a scapula fracture due to an accidental fall 10 months after surgery, and in Patient 4, who sustained a hip fracture 1 year after surgery. We consider both events to be unrelated to the surgical procedure. No other new symptoms or adverse events occurred during the follow-up period. We did not observe an increase in the severity of the dyskinesia during the 18-month follow-up period (*Table 2*). Overall, no changes were observed between the pre- and postsurgical neuropsychological evaluations. Patient 2 showed an improvement in visuomotor coordination and verbal and semantic fluency after surgery.

Evolution of Rating Scale Scores during the Initial 18 Months after Transplantation

The major scores in the "off" state for the six patients are listed in *Table 2*. The changes in the blinded UPDRS III scores in the "off" state, which is the main objective measure of treatment efficacy in this study, are plotted in *Figure 2A*. The best clinical results of CB transplantation were observed at 6 months after surgery. Three patients (Patients 2, 4, and 5) showed major improvements, as evidenced by clear reductions on the UPDRS motor subscale in the "off" state (35–74%) and in the other scores, including the L-dopa-equivalent dose (Patients 2 and 4) and the time in the "off" state. Two patients (Patients 1 and 6) had moderate improvement (>25% reduction on UPDRS III subscale), whereas one patient (Patient 3) did not improve at all. Two years after transplantation, Patient 3 underwent surgical implantation of electrodes for the administration of bilateral pallidal high-frequency stimulation, which led to significant clinical improvement. With the exception of Patients 3 and 5, different degrees of improvement in the UPDRS subscales were maintained at 18 months postoperatively (*Fig. 2A*). Because Patient 5 received needle tracks bilaterally but the requisite CB cell aggregates were successfully delivered only to the right hemisphere (see Patients and Methods), we evaluated (in addition to the overall UPDRS III subscale) the motor state of the left and right limbs separately (*Fig. 2B*). At 6 months after surgery, clinical improvement in Patient 5 was evident only in the left hemibody (contralateral to the implanted hemisphere), although preoperatively it had been in worse condition than the right hemibody. Slight improvement in the left limbs was still evident at 18 months after surgery, whereas the right limbs showed marked deterioration, possibly because of normal disease progression (*Fig. 2B*).

The boxplots shown in *Figure 3* summarize the evolution of the blinded UPDRS motor subscale in the "off" state observed

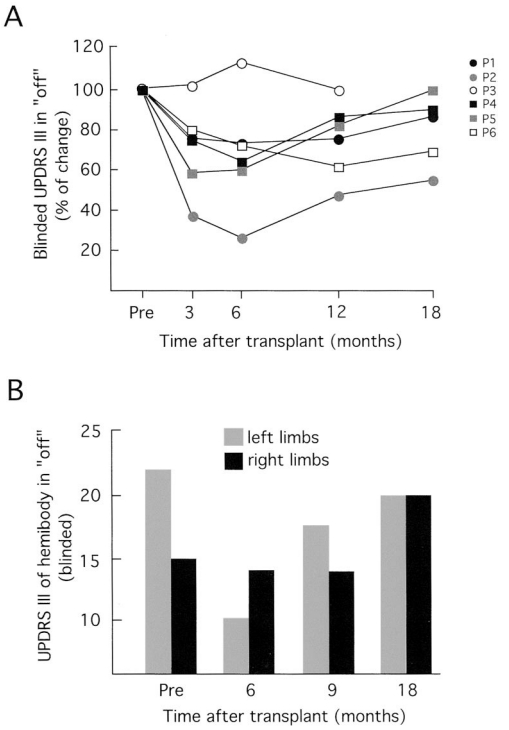


FIGURE 2. *Graphs illustrating the evolution of UPDRS motor subscale scores in patients who underwent transplantation.* A, *changes in UPDRS III in the "off" state (blinded) in six patients during the initial 18 months after transplantation. Patient 3 missed the evaluation at 18 months.* B, *blinded UPDRS motor subscale scores of the right and left hemibodies in Patient 5 evaluated separately.*

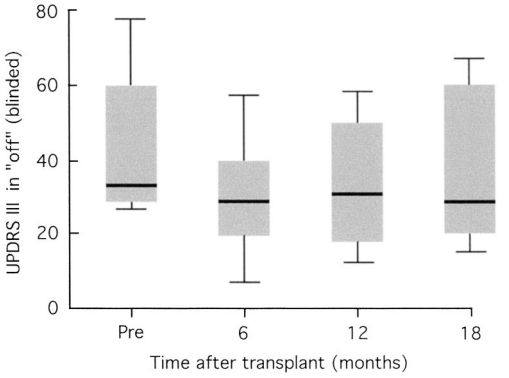


FIGURE 3. *Boxplot illustrating the distribution of the UPDRS motor subscale scores in the "off" state (blinded) of the patients studied (n = 6 preoperatively and at 6 and 12 mo; n = 5 at 18 mo). On the basis of Wilcoxon's test for paired samples,* P = *0.046 (6 mo),* P = *0.043 (12 mo), and* P = *0.068 (18 mo) compared with baseline* (Pre) *values.*

in patients during the 18 months of follow-up. Compared with the preoperative data, the distribution at 6 months is compacted and symmetrical around the median, with clear displacement toward the lower values. Although the median and some of the values remained low at 12 and 18 months (reflect-

ing those patients with sustained improvement after surgery), the progressive asymmetrical appearance of the distribution indicates the tendency of the scores of some patients to return to their preoperative levels.

Clinical Improvement, Patient Age, and Carotid Body Histology

Although the CB implants produced clinical improvement in most patients, the data in *Figures 2* and *3* demonstrate marked variability in patient outcomes. Despite the small number of patients in our study (which does not allow definitive conclusions to be drawn for the general patient population), we found the clinical efficacy of CB transplantation to be inversely related to patient age (*Fig. 4*). The loss of efficacy of CB implants observed in older patients may be a function of the histological state of implanted tissue, because we observed a decrease in the number of glomeruli and TH-positive cells in older patients (*Table 1*).

DISCUSSION

The goals of this study were to evaluate the feasibility, safety, and clinical efficacy of the autotransplantation of CB cell aggregates into the striata of patients with PD. CB transplantation was performed in a single, relatively simple surgical intervention that was tolerated well by all patients. In none of the six patients was there any evidence of serious complications and/or side effects during the 18 months after surgery. Five of the six patients who underwent transplantation manifested a measurable degree of clinical improvement evidenced by assessment according to the Core Assessment Program for Intracerebral Transplantation scales, particularly with regard to the blinded UPDRS motor subscale in the "off" state, which we considered the most objective measure in our study.

On average, the time course and the magnitude of clinical improvement observed in our study are comparable to those reported in previous studies in which grafts of fetal mesencephalic neurons were used (8). The clinical outcomes of patients who underwent additional caudate implantation (Patients 5 and 6) did not differ significantly from those of patients who underwent standard putaminal grafting. This finding might indicate that nigral projections to the caudate nucleus were not damaged extensively in our patients with PD. Similarly to studies with fetal cells, the efficacy of CB grafts varied among the patients studied. At 1 year after transplantation, the clinical benefit, as assessed by the decrease in the UPDRS III "off" state scores, ranged from approximately 60% to an absence of effect. None of our patients reported the appearance of motor dyskinesias. A major difference between our procedure and that of studies in which grafted mesencephalic neurons were used is the relatively small number of dopaminergic CB cells transplanted, which we roughly estimated to be approximately 10,000 per track versus the several hundred thousand cells grafted in the trials in which fetal neurons were used. As supported by our parallel studies in animal models of PD (20, 33), the beneficial effects of CB implantation are due predominantly to the trophic action of the CB cells on intrinsic nigrostriatal neurons rather than to the direct release of dopamine from the graft. Adult rodent CB cells express high levels of glial cell line-derived neurotrophic factor, and the implants of CB cell aggregates survive in the rat brain for almost the entire life of the animal (33). Thus, the decline in graft efficacy with time observed in our patients might reflect the progression of the disease, which could be retarded in some cases by CB autotransplantation, rather than the death of the transplanted cells.

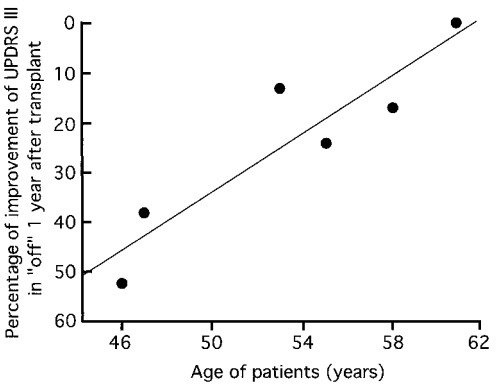


FIGURE 4. *Graph illustrating the relationship between the percentage of improvement of the blinded UPDRS motor subscale in the "off" state at 1 year after transplantation (ordinate) and patient age (abscissa). A linear regression fit* ($r = 0.92$) *is superimposed on the data points.*

The main factor that influenced clinical outcome in this study was the age of the patient. Likewise, a recent study in which grafted fetal mesencephalic neurons were used reported that clinical improvement was appreciable only in patients younger than 60 years of age (8). In our study, age not only may influence the responsiveness of brain parenchyma to the grafting of foreign tissue but also may affect the histological quality of the transplanted CB tissue. Although the number of patients we studied is small, the CB tissue of older patients clearly contained fewer glomeruli and a smaller percentage of TH-positive cells. The CB parenchyma is known to become less glandular and more fibrous with age, and, like other peripheral neuronal or paraneural tissues, CB tissue may be affected by PD. In fact, it has been suggested that the respiratory abnormalities commonly observed in patients with PD may be a result of an impaired response to hypoxia caused by CB dysfunction (23). This possibility, however, must be considered cautiously, because patients with PD receive high doses of dopaminergic agents, which are known to depress CB function (1, 27, 37). CB cells seem to be particularly resistant because, as indicated above, they survive in the rat brain for almost the entire life of the animal, and, in contrast to substantia nigra neurons, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine does not destroy them (33). Further experimental evidence is necessary to determine whether the survival of CB cells is compromised in patients with PD.

The results of our trial are tempered by the study design, which may be susceptible to patient bias. In fact, a recent double-blinded, controlled trial of human fetal transplantation (8) provided evidence of a placebo effect, because some sham-operated patients reported positive changes in a global rating scale. The main measure in our study was the blinded UPDRS III subscale, which is considered to be a scale that is less susceptible to observer bias. Therefore, it is unlikely that the clinical improvements observed in our most responsive patients can be attributed to patient or observer bias. In addition, we observed a clear correlation between the magnitude of clinical improvement and objective variables such as the age of the patient and CB histology. The clinical course of Patient 5, who received needle tracks bilaterally but CB grafts more abundantly in the right hemisphere, indicates a direct effect of CB cells, because the clinical improvement was observed predominantly on the contralateral side of the brain. The clinical evidence also indicates that small lesions in the putamen produced by the needle track in Patient 5 were not responsible for significant clinical improvement.

CB autograft is a viable, relatively simple and safe procedure that produces clinical amelioration in patients with PD. More prolonged follow-up and additional studies of the amount and the location of transplanted CB tissue, complemented by ^{18}F-labeled dopa positron emission tomographic scans, are required to optimize the procedure and to establish its general applicability for the treatment of PD.

REFERENCES

1. Benot AR, López-Barneo J: Feedback inhibition of Ca^{2+} currents by dopamine in glomus cells of the carotid body. **Eur J Neurosci** 2:809–812, 1990.
2. Choi-Lundberg DL, Lin Q, Chang YN, Chiang YL, Hay CM, Mohajeri H, Davidson BL, Bohn MC: Dopaminergic neurons protected from degeneration by GDNF gene therapy. **Science** 275:838–841, 1997.
3. Defer GL, Geny C, Ricolfi F, Fenelon G, Monfort JC, Remy P, Villafane G, Jeny R, Samson Y, Kéravel Y, Gaston A, Degos JD, Peschanski M, Cesaro P, Nguyen JP: Long-term outcome of unilaterally transplanted parkinsonian patients: Part I—Clinical approach. **Brain** 119:41–50, 1996.
4. Dunnett SB, Björklund A: Prospects for new restorative and neuroprotective treatments in Parkinson's disease. **Nature** 399[Suppl]:A32–A39, 1999.
5. Espejo E, Montoro R, Armengol JA, López-Barneo J: Cellular and functional recovery of parkinsonian rats after intrastriatal transplantation of carotid body cell aggregates. **Neuron** 20:197–206, 1998.
6. Freed CR, Breeze RE, Rosenberg NL, Schneck SA, Kriek E, Qi JX, Lone T, Zhang YB, Snyder JA, Wells TH, Ramig LO, Thompson L, Mazziotta JC, Huang SC, Grafton ST, Brooks D, Sawle G, Schroter G, Ansari AA: Survival of implanted fetal dopamine cells and neurologic improvement 12 and 46 months after transplantation for Parkinson's disease. **N Engl J Med** 327: 1549–1555, 1992.
7. Freed CR, Breeze RE, Rosenberg NL, Schneck SA, Wells TH, Barrett JN, Grafton ST, Huang SC, Eidelberg D, Rottenberg DA: Transplantation of human fetal dopamine cells for Parkinson's disease: Results at 1 year. **Arch Neurol** 47:505–512, 1990.
8. Freed CR, Greene PE, Breeze RE, Tsai WY, DuMouchel W, Kao R, Dillon S, Winfield H, Culver S, Trojanowski JQ, Eidelberg D, Fahn S: Transplantation of embryonic dopamine neurons for severe Parkinson's disease. **N Engl J Med** 344:710–719, 2001.
9. Gash DM, Zhang Z, Ovadia A, Cass WA, Yi A, Simmerman L, Russell D, Martin D, Lapchak PA, Collins F, Hoffer BJ, Gerhardt GA: Functional recovery in parkinsonian monkeys treated with GDNF. **Nature** 380:252–255, 1996.
10. Gibb WR, Lees AJ: The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. **J Neurol Neurosurg Psychiatry** 51:745–752, 1988.
11. Honda Y: Respiratory and circulatory activities in carotid body-resected humans. **J Appl Physiol** 73:1–8, 1992.
12. Kordower JH, Emborg ME, Bloch J, Ma SY, Chu Y, Leventhal L, McBride J, Chen EY, Palfi S, Roitberg BZ, Brown WD, Holden JE, Pyzalski R, Taylor MD, Carvey P, Ling Z, Trono D, Hantraye P, Deglon N, Aebischer P: Neurodegeneration prevented by lentiviral vector delivery of GDNF in primate models of Parkinson's disease. **Science** 290:767–773, 2000.
13. Kordower JH, Freeman TB, Snow BJ, Vingerhoets FJ, Mufson EJ, Sanberg PR, Hauser RA, Smith DA, Nauert GM, Perl DP, Olanow W: Neuropathological evidence of graft survival and striatal reinnervation after the transplantation of fetal mesencephalic tissue in a patient with Parkinson's disease. **N Engl J Med** 332:1118–1124, 1995.
14. Lang AE, Lozano AM: Parkinson's disease: First of two parts. **N Engl J Med** 339:1044–1053, 1998.
15. Lang AE, Lozano AM: Parkinson's disease: Second of two parts. **N Engl J Med** 339:1130–1143, 1998.
16. Langston JW, Widner H, Goetz CG, Brooks D, Fahn S, Freeman T, Watts R: Core Assessment Program for Intracerebral Transplantation (CAPIT). **Mov Disord** 7:2–13, 1992.
17. Lindvall O, Brundin P, Widner H, Rehncrona S, Gustavii B, Frackowiak R, Leenders KL, Sawle G, Rothwell JC, Marsden CD, Björklund A: Grafts of fetal dopamine neurons survive and improve motor function in Parkinson's disease. **Science** 247:574–577, 1990.
18. Lindvall O, Widner H, Rehncrona S, Brundin P, Odin P, Gustavii B, Frackowiak R, Leenders KL, Sawle G, Rothwell JC, Björklund A, Marsden CD: Transplantation of fetal dopamine neurons in Parkinson's disease: One-year clinical and neurophysiological observations in two patients with putaminal implants. **Ann Neurol** 31:155–165, 1992.
19. López-Barneo J, Pardal R, Ortega-Sáenz P: Cellular mechanism of oxygen sensing. **Annu Rev Physiol** 63:259–287, 2001.
20. Luquin MR, Montoro RJ, Guillén J, Saldise L, Insausti R, Del Río J, López-Barneo J: Recovery of chronic parkinsonian monkeys by autotransplants of carotid body cell aggregates into putamen. **Neuron** 22:743–750, 1999.
21. MacGregor KH, Gil J, Lahiri S: A morphometric study of the carotid body in chronically hypoxic rats. **J Appl Physiol** 57:1430–1438, 1984.
22. Mínguez A, López-Barneo J, Arjona V, Montoro RJ, Escamilla F, Ortega A, Toledo-Aral JJ, Pardal R, Méndez-Ferrer S, Martín JM, Pérez P, García T: Transplantation of carotid body cell aggregates in patients with Parkinson's disease: A pilot study. **Parkinsonism Relat Disord** 7:S82, 2001 (abstr).
23. Onodera H, Okabe S, Kikuchi Y, Tsuda T, Itoyama Y: Impaired chemosensitivity and perception of dyspnoea in Parkinson's disease. **Lancet** 356:739–740, 2000 (letter).
24. Pardal R, Ludewig U, García-Hirschfeld J, López-Barneo J: Secretory responses of intact glomus cells in thin slices of rat carotid body to hypoxia and tetraethylammonium. **Proc Natl Acad Sci U S A** 97:2361–2366, 2000.
25. Peschanski M, Defer G, N'Guyen JP, Ricolfi F, Monfort JC, Remy P, Geny C, Samson Y, Hantraye P, Jeny R, Gaston A, Kéravel Y, Degos JD, Cesaro P: Bilateral motor improvement and alteration of L-dopa effect in two patients with Parkinson's disease following intrastriatal transplantation of fetal ventral mesencephalon. **Brain** 117:487–499, 1994.
26. Piccini P, Brooks DJ, Björklund A, Gunn RN, Grasby PM, Rimoldi O, Brundin P, Hagell P, Rehncrona S, Widner H, Lindvall O: Dopamine release from nigral transplants visualized in vivo in a Parkinson's patient. **Nat Neurosci** 12:1137–1140, 1999.
27. Röggla G, Weber W, Röggla M: Parkinson's disease and impaired chemosensitivity to hypoxia. **Lancet** 356:2099, 2000 (letter).
28. Rosenthal A: Autotransplants for Parkinson's disease? **Neuron** 20:169–172, 1998.
29. Rosenthal A: The GDNF protein family: Gene ablation studies reveal what they really do and how. **Neuron** 22:201–203, 1999.
30. Schaltenbrand G, Wahren W: *Atlas for Stereotaxy of the Human Brain*. New York, Thieme Medical Publishers, 1977, ed 2.

31. Schumacher JM, Ellias SA, Palmer EP, Kott HS, Dinsmore J, Dempsey PK, Fischman AJ, Thomas C, Feldman RG, Kassissieh S, Raineri R, Manhart C, Penney D, Fink JS, Isacson O: Transplantation of embryonic porcine mesencephalic tissue in patients with PD. **Neurology** 54:1042–1050, 2000.
32. Spencer DD, Robbins RJ, Naftolin F, Marek KL, Vollmer T, Leranth C, Roth RH, Price LH, Gjedde A, Bunney BS, Sass KJ, Elsworth JD, Kier EL, Makuch R, Hoffer PB, Redmond DE: Unilateral transplantation of human fetal mesencephalic tissue in the caudate nucleus of patients with Parkinson's disease. **N Engl J Med** 327:1541–1548, 1992.
33. Toledo-Aral JJ, Méndez-Ferrer S, Pardal R, Echevarría M, López-Barneo J: Trophic restoration of the nigrostriatal dopaminergic pathway in long-term carotid body-grafted parkinsonian rats. **J Neurosci** 23:141–148, 2003.
34. Toledo-Aral J, Méndez-Ferrer S, Pardal R, López-Barneo J: Dopaminergic cells of the carotid body: Physiological significance and possible therapeutic applications in Parkinson's disease. **Brain Res Bull** 57:847–853, 2002.
35. Widner H, Tetrud J, Rehncrona S, Snow B, Brundin P, Gustavii B, Björklund A, Lindvall O, Langston JW: Bilateral fetal mesencephalic grafting in two patients with parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). **N Engl J Med** 327:1556–1563, 1992.
36. Winter B: Bilateral carotid-body resection. **N Engl J Med** 283:661, 1970.
37. Zapata P, Zuazo A: Respiratory effects of dopamine-induced inhibition of chemosensory inflow. **Respir Physiol** 40:79–92, 1980.
38. Zurn AD, Widmer HR, Aebischer P: Sustained delivery of GDNF: Towards a treatment for Parkinson's disease. **Brain Res Brain Res Rev** 36:222–229, 2001.

Acknowledgments

We thank Dr. Gregory Gasic for comments on the manuscript. Our research was supported by grants from Consejería de Salud of the Andalusian Government, Fundación La Caixa, and Fundación Ramón Areces. SMF is a predoctoral fellow of the Spanish FPI Program. JLB received the "Ayuda a la Investigación 2000" of the Juan March Foundation.

COMMENTS

This interesting article describes six patients with advanced Parkinson's disease (PD) who were treated with implanted bilateral autografts of carotid body (CB) cells. CB cells are paraneurones derived from the neural crest similarly to chromaffin cells in the adrenal medulla. Within the CB are dopamine-producing glomus cells. Glomus cells are useful because they not only produce dopamine but also survive well in low-oxygen environments. Unilateral resection does not produce adverse side effects, and because CB cells are not neurons, surgical removal does not result in cell death as a result of axonotomy. The biggest advantage is that the tissue can be obtained by autografting. The disadvantages of this tissue are that it cannot be cultured in great quantities, its electrophysiological behavior in the host is entirely unknown, the parameters of dopamine release are unknown, and its long-term survivability in the brain has not been established. One wonders how useful these cells can be when implanted into the brain if dopamine is released predominantly under hypoxic conditions. Moreover, there is some indirect evidence that these cells are also affected in patients with PD (6).

The authors have attempted to evaluate these patients in a blinded manner on the basis of assessment conducted by an independent neurologist who graded videotapes that were presented in a random fashion. Although this attempt to obtain unbiased, semiquantitative data is reasonable, it does compromise the data in that rigidity cannot be evaluated, thus removing one of the major components of Unified Parkinson's Disease Rating Scale Grade III. It also does not get around the placebo effect of surgery, and how well the patients were blinded is also unknown. Thus, if Patient 5 knew that needle tracts on the left did not contain adequate tissue deposits, then interpretation of the finding would be difficult. The overall pattern of improvement at 6 months and gradual deterioration thereafter is reminiscent of the adrenal medullary data (3, 7). The authors think that the deterioration represents a continuation of the disease, but this deterioration is far more rapid than one would anticipate in cases of natural progression. The parallels to adrenal medullary studies are striking.

The primary studies on which this article is based involve two subjects that have been approached differently (5). The monkeys did not survive long enough and were not tested sufficiently to be sure that their recovery was not a nonspecific effect. The number of surviving glomus cells was two orders of magnitude less than that observed in fetal tissue. In studying chromaffin cell grafts, my colleagues and I found that what correlated best with clinical improvement was not the amount of tissue or the characteristics of the tissue as much as the characteristics of the host (i.e., residual host tyrosine hydroxylase-positive buttons in the striatum) (1). Although the chromaffin cells did not survive well in the brain, they did have the effect of stimulating the brain's natural reparative mechanism (4). This effect is not specific for chromaffin cells and can be demonstrated to occur experimentally, at least transiently, for a variety of different tissues (2). A different mechanism may be at work here. That these cells can produce glial cell line-derived neurotrophic factors is interesting. If this can be documented to occur for a long period of time, and if the amounts released are sufficient, then neuroprotection could be a possibility. Of course, this hypothesis would have to be proved in the laboratory.

The technical aspects of this study are left open. Thus, it remains unclear whether tissue treatment with trypsin and collagenase is necessary to achieve the best cellular isolation and survival or whether grafting needs to be performed in both the caudate and the putamen. It does seem to be relatively clear that old age and lack of glomus cells should be contraindications to performing this type of surgery. Overall, the benefits are relatively small, and the uncertainty concerning techniques, suitability of tissue, storage of tissue, for example, suggest the need for much greater laboratory work before further clinical studies are undertaken. Additional clinical studies are certainly necessary, because this surgery is not ready to be studied in a randomized, blinded trial.

Short-term improvement can be achieved by using a variety of injury techniques, and interpretation of the data must be performed carefully. These studies, although interesting, leave many questions unanswered, and I think that the best place to answer these questions is in the laboratory and not in the operating room. This technique must compete with other,

apparently more successful transplantation techniques, as well as deep brain stimulation, to prove its clinical relevance.

Roy A.E. Bakay
Chicago, Illinois

1. Bakay RAE, Watts RL, Freeman A, Iuvone PM, Watts N, Graham SD: Preliminary report on adrenal-brain transplantation for parkinsonism in man. **Stereotact Funct Neurosurg** 54–55:312–323, 1990.
2. Bankiewicz KS, Plunkett RJ, Kophin IJ, Jacobowitz DM, London WT, Oldfield EH: Transient behavioral recovery in hemiparkinsonian primates after adrenal medullary allografts. **Prog Brain Res** 78:543–549, 1988.
3. Goetz CG, Olanow CW, Koller WC, Penn RD, Cahill D, Morantz R, Stebbins G, Tanner CM, Klawans HL, Shannon KM: Multicenter study of autologous adrenal medullary transplantation to the corpus striatum in patients with advanced Parkinson's disease. **N Engl J Med** 320:337–341, 1989.
4. Kordower JH, Cochran E, Penn RD, Goetz CG: Putative chromaffin cell survival and enhanced host-derived TH-fiber innervation following a functional adrenal medulla autograft for Parkinson's disease. **Ann Neurol** 29:405–412, 1991.
5. Luquin MR, Montoro RJ, Guillen J, Saldise L, Insausti R, Del Rio J, Lopez-Barneo J: Recovery of chronic parkinsonian monkeys by autotransplants of carotid body cell aggregates in putamen. **Neuron** 22:743–750, 1999.
6. Onodera H, Okabe S, Kikuchi Y, Tsuda T, Itoyama Y: Impaired chemosensitivity and perception of dyspnoea in Parkinson's disease. **Lancet** 356:739–740, 2000.
7. Watts RL, Bakay RAE: Autologous adrenal medulla-to-caudate grafting for parkinsonism in humans and in nonhuman primates. **Front Neuroendocrinol** 12:357–378, 1991.

I read with interest this article by Arjona et al. The authors have conducted a pilot study in which they performed autotransplants of human CB cell aggregates in six individuals with PD. This small, open label trial without controls was designed to evaluate the feasibility, safety, and clinical efficacy of such autotransplants. The authors conclude that CB autografts are a "relatively simple, safe, and viable therapeutical approach for the treatment of patients with advanced PD." Furthermore, they suggest that the mechanism of action is "due predominantly to the trophic action of the CB cells on intrinsic nigrostriatal neurons rather than to the direct release of dopamine from the graft." I find the results of the study intriguing and agree with the authors that the need for further research along these lines is indicated. I must confess, however, that I am somewhat skeptical regarding the ultimate usefulness of such transplants. My bias is that successful transplants depend on the release of dopamine. In particular, *Figure 2A* demonstrates a time course with maximal improvement at 6 months, with a trend toward baseline at 12 to 18 months. This temporal sequence is reminiscent of the original adrenal transplant data and mimics to some extent what my colleagues and I saw in our placebo group when we transplanted fetal mesencephalic tissue in a blinded, controlled study. However, the lack of improvement in their one patient who received tissue from fibrotic CB cells and the asymmetric response in the patient who essentially received a unilateral transplant argue against my bias and suggest that a true effect was observed in this study. I also found the age-related effect to be interesting. As the authors point out, this finding is in agreement with the observation my colleagues and I made that our young patients fared better with fetal cell transplants. Secondary analysis of our material has suggested, however, that preoperative dopamine responsiveness, not age, is the independent variable of interest. If, in fact, the mechanism of action with CB cell aggregates is not dopamine release but the release of trophic factors, then perhaps age truly is the independent variable at play. The authors achieved a low complicate rate. Of interest, however, is that they note a few complications related to falls that were thought to be unrelated to the surgical procedure. I wonder whether these falls truly were unrelated to surgery. My experience has been that patients who undergo experimental treatments push themselves more in the ensuing months because they believe that their conditions are truly improving. It may be that the increased falls noted in this population were in fact an indirect complication of experimental surgery. Nonetheless, I find the data intriguing and look forward to further research from this group.

Robert E. Breeze
Denver, Colorado

The authors describe their interesting work regarding the safety, feasibility, and impact of CB glomus cell autotransplantation in the striata of six patients with advanced PD. This concept is innovative and bypasses all the caveats of immunosuppression necessary until now in the field of brain repair. Furthermore, it does not involve as much ethical consideration as does embryonic cell transplantation. However, the use of an extraneural source of dopaminergic cells in the treatment of patients with PD must be considered carefully. Indeed, the experience with adrenal gland cell transplantation was disappointing and was completely abandoned a few years ago because of lack of graft survival and long-term therapeutic effects. Nevertheless, in this preliminary clinical trial, the authors demonstrate that CB glomus cells have interesting characteristics that could help them survive and become integrated into the human brain better than adrenal gland cells. These findings are highly interesting, but in the future, if a Phase II clinical trial is proposed, positron emission tomographic studies should be mandatory to obtain more objective information regarding the striatal dopaminergic activity as well as the long-term survival of grafted tissue.

Jocelyne Bloch
Nicolas de Tribolet
Geneva, Switzerland

This interesting article reports six patients with PD who were treated with autologous transplantation into the striatum using tissue from the CB of the patients. The well-founded reasoning underlying the study and the general design of the surgical procedure are analogous to those of a pilot study on autografting published in 1985 (1), in which chromaffin tissue from the suprarenal gland of the patients was used. In the present study, rewarding clinical effects are reported in five of the six patients, according to the clinical rating scale used (e.g., Unified Parkinson's Disease Rating Scale Grade III). In three of the patients, the effects have lasted for at least 1 year. Some interesting remarks the authors make deserve attention, such as that mild trypsination of the small solid grafts did not seem to improve graft survival. Another noteworthy comment relates to the hypothesis that the com-

paratively poor oxygenation of CB cells in normal tissue would make this tissue especially suitable for grafting into the cell-depleted striatum, which also is poorly oxygenated. Moreover, the authors postulate that the rewarding effects of the CB grafts might be due to the pronounced expression of glial cell line-derived neurotrophic factors characterizing the CB cells rather than to reinnervation.

Erik Olof Backlund
Linköping, Sweden

---

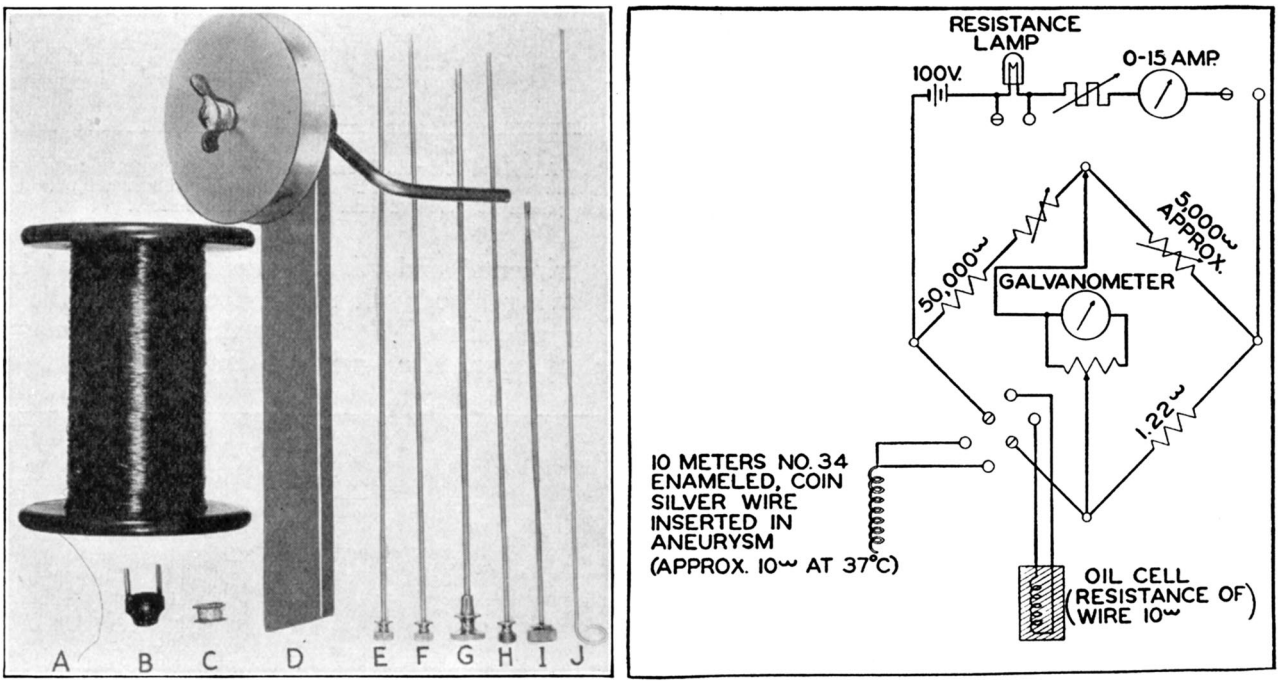
1. Backlund EO, Granberg PO, Hamberger B, Knutsson E, Mårtensson A, Sedvall G, Seiger Å, Olson L: Transplantation of adrenal medullary tissue to striatum in parkinsonism: First clinical trials. **J Neurosurg** 62:169–173, 1985.

Arjona et al., an expert team of basic scientists, neurologists, and neurosurgeons, have conducted a pilot study of the autotransplantation of human CB cells into the striata of six patients with PD. These investigators compose one of the preeminent groups in the neurobiology of transplantation in animal models of PD. They have conducted fundamental studies in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine primates that have shown some striking benefits in these animals.

This effort represents a novel approach to focal dopamine replacement therapy to help restore the neurological circuits that degenerate in parkinsonian brain. They conducted their study with the use of validated scales and have included excellent follow-up and documentation of the patients' progress. They argue that the CB cells have a number of features that make them particularly well suited for grafting, including their ability to release large amounts of dopamine and their proved ability to restore parkinsonian function in animal models. The main findings of the study are that after bilateral transplantation, five of the six patients demonstrated significant improvement 6 months after surgery, but that this benefit waned with time. Furthermore, the patient with fibrotic CB received little benefit from the transplantation.

Studies of this type raise a number of important issues. First is the choice of these cells for use as autograft material in patients with PD. The multisystem and widespread degeneration of neurons in PD is well known, and it is likely that these autotransplants may have the same genetic lesions or may be exposed to the same environmental agents that cause PD. In this respect, these cells may not be the best sources for transplant tissue. Indeed, this may have been the case in the patient in whom the CB was fibrotic. Second, the issue of where to transplant and how many cells to transplant in humans requires further investigation. The advantages of autotransplantation obviate the need for immunosuppression, which has become an important issue in some other transplantation studies, particularly the human fetal and the xenograft transplantation trials using poor sign cells. Little is known regarding the long-term benefits, and indeed the survival, of the cell transplants. The observation that the benefits of the transplants waned over time indicates that more work is needed and that many important questions remain to be answered. These investigators have performed a wonderfully conducted study that will be an important milestone in the fields of neural transplantation and PD research.

Andres M. Lozano
Toronto, Ontario, Canada



Left, *materials and instrumentation historically associated with electrothermic coagulation of aortic aneurysms.* A, *enameled wire on spool;* B, *electrode cap;* C, *self-retaining skin retractor;* D, *hand reel for winding wire onto spools;* E, *trocar pointed stylet (adapted to* G*) for traversing firm tissues and piercing the sac of the aneursym;* F, *tapered blunt stylet (adapted to* G*) for traversing tissue of the lungs;* G, *14-gauge 6½-inch needle made of stainless steel;* H, *inner sheath for the needle* (G)*;* I, *wire passer (adapted to* H*); and* J, *a blunt stylet (adapted to* H*).* Right, *schematic diagram of the apparatus for controlling the temperature of the wire. This device was described in a report on early studies of electrothermic occlusion of aneurysms (both pictures* from, *Blakemore AH, King BG: Electrothermic coagulation of aortic aneurysms.* ***Jour AMA*** *111:1821, 1938).*