Intraarterial Autologous Implantation of Adult Stem Cells for Patients with Parkinson Disease

Augusto Brazzini, MD, Raúl Cantella, MD, Antonio De la Cruz, MD, Jorge Yupanqui, MD, Carlos León, MD, Tamara Jorquiera, MD, Mariana Brazzini, MD, Melitón Ortega, MD, and Luis N. Saenz, MD

PURPOSE: To evaluate the feasibility, safety, and effectiveness of intraarterial autologous implantation of adult stem cells for Parkinson disease (PD).

MATERIALS AND METHODS: From June 2006 to August 2008, 36 men and 14 women (mean age, 62.5 years ± 10.4; range, 38–81 y) with PD (mean duration, 9.3 years; range, 1–28 y) underwent autologous implantation of stem cells with superselective arterial catheterization. Patients were evaluated with clinical and neurologic examinations; internationally recognized scales for the evaluation of PD, disability, activities of daily living, depression, and quality of life (QOL); as well as videos, magnetic resonance (MR) imaging, and MR spectroscopy. Stem cells were implanted in the posterior region of the circle of Willis. Patients were evaluated according to clinical measures. Comparison was made versus data collected from all scales before treatment, as well as videos and spectroscopy in eight patients.

RESULTS: In a mean follow-up of 7.4 months ± 4.5 (range, 1–18 months), patients showed a median improvement of 51.1% and quartile deviation (QD) of 24.8% on the Unified PD Rating Scale. They showed significant improvement in disability, activities of daily living, depression, and QOL ($P < .5$). No complications were observed. In eight patients, follow-up MR spectroscopy revealed mean improvements in n-acetylaspartate/creatine ratio from 1.805 to 2.07 (12.8%) and from 1.25 to 1.88 (43.56%) in right and left basal ganglia, respectively, versus preprocedural values ($P < .05$).

CONCLUSIONS: Treatment of PD with intraarterial autologous implantation of adult stem cells is feasible and safe and results in improved severity of disease and QOL.

J Vasc Interv Radiol 2010; 21:443–451

Abbreviations: ADC = apparent diffusion coefficient, NAA = n-acetylaspartate, NUDS = Northwestern University Disability Scale, PD = Parkinson disease, QD = quartile deviation, UPDRS = Unified Parkinson Disease Rating Scale

HISTORICALLY, the standard treatment for Parkinson disease (PD) has been to enhance dopamine levels by administering levodopa, a dopamine precursor. However, this has adverse long-term side effects such as dyskinesias and "on–off" fluctuations. Moreover, because of the degenerative nature of the disease, it can only be controlled, not cured. Therefore, as dopamine replacement does not slow down the rate at which neurons are lost, it is necessary to increase dosages or introduce new medication, such as anticholinergic agents or selective monoamine oxidase B inhibitors (1,2). Other treatments include surgical procedures such as pallidotomy or deep brain stimulation (3). Pallidotomy is the destruction of a tiny part of the globus pallidus, reducing neuronal activity in that area, whereas deep brain stimulation works as a "pacemaker for the brain" by stimulating the affected neurons. Both procedures are intended to relieve movement symptoms.

Stem cells from bone marrow have shown the ability to differentiate into neurons and other tissues (4,5). Although these cells will migrate to sites of injury (6–8), they do so in very small quantities and are therefore unable to play a role in regeneration. In this study, we aimed to use this physiologic reality and enhance it. We did so by delivering large numbers of autologous stem cells to the affected area with the use of superselective arterial catheterization, with the goal of providing significantly higher concentrations of stem cells very close to the affected area than could be achieved with the venous peripheral approach. In addition, this is a minimally invasive procedure compared with the stereotaxic approach. We are reporting the results of this technique with regard to motor and central neural function, as well as quality of life (QOL).

From the Brazzini Institute of Radiology (A.B., R.C., A.D.l.C., J.Y., C.L., T.J., M.B., M.O., L.N.S.), Medical School of the University of San Martin de Porres (A.B., R.C., C.L., T.J.), Hospital Nacional Daniel A. Carrión (A.D.l.C.), Laboratorios Cantella (J.Y.), Instituto Peruano de Paternidad Responsable (C.L.), Hospital Guillermo Almenara Irigoyen (M.B.), and Hospital Nacional José Casimiro Ulloa (M.O.), Lima, Perú. Received November 26, 2007; final revision received November 18, 2009; accepted January 19, 2010. **Address correspondence to** A.B., Instituto Brazzini Radiólogos, Jorge Basadre 223 San Isidro, Lima 27, Perú; E-mail: research@brazzini.com

From the SIR 2008 Annual Meeting.

None of the authors have identified a conflict of interest.

DOI: 10.1016/j.jvir.2010.01.008

Table 1
Patient Demographics ($N = 53$)

Variable	Value
Male sex (%)	37 (69.8)
Age (y)	
Mean ± SD	61.8 ± 10.67
Median (range)	63 (38–81)
Duration of disease	
Mean ± SD	9.09 ± 5.397
Median (range)	8 (1–25)
Baseline UPDRS	
Mean ± SD	76.17 ± 37.92
Median (range)	68 (16–166)

MATERIALS AND METHODS

Institutional review board approval for treatment of patients with PD with autologous, nonmanipulated bone marrow stem cells was obtained based on the Declaration of Helsinki. In view of the innovative character of the procedure, a multispecialty committee and the institutional review board closely monitored the clinical records of each treated patient on a regular basis. A retrospective review was performed of all patients who underwent autologous stem cell transplantation as treatment for PD. The diagnosis of PD was made by a neurologist based on the United Kingdom PD Society Brain Bank diagnostic criteria. Patients who were not able to complete at least 1 month of follow-up for geographic reasons were excluded from this analysis. Demographics are included in **Table 1**. Inclusion criteria were age between 18 and 80 years and good general health state as evaluated by clinicians based on laboratory and imaging studies. In case of comorbidity, a specialist was consulted. Exclusion criteria were bone marrow depletion; blood dyscrasias; cardiac, respiratory, hepatic, or renal insufficiency; cancer; and infectious diseases. Any patient older than 75 years was evaluated for good general heath, no comorbidities, and a low grade of fragility to be considered for this treatment.

Primary endpoints were safety and clinical response according to internationally recognized scales including the Unified PD Rating Scale (UPDRS) (9), Hoehn and Yahr staging of PD (10), Schwab and England scale of activities of daily living (11), and Northwestern University Disability Scale (NUDS) (12); as well as QOL according to the eight-item version of the PD Questionnaire (PD Questionnaire–8) (13) and the Beck Depression Inventory II (14).

Secondary endpoints were functional and metabolic neural response as observed by magnetic resonance (MR) imaging of the brain with perfusion, apparent diffusion coefficient (ADC) map, and multivoxel cerebral spectroscopy evaluation.

Safety

Major complications were defined as illnesses or incidents that required medical treatment and/or hospitalization, or caused sequelae or death, that we determined to be related to the procedure. These events were to be reported to the internal ethics committee. After the procedure, all patients stayed overnight in the hospital for observation only.

Clinical Response on Internationally Recognized Scales

The UPDRS was developed in 1987 by a team of PD investigators as an overall assessment scale to quantify the signs and symptoms of PD (9). It evaluates four areas related to mentation, behavior, and mood (part I); activities of daily living (part II); motor examination (part III); and complications of pharmacologic therapy such as dyskinesias and clinical fluctuations (part IV). The UPDRS scores range from 0 (no disability or symptoms) to 199 (complete disability and severe symptoms).

The Hoehn and Yahr scale is a brief, single-item assessment of motor function (10). It is used to classify patients broadly and is rated from 0 (no signs of disease) to 5 (wheelchair-bound or bedridden unless aided).

The modified Schwab and England scale is used for the evaluation of activities of daily living and takes into account specific PD symptoms such as slowness. The Schwab and England scores range from 0% (normal) to 100% (severely affected) (11).

The NUDS (12) is a six-part scale that assesses a mixture of motor signs and functional status. The scores range from 0 (normal) to 50 (severely affected).

These scales were selected taking into account previous studies (13) to determine the improvement of PD after stem cell implantation in terms of biologic, physical, psychologic, and social parameters.

QOL

The PD Questionnaire–8 (14) is used for the assessment of health-related QOL in PD. This scale is derived from a well known measure for the evaluation of health-related QOL in PD. PD Questionnaire–8 scores range from 0 to 24, reflecting higher to lower health-related QOL. The Beck Depression Inventory II (15) is a patient-rated scale that has been validated in screening for depression in PD. It assesses severity of depressed mood on a score from 0 to 63, reflecting less to more depressed mood.

Functional and Metabolic Neural Response

Baseline evaluation by cerebral MR imaging was performed with turbo spin-echo and fast-field echo sequences. Sagittal images were acquired in T1 and axial and coronal images in T2. Axial images were obtained with fluid-attenuated inversion recovery and diffusion techniques. Images from all three axes were obtained after peripheral injection of 30 mL of paramagnetic contrast agent.

MR spectroscopy is a technique that analyzes protons within the brain tissue. It has been used in the evaluation of several neurologic disorders, including PD (16). It can provide information about various different metabolites, such as n-acetylaspartate (NAA), creatine, choline, lactate, glutamate, myoinositol, and alanine. This has clinical significance, as NAA is present in mature neurons and reflects neural viability and integrity. In addition, creatine is associated with energy homeostasis and energy reserves in the cytosol, and because its concentration is relatively constant, it is useful as a standard to compare with other metabolites. The reduction of these metabolites is associated with neuronal loss or damage of neurons in a specific area. For example, the ratio of NAA to creatine indicates internal metabolism (16–18).

Metabolites can be altered in many neurological conditions. For example, NAA is reduced in conditions in which neural loss is present. MR proton spectroscopy in early studies showed contradictions concerning metabolite ratios in PD compared with healthy controls or "Parkinson plus" syndromes. For example, O'Neill et al (19) have shown a reduction of NAA/creatine ratio in lentic-

ular nucleus in PD compared with controls. However, this might be attributable to different techniques. Recent studies have also demonstrated a reduction of NAA/creatine and choline/creatine ratios in motor cortex in de novo PD, indicating that other areas besides the basal ganglia are affected in PD (20,21). Interestingly, treatment with pergolide has improved choline/creatine ratios but not NAA/creatine ratios within these areas (19).

Even though diffusion-weighted studies have been shown to be useful for the assessment of nerve fiber anisotropy and cellular integrity in the context of stroke, they are also sensitive to the translational motion of water molecules in neural tissue. Neural loss can be associated with increased mobility of water molecules inside a tissue, resulting in increased ADC values. As PD is a neurodegenerative problem that affects neural tissue of a specific area, studies have suggested the use of this method in helping to diagnose PD when values are compared with those in healthy controls (1).

Multivoxel spectroscopy with short echo was performed in basal ganglia. Diffusion evaluates the random movement of intracellular water, based on a physical property called diffusion associated with the movement of water through neural membranes, and is presented as an ADC map. A decrease in ADC values is associated with ischemic lesions, and images that use ADC values allowed us to monitor the process of edema and neural damage in the affected region. In this case, ADC was evaluated in the lenticular nuclei. The normal value is 0.75 $mm^2/sec \pm 0.06$ (22,23).

Perfusion uses a paramagnetic contrast agent to evaluate the amount of blood that circulates in a specific area of the brain, yielding cerebral blood flow values. The normal value is 0.50 mL/g/min (24). In the present study, the basal ganglia and its flow was compared versus that in the occipital lobe because it is the least altered vascular zone of the brain (25). The ratio between them has a normal value of 1.

Procedure

Autologous stem cell implant.—The procedure was carried out according to the same technique for each patient by a multidisciplinary team composed of interventional radiologists who performed the implantation, neurologists in charge of clinical evaluation and follow-up, hematologists and clinical pathologists responsible for bone marrow aspiration and processing of stem cells, anesthesiologists who were involved in intraprocedural control, and physicians from the scientific department who were in charge of scale administration, video recordings, and data analysis. In addition, psychologists and physical therapists were involved for evaluation and therapy.

Prior to the procedure, a neurologist established a baseline for the patients' general health and disease state patients via clinically evaluation. Auxiliary examinations included complete blood count; measurement of glucose, blood urea nitrogen, and creatinine; and urinalysis to rule out coagulation problems, diabetes, renal disease, and infections. In addition, all patients received a full cardiologic evaluation, including electrocardiography and chest radiography. The six scales used in this study were assessed for baseline values, and baseline MR imaging, spectroscopy, and video assessments were made.

Bone marrow extraction and stem cell processing.—All patients were placed under monitored moderate sedation, controlled by the anesthesiologist. Bone marrow aspiration was performed by a medical team of hematologists and clinical pathologists on both iliac crests, extracting 300–400 mL, after which the patient remained under observation at the clinic for 2–3 hours. The clinical pathologist processed the sample, purifying and concentrating the stem cells according to a modification of the method of Rubinstein et al (26) to obtain the mononuclear fraction. After aspiration and purification of the stem cells, the interventional radiologists would receive 80–120 mL of purified stem cells in a sterile container, along with flow cytometry results ($CD34^+$), indicating stem cell concentration per microliter and viability.

Stem cell implantation.—As with aspiration, all patients were placed under monitored conscious sedation controlled by the anesthesiologist. With Seldinger technique, catheterization was carried out via the right femoral artery with placement of a 5-F Check-Flo catheter (0.038 inch; 11-cm hemostatic introducer sheath; Cook, Bloomington, Indiana). Diagnostic pancerebral angiography was performed with a 5-F HN1 and HN5 vascular catheters (0.035 inch × 100 cm and 0.038 inch × 100 cm, respectively; Cook). Cervical and intracranial arteries were studied to map out the anatomy and record anatomic variants, sinuosity, or atherosclerotic plaques.

After respective changes, the aortic arch was reached with 0.35-inch × 2.60-cm Road Runner guide wires (Cook). A Guidant Viking guide catheter (6 F × 100 cm; Boston Scientific) was placed consecutively in the vertebral arteries from one side and then the other, and then in both internal carotid arteries. A 2.3-F × 135-cm Embocath microcatheter (Biosphere Medical, Rockland, Massachusetts) or 1.7-F × 150-cm Target Excelsior SL 10 microcatheter (Boston Scientific) and a Silverspeed microwire (0.016 inch × 200 cm; Micro Therapeutics, Irvine, California) or Transend EX floppy wire (0.014 in × 205 cm; Boston Scientific) were placed to reach the basilar trunk, posterior cerebral arteries, and posterior part of the circle of Willis, from which originate the perforating arteries that irrigate the basal nucleus and the substantia nigra (27,28). If the patients showed evidence of arterial plaques, the infusion was made proximal to these lesions. The fentanyl dose was then increased by 5 mg/kg to lightly reduce blood pressure in hopes of improving the results of the implantation. The concentration of stem cells was diluted in NaCl 0.9% at 2 mL per 10 mL. The stem cells were then manually infused in small pulses for a period of approximately 90–120 minutes. The patients remained under observation for one night, received a neurologic examination, and were released from the hospital.

Follow-up

Follow-up consisted of several medical evaluations as well as imaging control. Neurologic evaluations were conducted during hospitalization at 12 and 72 hours after stem cell implantation, weekly for the first month, then monthly for 1 year after the procedure. Evaluation was performed with the aforementioned scales for PD at 1 and 2 weeks and 1, 3, 6, and 12 months after stem cell implantation. Video data were obtained with patient consent during follow-up. Nine patients had a control MR examination. Four patients received a second implant at 12 months and continued follow-up at 1 and 2 weeks and 1, 3, and 6 months after the second implantation.

Figure 1. Box-plot graphs show significant ($P < 0.05$) results for UPDRS **(a)**, Hoehn and Yahr **(b)**, NUDS **(d)**, PD Questionnaire–8 **(e)**, and Beck Depression Inventory II **(f)** scales, with higher scores indicating worse parkinsonism. For the Schwab and England activities of daily living scores **(c)**, higher scores indicate better performance in the activities of daily living.

Statistical Methods

Continuous variables were expressed as means ± SD or medians and quartile deviations (QDs). To determine clinical response and QOL (primary endpoints) according to scale evaluation, the nonparametric Wilcoxon matched-pairs signed-rank test was performed to compare status before stem cell implantation versus at last follow-up at the time of review. SPSS software was used for statistical analysis (version 15; SPSS, Chicago, Illinois). After two and then 20 patients had at least 1 month of follow-up, statistics and a global evaluation involving all variables and videos were reviewed by the internal ethics committee, who agreed to continue with the procedure.

RESULTS

Study Group

There were initially 77 patients with PD treated with superselective intraarterial autologous stem cell implants, but only 53 could follow controls at our facility; these comprised the study group and the others were excluded. Of those excluded, one had Parkinson plus syndrome (with supranuclear palsy), one died of unrelated causes, one stopped participating in controls, and 21 came from locations too far away from the study center to allow them to comply with follow-up.

The study group consisted of 53 patients (37 men and 16 women; mean age, 61.8 years; range, 38–81 y) treated for PD from June 2006 to January 2009 who received the implant and could comply with at least 1 month of follow-up. The patients' duration of disease before stem cell implantation ranged from 1 to 25 years. The mean age of onset was 52.7 years (range, 26–75 y). The mean follow-up period was 7.4 months ± 4.947 (range, 1–18 months). All patients had a diagnosis of PD by their neurologist confirmed at the neurologic evaluation before intervention. Six had early-onset PD. The time points compared were the ones before stem cell implantation and at the last follow-up available for each patient who received more than 1 month of follow-up. All scales were evaluated when patients were receiving medication and during their best time of the day in terms of PD symptoms.

Safety

None of the treated patients ($N = 77$) had major complications as a result of the implantation procedure, and all left the hospital after evaluation the next morning. Three patients had to be hospitalized after stem cell implantation (weeks to months after) for reasons not related to the implant. One had to have a prostatectomy for treatment of benign hypertrophy of the prostate. The second had a period of psychosis, but this patient had a history of moderate to severe anxiety, depression, and a polyneuropathy that was not reported although detected previously. The third patient had an intestinal obstruction. One additional patient died of a sudden heart attack 4 days after stem cell implantation. We determined that none of these incidences were caused by or related to the implant or implantation procedure. There were other intercurrences that affected some patients, such as common colds and stressful situations that caused a temporary deterioration of their clinical status.

Table 2
Measurements on Internationally Validated PD Symptom Scales before and after Autologous Stem Cell Implantation

Scale	Difference (after vs before)	Asymptotic significance (two-tailed)
UPDRS	−5.967	0.000
Hoehn and Yahr	−4.045	0.000
Activities of daily living	−5.392	0.000
NUDS	−5.700	0.000
Beck II	−5.135	0.000
PD Questionnaire–8	−4.763	0.000

Based on positive ranks.

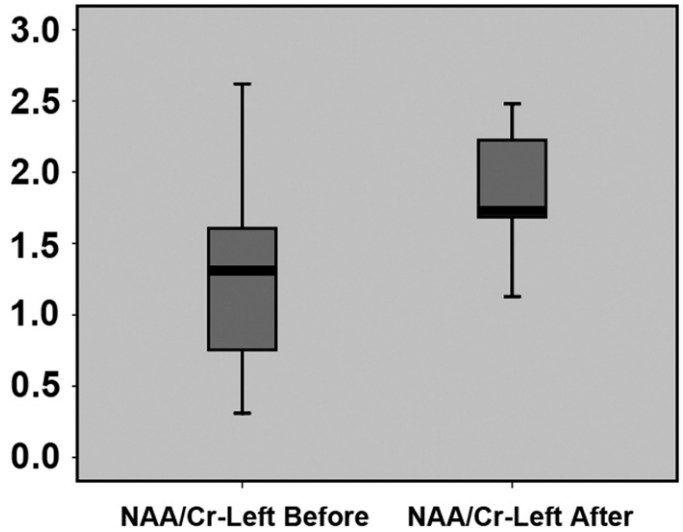

Figure 2. Box-plot graph describing statistically significant results on MR spectroscopy comparing values of NAA/creatine ratio in left basal ganglia.

Clinical Response on Internationally Recognized Scales

All 53 patients had UPDRS, Hoehn and Yahr, and Schwab and England scale evaluation, and 50 had a complete NUDS evaluation. The Beck II scale was completed by 43 patients and PD Questionnaire–8 by 45 patients. Results are shown in box-plot graphs comparing baseline and follow-up scores on each scale (**Fig 1**).

The UPDRS showed a median baseline score of 68 and QD of 25. At follow-up, the median UPDRS score 34 with a QD of 20. The scores improved at follow-up by a median of 45.5% and QD of 24.8% from baseline values.

Hoehn and Yahr scores showed a median baseline value of 3.0 with a QD of 1.0. At follow-up the median Hoehn and Yahr score was 2.0 with a QD of 0.5. The median showed no changes (0%; QD of 16.7%). However, a comparison of differences with the Wilcoxon test revealed a significant difference ($P < .01$).

The Schwab and England activities of daily living scale showed a median baseline score of 70 and QD of 20.0. At follow-up, the median Schwab and England score was 80.0 with a QD of 10, for a significant median improvement of 14.3%, with a QD of 14.2% ($P < .01$).

The NUDS showed a median baseline value of 16.5 and a QD of 9.25. At follow-up, the median value was 7.0 with a QD of 7.25. There was a significant median improvement of 38.2%, with a QD of 33.4% ($P < .01$).

QOL

QOL scales are designed for self-assessment and were not completed in patients who were unable to communicate adequately, as their scores would not be valid if they were based on answers from a caretaker or the impressions of the evaluator.

At baseline the PD Questionnaire–8 showed a mean value of 15.9 ± 7.0, with a median of 17.0 (range, 3–29) and a QD of 5.5. At follow-up, there was a mean PD Questionnaire–8 value of 9.8 ± 5.7, with a median of 9.0 (range, 1–24) and a QD of 4.0. There was a significant median improvement of 50% (range, −200% to 93.3%), with a QD of 21.4 ($P < .01$).

The Beck Depression Inventory II showed a mean baseline value of 20.38 ± 12.1, with a median of 18 (range, 2–50) and a QD of 9. At follow-up, there was a mean Beck II value of 10.27 ± 7.44, with a median of 7.5 (range, 0–27) and a QD of 6. There was a significant median improvement of 50% (range, −100% to 100%) with a QD of 17.3 ($P < .01$). All scales were evaluated with use of nonparametric Wilcoxon matched-pairs signed-rank tests (**Table 2**).

Neurologic evaluations revealed improvements in most patients. Many patients required a readjustment of medication from the first weeks after stem cell implantation because of an increase in dyskinesia and a greater time of drug effect. Most of these patients required a decrease of dosage (from 25% to 10% of the original dose), and some changed drugs (as prescribed by the neurologist).

Videos were used to record patient progress; they were used for medical evaluations and were also shown to patients, especially if, after scale assessment, we observed improvements that were not acknowledged by the patients and their families.

Functional MR and Metabolic Neural Response

For eight patients, we obtained control imaging evaluations at 4–9 months after intervention. These patients were between 39 and 72 years of age and had durations of disease ranging from 5 to 12 years. Conventional MR images revealed no signs of tumor or stroke after stem cell implantation.

Results in perfusion values showed changes in left basal ganglia from 0.44 mL/sec before treatment to 0.46 mL/sec after treatment (4.54%) and in occipital lobe from 0.42 mL/sec before treatment to 0.43 mL/sec after treatment (2.38%). For the right basal ganglia, we registered changes ranging from 0.43 mL/sec to 0.42 mL/sec (−2.32%). These dif-

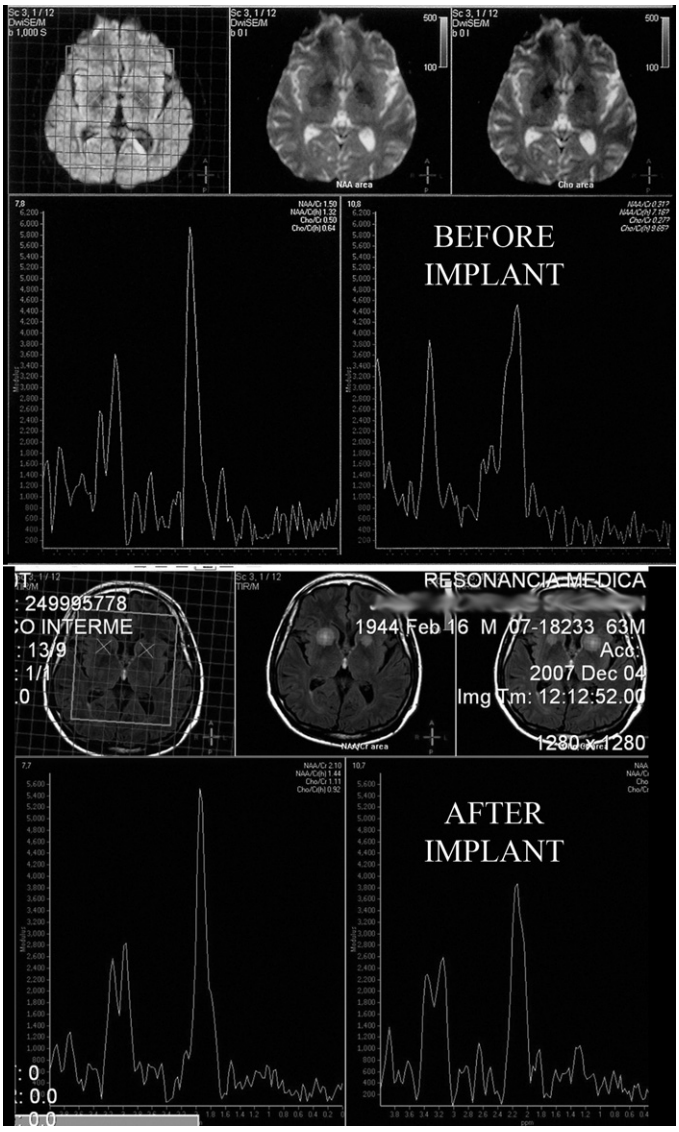

Figure 3. Spectroscopy results for a representative patient with PD. The area under the curve in spectroscopy represents the concentration of metabolites (in ppm) in a region analyzed by the voxel (square), which in this figure is the basal ganglia. The first large crest (right) corresponds to the presence of NAA (red arrow). In this case, it can be seen that the area under the curve from the spectroscopy 6 months after stem cell implantation is greater than in the previous study. Syngo software (Siemens, Iselin, New Jersey) was used to provide exact values.

ferences were not significant at $P = .446$, $P = .833$, and $P = .889$, respectively.

The perfusion index, obtained by comparison of basal ganglia perfusion versus occipital lobe perfusion, showed ratio changes in the right ganglia from 0.99 before treatment to 0.97 after treatment and in the left ganglia from 1.05 before treatment to 1.08 after treatment. Both differences were not significant ($P = .89$ and $P = .33$, respectively). In terms of mean diffusion values in left and right lenticular nuclei, we observed no differences after stem cell implantation.

As shown in **Figure 2**, in spectroscopy studies, the mean values of NAA/creatine ratio in both basal ganglia showed an increase; from 1.805 before treatment to 2.07 after treatment (12.8%) in the right basal ganglia ($P = .249$) and from 1.25 before treatment to 1.88 after treatment (43.56%) in the left basal ganglia ($P < .05$). The data from right basal ganglia was lower than those from the contralateral side. No major changes were observed in ADC or perfusion values. Both basal ganglia showed values below normal parameters. **Figure 3** shows representative results from one patient. **Figure 4** shows the overall distribution of the extent of improvement for all patients.

DISCUSSION

Treatment strategies for PD are directed toward improvement of symptoms and aimed at increasing dopamine in the substantia nigra. In addition, the degenerative characteristics of PD mean that symptoms can only be controlled temporarily. Our goals were to demonstrate that intraarterial autologous stem cell implantation is a safe procedure with good clinical response, which can improve QOL and achieve a functional and metabolic neural response.

All primary endpoints were achieved in this study, and significant differences were found between baseline and follow-up evaluations. **Figure 4** shows that more than half the patients showed more than a 50% improvement on UPDRS evaluation after stem cell implantation. The response of some patients to the implant could be observed at 24 hours. A peak improvement was observed at the first week, which then lessened slightly and later continued. However, in other cases the effect was not so immediate, with significant improvements observed after 1 or even 3 months after implantation. This difference may be related to different mechanisms of action of the stem cells. The more immediate responses may be a result of exchange of neurotransmitters and growth factors introduced by stem cells into the affected area. To the contrary, the slower effect at 3 months may be a result of stem cells integrating as neurons into the affected tissue, or because of improved perfusion caused by the angiogenic properties of stem cells (29).

Previous studies have found that dyskinesia can occur after transplantation, which can be alleviated by reducing drug doses (2,3,30). This may be because the implant may be repopulating the substantia nigra, increasing the endogenous production of dopamine. This finding confirms the results of clinical improvements in our patients.

Autologous stem cell implantation is a novel procedure, so we are aware of no references in the literature indicating

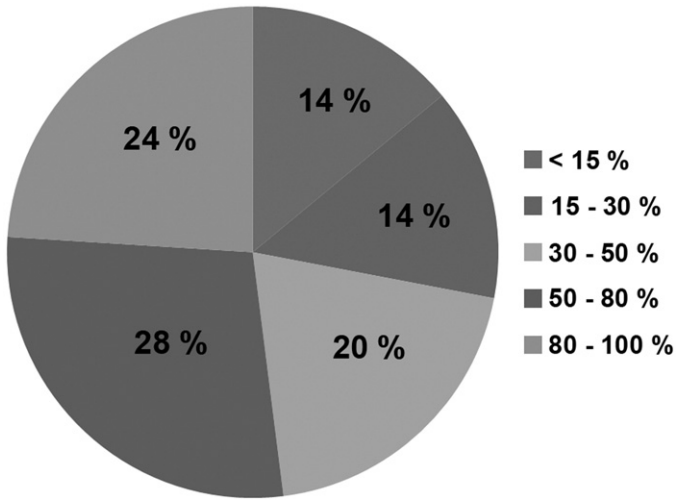

Figure 4. Pie chart shows the percentages of patients who exhibited different levels of improvement according to UPDRS evaluation.

the best moment to carry out a second implantation procedure, or even if this is necessary at all. Patients have reported no resistance to the possibility of receiving a second implant if it would reinforce the acquired improvements, or if these improvements stopped. In our observations, we have seen improvements as long as to 3–9 months after stem cell implantation. These improvements do continue, but in a less dramatic way. Two patients received a second implant at 12 months; both had stressful situations that had stopped their progress. They regained their improvements, which were maintained at 18 months after the first procedure. The rest of the patients showed maintained improvement without a second intervention. We must remember that PD is a progressive neurodegenerative disorder, and even if there were no improvements in symptoms, lack of symptomatic deterioration for more than 1 year is an important achievement.

Diffusion results showed that most values were closer to normal in the right basal ganglia, and after implantation, these values did not change. This suggests that fibers inside the lenticular nucleus remained in their normal state, suggesting that there was no modification in the area, nor edema or infarction, proving this therapy safe. We also compared NAA/creatine ratios of patients with PD before and after autologous stem cell implantation and observed an increase in mean values in both basal ganglia, but only the difference at the left basal ganglia was significant. This may be because the baseline left side mean value (1.205) was lower than normal (1.70 ± 0.56) (19). We demonstrated differences of as much as 260%, which increased preimplantation values to normal or higher. To our knowledge there is still no treatment that can improve these values. These differences can be related to the differentiation properties of adult stem cell and their capacity to stimulate regeneration from tissue progenitor cells (4), and we believe these changes suggest that there is greater integrity of neurons in the area of study. Moreover, these results correlate with the clinical improvements observed during follow-up, supporting the clinical response after treatment. However, we could not establish a relationship between the extent of improvement and variables such as age or age at onset of the disease. It is important to mention that most patients were taking medications for symptom control; however, one patient who had never taken medication showed clinical improvement and increased NAA/creatine values.

Examination of cerebral perfusion revealed positive changes in blood flow values in the left basal ganglia and occipital lobe after the procedure. The changes observed in the right basal ganglia could indicate lower blood flow, but they were all nonsignificant. No signs of recent stroke or other vascular changes from the previous MR imaging studies were observed. Even though no major changes were seen, stem cells have cytokine-mediated angiogenic properties that can positively influence damaged tissue areas after stress (29,31,32).

Combined as a whole, our MR imaging results show that the basal ganglia had no signs of ischemia; in fact, there were positive changes concerning integrity and functionality, which may indicate improved metabolism and synaptic interaction. These biochemical changes may also be related to the clinical improvement presented in the evaluation scales of the patients. Unfortunately, data from MR imaging analysis after implantation are insufficient as a result of the high cost of these examinations. We believe better interpretation of the data and clinical correlations may be achieved when we reach a greater quantity of MR follow-up controls.

The first and most important primary endpoint for treatment is safety. We need to find a nondetrimental way to use stem cells and take advantage of their regenerating capacity. Keeping in mind the first rule of medicine—"primum non nocere," or "first, do no harm"—in this protocol we did not use embryonic stem cells, avoiding two of their greatest controversies: the ethical problem of their use and their potential for tumor formation (33,34). We used adult stem cells taken from each patient in an autologous transplant. In this way, we avoided the use of immunosuppression to prevent implant rejection. The stem cells were not incubated with animal serum; they were only quantified via flow cytometry and then implanted within hours of their collection from the patient's own bone marrow. We did not use any method of promoting differentiation of stem cells because of a lack of certainty to which line of differentiation this stimulation might lead. We did not cultivate them, thereby avoiding any possible adverse effect on the patient because manipulated stem cells may not reflect normal plasticity. One study (35) has shown that implanting stem cells into the tissue itself through injection causes inflammation and consequently rejection. This protocol sought to implant stem cells in the most natural way, allowing the cells and the environment of the implantation site to determine differentiation. An advantage of this protocol is that superselective arterial catheterization is used to implant stem cells into the arteries that feed the substantia nigra. There is less chance of harm when

transplanting a tissue from one part of the body to another, especially given that mesenchymal stem cells are known to physiologically circulate in peripheral blood (36); our method insures only that they will be at a greater concentration. This way, the target region can have the greatest possible concentration of stem cells, just as noninvasive superselective intraarterial thrombolysis or hepatic selective chemotherapy have better results than systemic intravenous injection. In addition, it has been proven that stem cells stay in circulation for no more than 1 hour, decreasing the opportunity for adverse events (37). They can also distribute into a wide variety of tissues (36) and can pass through the blood–brain barrier (37). Our objective was to insure that stem cells were able to reach the substantia nigra and differentiate into dopaminergic cells capable of restoring normal function.

These results demonstrate that mesenchymal stem cells from bone marrow, without genetic manipulation or cultivation, have an effect on the function of the substantia nigra of patients with PD. Secondary endpoints showed statistical significance between pretransplantation and follow-up spectroscopy measurements in the left basal ganglia; however, ADC and perfusion measurement comparison between pretransplantation and follow-up values were nonsignificant.

We are aware of the importance of long-term follow-up (6) and that blind clinical trials are the best way to prove causality. However, because this is an original experiment, we have decided to share our findings, which suggest a safe and effective therapy with interventional radiologic techniques to treat an aggressive and progressively degenerating disease of the nervous system. Our findings show this is a feasible procedure that results in clinical recovery of extrapyramidal symptoms that is maintained over time, as well as neurologic recovery as shown on MR imaging. These improvements also have a positive effect on the emotional stability of the patient. Moreover, some patients have returned to their social and professional activities, confirming that this therapy has an important and integral impact on the QOL of patients with PD, as well as their families.

Acknowledgments: The authors thank Ausberto Chunga, María Colquicocha, Diana Vizarreta, Ofelia Huamán, Salomé Reyna, Maria del Carmen Bello, Juan Francisco Altamirano, María Palomino, and Ana Rosa Montes for their contributions.

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