Open-labeled study of unilateral autologous bone-marrow-derived mesenchymal stem cell transplantation in Parkinson’s disease

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Parkinson’s disease (PD) is a progressive neurodegenerative disease for which stem cell research has created hope in the last few years. Seven PD patients aged 22 to 62 years with a mean duration of disease 14.7 ± 7.56 years were enrolled to participate in the prospective, uncontrolled, pilot study of single-dose, unilateral transplantation of autologous bone-marrow-derived mesenchymal stem cells (BM-MSCs). The BM-MSCs were transplanted into the sublateral ventricular zone by stereotaxic surgery. Patients were followed up for a period that ranged from 10 to 36 months. The mean baseline “off” score was 65 ± 22.06, and the mean baseline “on” score was 50.6 ± 15.85. Three of 7 patients have shown a steady improvement in their “off”/“on” Unified Parkinson’s Disease Rating Scale (UPDRS). The mean “off” score at their last follow-up was 43.3 with an improvement of 22.9% from the baseline. The mean “on” score at their last follow-up was 31.7, with an improvement of 38%. Hoehn and Yahr (H&Y) and Schwab and England (S&E) scores showed similar improvements from 2.7 and 2.5 in H&Y and 14% improvement in S&E scores, respectively. A subjective improvement was found in symptoms like facial expression, gait, and freezing episodes; 2 patients have significantly reduced the dosages of PD medicine. These results indicate that our protocol seems to be safe, and no serious adverse events occurred after stem-cell transplantation in PD patients. The number of patients recruited and the uncontrolled nature of the trial did not permit demonstration of effectiveness of the treatment involved. However, the results encourage future trials with more patients to demonstrate efficacy. (Translational Research 2010;155:62–70)

Abbreviations: 7-AAD = 7- amino actinomycin D; ADL = activities of daily living; bFGF = basic fibroblast growth factor; BM-MSC = bone-marrow-derived mesenchymal stem cell; COA = certificate of analysis; CT = computed tomography; DA = dopamine; DBS = deep brain stimulation; DPBS = Dulbecco’s phosphate buffered saline; EDTA = ethylenediaminetetraacetic acid; FBS = fetal bovine serum; H&Y = Hoehn and Yahr; IEC = Institutional Ethics Committee;
Parkinson’s disease (PD) is a progressive neurodegenerative disease. The clinical features occur as a consequence of the degeneration of dopaminergic nigrostriatal neurons. The primary symptoms of PD include tremor, rigidity, bradikinesia, and postural instability. Additional symptoms, such as motor fluctuations, dyskinesias, dementia, dystonia, and a range of non-motor symptoms, emerge as the disease progresses and at times may dominate the clinical picture.1 PD causes a significant decline in the quality of life for patients and is a significant economic burden to caregivers and society.2 The rate of clinical deterioration is rapid in the early phase with a decline of approximately 8 to 10 points on the Unified Parkinson Disease Rating Scale (UPDRS) in the 1st year.3,4 Effective symptomatic treatment for PD has been available since the introduction of L-dopa more than 40 years ago. Every year, additional drugs have been added to the PD armamentarium, but predominantly they remain focused on the symptomatic treatment. One school promulgates that drug treatment should be delayed until the symptoms of PD significantly limited the patient’s ability to function at work or socially.5 The rationale for this is reasonable: The treatments available currently are only symptomatic and cannot modify the course of the disease, so a delay in L-dopa therapy can also help in delaying the troublesome motor complications like dyskinesias and motor fluctuations, which inadvertently develops from prolonged L-dopa use. In general, it is also known that the efficacy of medical treatment declines as the disease progresses. The clinical onset of PD motor features is directly associated with a series of functional changes in basal ganglia circuits and their target projections.6 The output of basal ganglia through the nigrostriatal pathway becomes abnormal, and clinical features of PD appear when striatal dopamine levels decrease to less than 7% in 1-methyl 4 phenyl 1,2,3,6 tetrahydropyridine-treated nonhuman primates.7,8 The corresponding figure in humans is not known but may be approximately 20% to 30%. Depleted dopamine levels cause increased neuronal activity in the subthalamic nucleus that drives the globus pallidum pars interna and substantia nigra pars reticulata through its glutamatergic and potentially toxic excitatory connections, and it also enhances corticostriatal excitatory activity.9 Even though the dopamine levels start to decrease, it takes a while for the development of clinically evident symptoms based on compensatory mechanisms. These compensatory mechanisms include increased striatal dopamine turnover and receptor sensitivity, upregulation of striatopallidal enkephalin levels, increased subthalamic excitation of the globus pallidum pars externa, and maintenance of cortical motor area activation.10,11 For the last 2 decades, it was believed that regeneration was never possible in the brain. However, recent discoveries by neuroscientists have changed this dogma. The presence of stem cells in the subventricular zone (SVZ) below the lateral ventricles and their propensity to migrate to the traumatized or degenerated areas of the nervous system have been proved beyond doubt.12 Many studies using different kinds of stem cells have shown that they can migrate and differentiate into dopaminergic neurons.13,14

In this pilot study, we have included 7 PD patients for bone-marrow-derived mesenchymal stem cell (BM-MSC) transplantation. The clinical study was designed to ascertain the safety and feasibility of BM-MSCs as a possible therapeutic strategy for PD. Ten to 36 months of follow-up after BM-MSC transplantation have also been described.

METHODS
A pilot clinical study was designed to ascertain the safety and feasibility of BM-MSCs in PD patients. As
a mandatory procedure according to the Indian National Stem Cell Guidelines, necessary accreditation was obtained from regulatory bodies for stem cell manufacturing, research, and therapy. Similarly, according to the National Guidelines, clinical protocol was first approved by the Institutional Committee for Stem Cell Research and Therapy and followed by the Institutional Ethics Committee (IEC). Informed consent was taken from every patient who participated in the study. Any deviations, dropouts, and adverse events were documented and informed to the IEC.

**Study design.** The study was performed as a prospective, 1-year, single-dose, uncontrolled, pilot study of autologous BM-MSCs unilaterally transplanted in patients with advanced PD (2 or more classic symptoms).

**Patient selection.** Seven PD patients aged between 22 and 62 years were enrolled to participate in the study. The criteria to include were at least 2 cardinal features of PD (tremor, rigidity, or bradykinesia) and a good response to L-dopa at the time of diagnosis, as well as intact higher mental functions to understand the requirements of therapy, procedures, investigations, interventions, and follow-up visits. A written informed consent was obtained from all the patients, and they were informed about the procedure, risks, benefits, complications, and long-term outcomes. Patients who suffered from neurodegenerative disorders other than PD with a history (within 1 year) of psychiatric illness that prevented them from giving informed consent or suffering from preexisting medical conditions, such as bleeding disorders, sepsis, hemoglobin <10 g/dL, serum creatinine >2 mg/dL, or serum total bilirubin >2 mg/dL, were excluded from study. At the time of obtaining informed consent, they were also screened for infection with HIV, Hepatitis B, Hepatitis C, cytomegalovirus, or syphilis using the reverse transcriptase-polymerase chain reaction (RT-PCR) method and excluded if found positive.

**Randomization.** No randomization was performed because this study was an open-label design. The neurosurgeon was aware of the treatment regimen of all the patients. Neurologic evaluation and clinical rating scales and scores were documented by an independent investigator.

**Isolation of MSCs.** BM-MSCs were isolated and expanded using a modification of methods previously reported. Briefly, 60 mL of bone marrow was aseptically aspirated from the iliac crest of all patients under deep sedation. Henceforth, all processing of the samples were performed inside a class 100 biosafety hood in a class 10,000 cyclic guanosine monophosphate facility. The bone marrow was diluted (1:1) with Knockout Dulbecco’s Modified Eagle’s Medium (KO-DMEM) (Invitrogen), and basic fibroblast growth factor (bFGF; 2 \( \mu \)g/mL). FBS that was used in the media was of Australian origin and as per the U.S. Food and Drug Administration guidelines. The nonadherent cells were removed after 48 h of culture and were replenished with fresh medium. Subsequently, the medium was replenished every 48 h.

**Subculturing and expansion of MSCs.** Once the cells became confluent, they were dissociated with 0.25% trypsin/0.53 mmol/L ethylenediaminetetraacetic acid (EDTA) (Invitrogen) and further upscaled and expanded to provide the required number of cells to the patient. Briefly, trypsinized cells were reseeded at a density of 5000 cells per cm\(^2\) in 1 cell stack (Corning Inc., Corning, NY). After 5 days in culture, the cells reached 90% confluence and were ready for transplantation.

**Preparation of cells for transplantation.** In all, 80% confluent single cell stacks were selected for transplantation. Each single-cell stack was washed twice with Dulbecco’s phosphate buffered saline (DPBS). Then, 0.25% trypsin-EDTA was added to harvest the cells. Culture medium was added to neutralize the action of trypsin. The cell suspension was centrifuged, and the cell pellet was washed 5 times with DPBS and once with normal saline in order to remove traces of FBS. The entire cell pellet was resuspended in 1 mL saline and then loaded into a syringe for transplantation.

**Quality control testing.** Before releasing the cells for transplantation, in-process testing of the cells was performed for cell surface markers analysis CD45, CD73, and CD90 (BD Pharmingen, San Diego, Calif).

Karyotyping was performed by visualizing chromosomes using the standard G-banding procedure and reported according to the International System for Human Cytogenetic Nomenclature. The endotoxin level was tested using the limulus amebocyte lysate test and mycoplasma using PCR-enzyme-linked immunosorbent assay was performed. At any step, if any sample was detected to be positive, it was discarded immediately and appropriately.

**End-product testing.** The final cell suspension that was provided to the clinician for transplantation was again
tested for cell-surface marker analysis as mentioned above. In addition, karyotyping, endotoxin, and mycoplasma were also performed as mandatory quality testing. Cell viability was measured by flow cytometry using 7-amino actinomycin D (7AAD). A certificate of analysis (COA) was prepared and cells were released along with COA for transplantation.

Transplantation protocol and surgical procedure. Stereotaxy. All surgical procedures were performed by the same neurosurgeon to minimize variation. Surgery was performed under local anesthesia, and in case of uncooperative patients an anesthetist was kept in standby for induction. A Cosman–Roberts–Wells stereotactic frame was fixed over the head with pins after injecting local anesthetic. A computed tomography (CT) scan was performed on the patient. Standard CT imaging with OM line as reference line, 2-mm sections were taken. An anterior commissure/posterior commissure line was identified. Subfrontal and SVZ lateral to the frontal horn of the lateral ventricles was taken as target on both sides, and target coordinates were calculated. The cells were transplanted into the lateral walls of the lateral ventricles of the left/right cerebral hemisphere, which represented the side of the body with maximum bothersome symptoms. The MSCs were transplanted in the subventricular zone through a precoronal burr hole with stereotactic or neuronavigational assistance. The dose was 1 million cells/kg body weight. Postoperatively, antiparkinsonian medications were reinstituted at preoperative doses and manipulated only in the case of inadequate symptom control or adverse events.

Evaluations. Clinical evaluations were performed as baseline at the time of induction into the study and at 3, 6, 9, and 12 months after transplantation. All evaluations were performed by an independent evaluator who played no other role in the study. Evaluations included the UPDRS, performed in the practically defined “off” state (approximately 12 h after the last evening dose of medication) and in the best “on” state (peak response, approximately 1 h after administration of morning medication). Dyskinesias were assessed at the beginning and end of the study by a rater in the practically defined “off” and best “on” states. Patients’ quality of life was assessed by Hoehn and Yahr (H&Y) scale and Schwab and England (S&E) score.

Outcome measures and statistical analysis. The primary outcome measure in the study was the change between baseline and final visit in UPDRS (range, 0–147; 0 was the best and 147 worst) in the “off” state and the “on” state. Secondary end points included H&Y score ranging from stage 0 (best/unilateral) to stage 5 (worst/bilateral), S&E score ranging from 100% (best) to 0 (worst), and symptomatic improvement from baseline.

RESULTS

MSCs were isolated from bone marrow of Parkinson’s disease patients and were cultured until sufficient numbers were obtained. In this protocol, 1 million cells per kg body weight were transplanted through stereotaxic surgery. An adequate number of cells was obtained at passage 1 or 2. BM-MSCs were tested for quality control and found clinically eligible (Fig 1). Each batch of cells was subjected to endotoxin testing and sterility testing, were found to be negative for mycoplasma, and were karyotypically normal. Immunophenotypic analysis showed that they were positive for CD73 and CD90 and were negative for CD45 (Fig 2).

Seven patients were enrolled into this study according to the protocol. They underwent intracerebral transplantation of autologous BM =−MSCs and were followed up over a period of 12–36 months. Final clinical evaluation was performed in a period that ranged from 10 to 32 months after transplantation. Patient demographics and time of last evaluation have been presented. Patients who participated in this study had a mean disease duration of 14.7 years. Surgical procedures were well
tolerated and all were discharged from the hospital within 3 to 5 days (Table I).

All the patients enrolled in the study were males. This reflects the fact that PD is more prevalent in the male sex with the occurrence in men being higher than that for women. \(^{18}\) Most patients were middle aged at the time of transplantation (mean age, 55.4 ± 15.2 years); the oldest was 62 years and the youngest was 21 years. They suffered from Parkinsonian symptoms a mean duration of 14.7 ± 7.6 years. The UPDRS was used, which

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**Fig 2.** Immunophenotyping of BM-MSCs derived from all 7 patients. Cells were cultured for passage 2, harvested, and labeled with antibodies against human antigen CD45, CD73, and CD90, and they were analyzed by fluorescence-activated cell sorting. The viability of the cells was tested by 7AAD markers. (Color version of figure is available online.)
Table I. Baseline characteristics of the enrolled patients

<table>
<thead>
<tr>
<th>Patient Demographics</th>
<th>Total Group</th>
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<tbody>
<tr>
<td>Number of patients</td>
<td>7</td>
</tr>
<tr>
<td>Sex</td>
<td>Males</td>
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<tr>
<td>Mean age at the time of surgery (years) (± SD)</td>
<td>55.4 ± 15.4</td>
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<tr>
<td>Mean years suffering from PD (year) ± SD</td>
<td>14.7 ± 7.6 years</td>
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<tr>
<td>Mean baseline UPDRS score—“off” period ± SD</td>
<td>65 ± 22.1</td>
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<tr>
<td>Mean baseline UPDRS score—“on” period ± SD</td>
<td>50.6 ± 15.9</td>
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<tr>
<td>Mean baseline H&amp;Y score ± SD</td>
<td>2.785 ± 1.1</td>
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<tr>
<td>Mean baseline S&amp;E ADL score ± SD</td>
<td>60% ± 23</td>
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assessed 4 different parameters such as (1) mentation, behavior, and mood; (2) activities of daily living; (3) motor skills; and (4) complications of therapy. The primary outcome measure was improvement in UPDRS in “off” and “on” periods. The “off” period evaluations were performed when patients had been withdrawn overnight from antiparkinsonian medications for approximately 8–10 h. “On” period evaluation was performed when during periods of maximum symptomatic benefit, which was approximately 1 to 2 h after the first morning dose of medication. The mean baseline “off” score was 65 ± 22.0; the best score was 34 and the worst score was 96. The mean baseline “on” score was 50.6 ± 15.9; the best score was 43 and the worst score was 73. Three patients who improved showed a steady improvement in their “off”/“on” scores. The mean “off” score at their last follow-up was 43.3, with an improvement of 22.9% from the baseline. The mean “on” score at their last follow-up was 31.7, with an improvement of 38%. We have observed marginal clinical benefit after a follow-up of 12–36 months in at least 3 of 7 patients with PD, who underwent BM-MSC transplantation according to the protocol. Despite the small numbers, an improvement was observed in total UPDRS scores during “off” and “on” periods, S&E score, and activities of daily living (ADL) scores (Fig 3).

Among the patients in whom improvement was seen, the total UPDRS “off” score improved by 22.1 ± 5.8 % and the “on” score by 38 ± 19.8 %. The mean H & Y score (used for evaluating the secondary outcome measures) was 2.7 with a low of 1.5 and a high of 5. The mean H&Y score at last follow-up was 2.5 thus virtually showing no change from the baseline. As a secondary outcome measure S&E score showed 14% improvement at last follow-up. In addition, patients subjectively reported marginal improvement in symptoms, overall well being, facial expression, gait and reduction in freezing episodes which never got benefited from traditional modes of therapy. Even we were able to marginally reduce the dosage of anti-parkinsonian medicines.

Fig 3. UPDRS score measured at baseline on and follow-up “on,” baseline “off” and follow-up “off” period. Graph showed that improvement in primary outcome was observed in total UPDRS scores during “off” and “on” periods as well as S&E and ADL scores. (Color version of figure is available online.)

Imaging with MRI of the brain was done at base line and at last follow-up. There were no parenchymal changes or evidence of tumor formation at the end of the follow-up period. There were no significant changes whatsoever in the reports of any patient. The needle tracts were not visualized as the scans were done at an interval of 12 months (Fig 4).

We successfully reduced the strength and frequency of the dose of L-dopa in 2 patients since the 3rd month follow-up (Syndopa CR 110 mg every 6–8 h) and they continued to remain stable.

DISCUSSION

Currently, the available modes of treatment for PD are medical, and the most commonly used medicine is L-dopa in various forms. Surgically creating a lesion in the thalamic nuclei (thalamotomy) and in the internal segment of the globus pallidum (pallidotomy) is in vogue. Deep brain stimulation (DBS) is an alternative surgical treatment that involves the implantation of microelectrodes and delivery of high-frequency stimulation through an implantable pulse generator placed subcutaneously. DBS of the subthalamic nucleus provides remarkable benefits in patients for whom medical therapy is ineffective. However, they only alleviate the symptoms and none of them offer a cure for the disease. Such pharmacologic replacement does not address the etiology of the disease and does not provide a permanent redress of the pathophysiology or forestall progression of the degenerative process. Stem cells are a promising candidate for dopamine (DA) regeneration. BM-MSCs have the potential to differentiate into the different lineages without being teratogenic.

Our results confirm the marginal improvement in the symptomology and quality of life after treatment with MSCs. This study represents the longest follow-up of patients with PD who have undergone unilateral BM-MSC transplantation. Our results are strikingly similar to the study performed by Hauser et al using fetal
nigral transplantation, where the UPDRS “off” scores were decreased by 18% (in 1 year follow-up) and 26% (in 2 years follow-up) compared with 22% in our study.

A trend of marginal deterioration in symptoms after initial improvement was observed in 25% of patients after 12–18 months of follow-up. This might be caused by the continued degeneration on the nongrafted side. Some studies have shown encouraging results where bilateral transplantation of mesencephalic tissue have been performed.23 These reports have encouraged us to undertake bilateral grafting of MSCs in future studies. Wenning et al21 reported an increased uptake of fluodopa on positron emission tomography (PET) in the putamen, by 68% after transplantation. Similarly, 61% uptake after 12 months was reported by Hauser et al.22 However, we could not support our results with fluorodopa uptake because a PET scan facility was not available in the hospital. The autopsy studies reported previously also support robust graft survival prominent neuritic outgrowth and extensive reinervations in an organotypic pattern.13,23-26 In this study, we cannot exclude the possibility of a placebo effect as it was an open-label study. However, the persistence of clinical improvement through 20–26 months solely caused by a placebo effect seems unlikely. Transplantation at different targets including bilateral hemispheres, different doses, and the role of booster dose needs to be explored in the future. Moreover, an improvement in dyskinesias was observed in some of our patients.

BM-MSCs were transplanted into the lateral walls of the lateral ventricles of the left/right cerebral hemisphere. This site was chosen because along much of the lateral walls of the lateral ventricles lies the largest germinal zone of the adult mammalian brain, which is called the SVZ.27 Studies have shown that in adult mammals, new neurons are born in the SVZ and migrate anteriorly into the olfactory bulb, where they mature into local interneurons.28-30 In some studies, SVZ neural stem cells have been grown in culture with epidermal growth factor, bFGF, or a combination of these.31-33 The SVZ as such represents an important reservoir of progenitors in the adult brain harboring cell populations that help in neuroregeneration.

The mechanism responsible for this benefit is not exactly known, but it may be caused by more normal DA regulation as a result of the survival and functioning of transformed DA neurons and their terminals. Previous studies have shown extensive DA transporter staining, which provides evidence of an increased number of DA terminals that may have the capacity to store DA and buffer fluctuations in striatal DA concentrations associated with development of dyskinesia.34 Marginal dose reduction could be another contributory factor. This study is the first report to demonstrate beneficial effects of BM-MSCs in Parkinson’s disease patients. BM-MSCs showed differentiation into DA neurons, and a detectable level of DA was observed in the culture media of differentiated cells.35 Moreover, a significant behavioral improvement in PD rat models 3 months posttransplantation was also observed.35,36 This proves that BM-MSCs have a potential to differentiate and exhibit several traits of DA precursors, which on transplantation in animal model, induce behavioral improvements in the hemiparkinsonian rat.36 These results were corroborated by Trzaska et al.37 who demonstrated that adult mesenchymal stem cells indeed show DA

Fig 4. Imaging with MRI shown at the baseline before stem cell transplantation (A) and after 12-month follow-up post-stem-cell transplantation (B). Cells were transplanted at SVZ. There are no perenchymal changes and no abnormal evidence post-stem-cell transplantation. There are no significant changes of any patient.
phenotypes. An immunohistochemical analysis revealed that the BM-MSCs were present more than 130 days after transplantation, and they showed integration into brain parenchyma, survival, and even migration toward the ipsilateral nigra. However, the specific mechanism by which the beneficial behavior effect was accomplished in animal models and also in our study is still difficult to interpret. Several likely mechanisms have been postulated recently, and some of them have explained that transplanted BM-MSCs perhaps exhibit or secrete neurotrophic factors. Some suggested the possibility of immunomodulation of host response to the lesion, and few implicated that transplanted BM-MSCs enhance endogenous neurogenesis. In our study, we cannot rule out any possible mechanisms that have been suggested above. Nevertheless, more studies need to be conducted to address and elucidate the possible mechanism of action so that better treatment options are available in future.

CONCLUSION

This study establishes the immediate and short-term safety of autologous BM-MSCs in the unilateral transplantation therapy of PD. The clinical improvement is only marginal; however, most patients experienced subjective well-being, without any notable adverse side effects. The exact mechanism of action is not clearly understood, which warrants elaborate studies with placebo control and bilateral transplantation with longer patient follow-up. Studies in this direction are currently being conducted at our center.

REFERENCES