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Evaluation of Human Embryonic Stem Cell Therapy in Patients with Parkinson's Disease

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Abstract

Parkinson's disease (PD) is clinically characterized by resting tremor and bradykinesia. We have previously reported the efficacy and safety of hESCs in a PD patient. Currently, we are evaluating the efficacy of the hESC therapy using the Unified Parkinson Disease Rating Scale (UPDRS) in PD patients both before and after therapy. The study included nine PD patients aged between 52 - 74 years who had undergone treatment with anti-Parkinson's medications prior to initiation of hESC therapy. We categorized patients as; W1 = number of patients staged by symptoms that score less than the highest possible grade (HPG) at the time of admission (baseline, BL) and reached HPG at the end of therapy (ET) and W2 = number of patients staged by symptoms that scored less at ET by at least one grade less of UPDRS as compared to the BL scores. All patients showed improvement following the hESC therapy. No adverse events were reported in the current study. After hESC therapy, remarkable improvement was seen in the perfusion in cerebral and cerebellar regions in the SPECT scan. hESC therapy could be a prospected future therapy for improving the quality of life of PD patients in addition to slowing down the progression of PD.

Keywords: *Cell Transplantation; Dopamine; Embryonic Stem Cells (ESCs); Parkinson's Disease (PD); Unified Parkinson Disease Rating Scale (UPDRS)*

Introduction

Parkinson's disease (PD), a slowly progressing neurodegenerative disorder, is clinically defined by the presence of bradykinesia (slowness of movement), resting tremor, rigidity and postural instability [1]. So far, the research has discovered that the deficiency of dopamine neurons (DA) in the corpus striatum and the substantia nigra [2] of the brain is the leading pathological cause of this progressive disease. Presence of Lewy bodies in the SN compacta (SNc) neurons and other parts of the brain is the pathologic marker for PD [3,4].

According to the mortality report of the Centers for Disease Control and Prevention (CDC) for 2011, PD is the 14th leading cause of deaths in the US [5]. Interplay of certain genetic and environmental factors are found to impact the idiopathic PD [6]. Studies have shown that mutation in the genes including PINK1 gene, PARKIN gene and FBXO7 gene can cause PD [7,8]. Environmental factors like pesticides exposure, rural living, and exposure to other toxins also influence the occurrence of PD. A family history of PD and a previous history of depression could be a precursor and risk factor for the onset of PD [9].

The use of dopaminergic drugs is the basic treatment for PD that includes Levodopa, Carbidopa, Benserazide and Selegiline. However, these therapies are known to provide only temporary relief from symptoms and do not halt the progression of the disease [4]. These dopaminergic therapies could not provide an acceptable quality of life to the PD patients for years as the therapeutic effects weaned off with the time. In addition, these therapies can lead to the development of treatment-resistant symptoms such as cognitive decline, depression, medication-induced psychosis and autonomic dysfunction [3].

Several agents including Rasagiline, Minocycline, and Creatine have been identified to be promising in providing a certain neuroprotection for PD patients. But presently, these agents are under phase III trials [10]. Advances in developing surgical therapies for PD have led to the emergence of deep brain stimulation (DBS) [11]. However, current guidelines from the UK National Institute for Clinical Excellence (NICE) recommend the use of DBS only in patients who could not be managed with pharmacological treatment and have lengthening off- periods [10].

In the past few decades, cell replacement therapy has gained a wide acceptance to be used as a therapeutic option for PD. Past research has investigated the potential of mesenchymal stem cells (MSCs), induced-pluripotent stem cells (iPSCs), neural stem cells (NSCs), dopaminergic neurons differentiated from fetal brain and embryonic stem cells (ESCs) in treating PD [12, 13]. Although, the transplantation fetal midbrain brain cells have shown symptomatic improvement in PD patients, but the treatment cannot be considered significant. It is reported to be associated with recurrence of dyskinesia, spread of the pathology even after transplantation and immunograft rejection. Thus, this therapy is not yet recommended for PD patients. The use of iPSCs is still limited to animal models; no clinical trial has been conducted till date to assess its use in humans. Human MSCs (derived from bone marrow and adults) have been reported to possess limited ability to differentiate into dopaminergic neurons [14,15]. Clinical and preclinical studies have been conducted using the embryonic stem cells (ESCs) and have appeared to be beneficial [16-18].

We have recently published a case report of a patient with PD treated with human embryonic stem cell (hESC) therapy [19]. This study is aimed at evaluating the efficacy of hESC therapy in patients with PD by scoring them with the Unified Parkinson's Disease Rating Scale (UPDRS), both before and after therapy.

Materials and Methods

Cell Culture and differentiation

The hESCs used in our study are cultured and maintained as per our proprietary in-house technology (United States Granted Patent No US 8592, 208, 52) in the Good Laboratory Practice (GLP), Good Manufacturing Practice (GMP) and Good Tissue Practice (GTP) certified laboratory. The two cultured cell lines (neuronal and non-neuronal) were obtained from a single, spare, expendable, two-cell blastocyst obtained during the natural *in vitro* fertilization (IVF) process with consent from the donor. The details on the composition, methods of preparation and methods of using hESCs have been elaborated elsewhere [20]. We have assessed the safety of hESCs in patients with PD and other diverse irreversible/incurable medical conditions such as spinal cord injury, cerebral palsy, non-healing wounds, Becker's Muscular Dystrophy and many more [21].

Study Population

The study included patients aged between 52 to 74 years who were ready to give a written and video consent and had previously been treated with anti-Parkinson's medications. The study was approved by an Independent Institutional Ethics Committee (IEC) and was conducted in accordance to the Declaration of Helsinki [22].

Study Design

All the patients, who were either referred to our facility or came to our facility undiagnosed, were investigated with the routine diagnostic procedure. The diagnosis of PD was confirmed clinically and supplemented by single photon emission computed tomography (SPECT) and magnetic resonance imaging (MRI). All the patients were assessed for hypersensitivity reactions to hESC by a subcutaneous (s.c.) test injection of 0.25 mL hESC.

Dosage Schedule

Subsequent to the hypersensitivity testing, patients entered the treatment session that lasted for 8 to 12 weeks. During the treatment session, 0.25 mL hESCs were administered intramuscularly (twice daily) to "prime" the body, 1 mL hESCs (≤ 16 million cells) were administered intravenously (IV, twice every 7 days) to "home in" to the required area and 1 to 5 mL hESCs were administered via any of the supplemental routes like brachial plexus injection, caudal and epidural injection (every 7 days). All the patients received nasal sprays of 1 mL hESCs (≤ 3.5 million cells) twice a week to enhance the absorption of hESCs to the brain via the nervus olfactorius.

Analysis

All the patients were scored with UPDRS before the start of the therapy and at the end of therapy (ET). An improvement in the symptoms was determined on the basis of two criteria referred to as W1 and W2, where W1 = number of patients staged by symptoms that scored less than the highest possible grade (HPG) at the time of admission (baseline; BL) and reached HPG at the ET; and W2 = number of patients staged by symptoms that scored later less by at least one grade of UPDRS as compared to the scores at BL.

SPECT Scan

All patients underwent the SPECT scan (Millennium MG, GE) before or within 7 to 10 days of hESC therapy initiation and thereafter at the ET. Before the SPECT scan, the patients were administered with an IV injection of hexamethyl propylene aminoxime (HMPAO) into antecubital vein. Subsequently, the patients were placed in a supine position with the orbitomeatal line positioned vertically centered in the field of view. Dual Digital Correlated Signal Enhancement (CSE) detectors, energy window between 55 keV and 540 keV and a gamma camera with a crystal thickness of 8.5 mm were used to obtain the SPECT scan. Improvement in the patient's condition was characterized with mild improvement showing 10% - 30%, moderate improvement with 30%-60% changes and significant improvement showing 60 - 90%.

Results

Patients

A total of nine patients aged 52 to 74 years were enrolled in this study. The number of days in T1 varied from 11 days to 190 days.

Patients that scored less than HPG at BL and reached HPG afterwards

Number of affected parameters varied among the patients. Of the 9 patients, 5 moved from scores less than HPG at BL to HPG at the ET. One patient had 50 affected parameters, of which 17 were scored as less than HPG at BL. At end of T1, the patient showed an improvement in 4 of the 17 parameters table 1.

Patients	Total Affected Parameters*	< HPG	HPG; n (%)
1	54	27	0
2	55	50	11 (22)
3	50	17	4 (23.5)
4	54	17	1 (5.9)
5	50	33	0
6	55	18	0
7	55	40	3 (7.5)
8	59	18	2 (11.1)
9	52	14	0

Table 1: *Number of Parameters that Scored Less Than the Highest Possible Grade (< HPG) at Baseline and Reached.*

Patients that scored differently after hESC therapy by at least one score of UPDRS

Of 9 patients, 7 scored differently by at least one grade. One patient had 50 parameters scored less than HPG at BL. At end of T1, 32 parameters improved by at least one grade table 2.

Parameters		Baseline		End of Therapy	
	Affected Patients	HPG	< HPG	Reached HPG	Changed by at least One UPDRS Grade
Mental impairment	9	6	3	0	1
Thought disorder	9	7	2	0	1
Depression	6	2	4	1	2
Motivation/Initiative	9	6	3	1	2
Speech	9	3	6	0	1
Salivation	9	7	2	1	2
Swallowing	9	8	1	1	1
Handwriting	9	1	8	0	2
Cutting food	9	1	8	0	1
Dressing himself	9	2	7	0	2
Hygiene	9	4	5	0	0
Turning in bed	9	4	5	0	1
Falling often	9	4	5	0	2
Freezing	9	3	6	0	3
Walking	9	1	8	2	5
Tremor	9	5	4	0	1
Sensory symptoms	9	8	1	0	0
Speech	9	3	6	1	3
Hypo mimic (e.g. less facial expression)	9	2	7	0	3
Tremor at rest: Face with lips and chin	8	6	2	0	1
Hand: right	7	3	4	0	2
Hand: left	7	3	4	0	2
Foot: right	7	4	3	0	0
Foot: left	7	4	3	0	0
Action tremor: right	9	4	5	0	2
Action tremor: left	8	3	5	0	2
Rigidity: neck	9	4	5	0	1
Upper extremity: right	9	6	3	0	1
Upper extremity: left	9	6	3	0	1
Lower extremity: right	9	5	4	0	2
Lower extremity: left	9	5	4	0	2
Finger tapping: right	9	3	6	1	3
Finger tapping: left	9	4	5	0	2
Hand grip: right	9	4	5	1	2
Hand grip: left	9	5	4	1	2
Hand pronation/supination: right	9	3	6	1	2
Left	9	5	4	0	2
Leg agility: right	9	2	7	0	2
Leg agility: left	9	2	7	0	2
Arising from chair	9	4	5	0	2
Posture	9	4	5	0	0
Gait	9	1	8	0	1
Postural stability	9	2	7	0	1
Bradykinesia	9	0	9	0	1
Dyskinesia (duration)	9	6	3	1	2
Dyskinesia (disability)	8	5	3	1	2
Dyskinesia (pain)	8	6	2	2	2
Early morning dystonia	9	8	1	1	1
"Offs" (predictable)	9	6	3	1	1
"Offs" (unpredictable)	9	8	1	1	1
"Offs" (sudden)	9	8	1	0	1
"Offs" (duration)	9	8	1	0	1
Anorexia, nausea, vomiting	9	8	1	1	1
Sleep disturbances	9	5	4	2	2
Symptomatic orthostatic	7	7	0	0	0
Blood pressure: seated	1	1	0	0	0
Blood pressure: supine	1	1	0	0	0
Blood pressure: standing	1	1	0	0	0
Weight	1	1	0	0	0
Pulse: seated	1	1	0	0	0
Pulse: standing	1	1	0	0	0

Table 2: *Number of Patients who scored Less Than the HPG at Baseline and Reached HPG at End of Therapy or Scored Differently after Therapy by at least One Grade of the UPDRS Score.*

Parameters that scored less than HPG at BL and reached HPG afterwards

The scores for patients were categorized based on the affected parameters. Bradykinesia, an indicative and frequent symptom of PD, was scored to be less than HPG in all the patients at BL and no patient reached HPG at ET. Resting tremor was scored as less than HPG in 2 patients at BL, none of them reached HPG at ET. The scores for all other parameters are presented in table 1.

Parameters that scored differently after therapy by at least one score

The change in UPDRS score by at least one grade based on affected parameters is presented in table 2. At BL, bradykinesia was scored to be less than HPG in all the patients and after hESC therapy, one patient had changed by a single score. Tremor at rest was scored to be less than HPG in 2 patients at BL, of which one patient showed improvement by one grade at ET.

SPECT Scan

Before therapy, mild to severe hypo perfusion was observed in bilateral fronto-parieto-temporal regions, bilateral basal ganglia and bilateral cerebellar regions in all nine patients. After hESC therapy, the SPECT scan findings showed significant improvement in the perfusion in cerebral and cerebellar regions. Blood circulation in other regions including fronto-parieto-temporal regions and bilateral basal ganglia was reduced to minimal hypo perfusion. Figure 1 and 2 presents the before and after therapy observations in SPECT scan of two study patients.

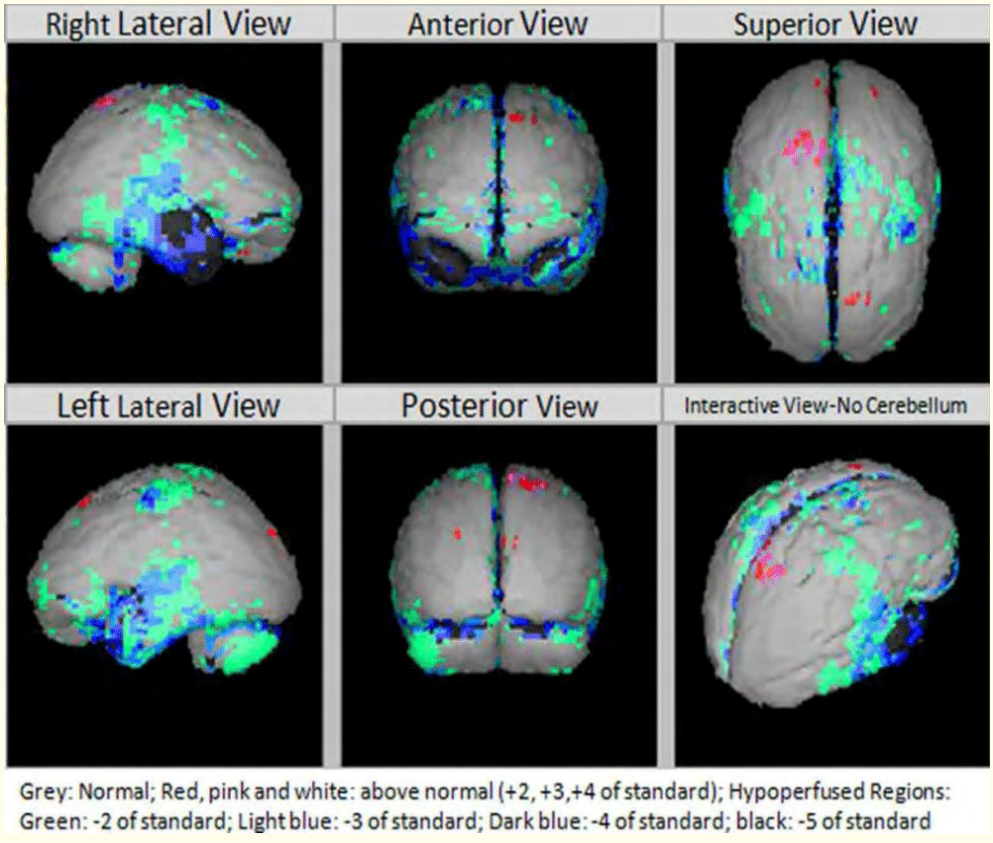

Figure 1: *SPECT Scan of a Parkinson's disease Patient before Therapy Showing Hypo perfused Regions.*

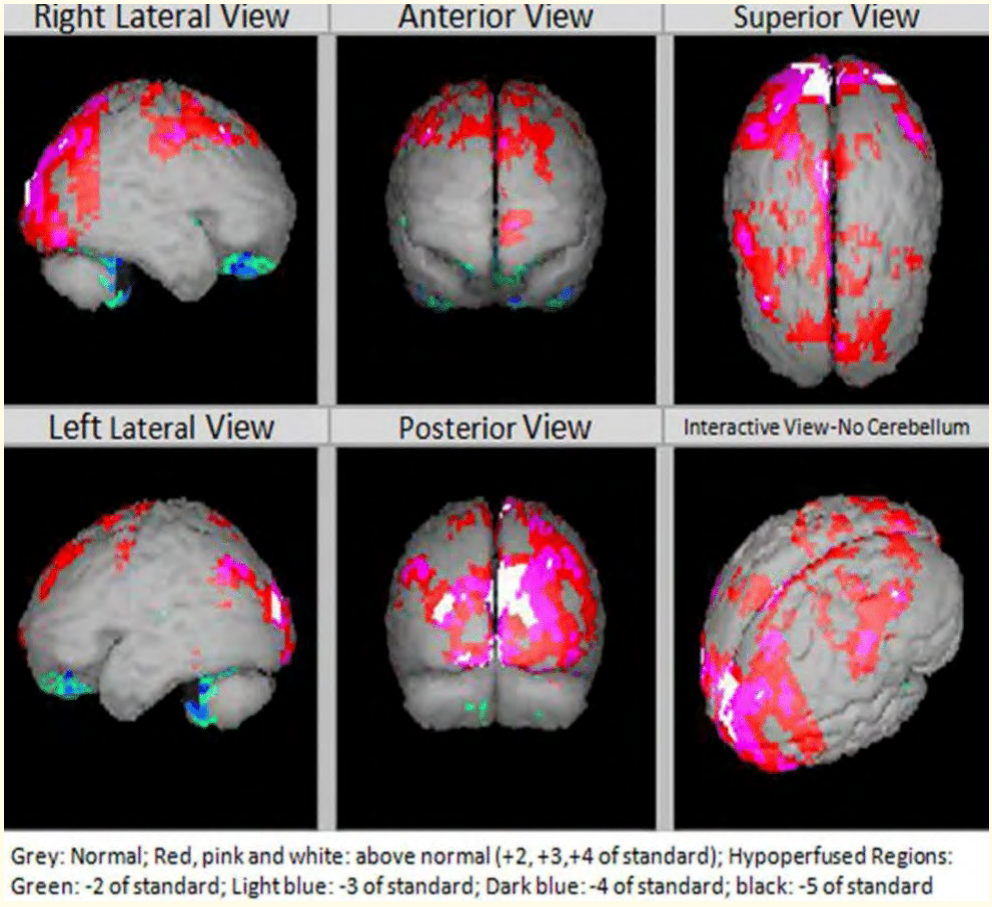

Figure 2: *SPECT Scan of the same Parkinson's disease Patient after Therapy Showing Improved Perfusion.*

Discussion

PD is characterized by the selective degeneration of DA neurons and the replacement therapies have targeted at restoring these neurons in the corpus striatum [17,23,24]. Stem cell therapy has raised a hope of being used as a viable therapy for slowing down the progression of PD in addition to improve the quality of life (QoL) of patients [25].

Several animal and human studies have been conducted with transplantation of human fetal DA neurons in PD patients. These studies have found that the use of fetal derived neurons might offer long-term clinical benefits in some patients with PD [2,26,27]. But, the use of fetal cells is associated with ethical concerns [28].

hESC as a replacement therapy is an option due to its potential in treating incurable conditions that are left untreated with the present medical or surgical therapies. Most of the studies conducted using hESCs have derived these cells from the inner mass cells of the blastocyst at 8-cell stage [29,30]. The hESC-line used in our study was derived from a 2-cell staged blastocyst/oocyte discarded during a legal regular *in vitro* fertilization after due consent from the donor [20]. At this stage, the human cells have not yet developed any antigenic properties, thus do not lead to immunologic reactions, but possess the omnipotence of embryonic stem cells.

Several studies in animal models have shown the ability of hESC derived DA neurons in improving the motor symptoms [17, 18]. A study by Freed., *et al*. attempted to assess whether the transplantation of human embryonic DA neurons in human brain could improve the condition of patients with severe PD. Bradykinesia was found to improve in younger patients and rigidity also improved in both the young and older patients. But, the study concluded that the transplants resulted only few clinical benefits in younger patients [16].

A phase I trial by Feldman., *et al.* was conducted to assess the safety of hESC in the treatment of Amyotrophic Lateral Sclerosis [7], another neurodegenerative disease. Researchers concluded that in addition to treat ALS with hESC, these cell-lines could also be used for the treatment of other neurodegenerative diseases like multiple sclerosis, Alzheimer's disease and PD [22]. The studies till date have fundamentally focused on developing neural progenitor cells (NPCs) from hESCs which are then differentiated into DA neurons *in vitro*. These neurons are afterwards transplanted into animal models or patients in clinical studies. However, in our study we have injected hESC directly via different routes into the patients with PD. Previous studies have prospected that various paracrine factors (cytokines, chemokines) released at the site of injury could potentially communicate with the stem cells and result in regeneration [31]. Thus, hESCs used in our study could have also resulted in regeneration of DA neurons in a similar manner.

In our previous study, we evaluated hESC therapy in a single patient with PD. We reported a remarkable clinical improvement in this patient [19]. In this study, we assessed the efficacy of hESCs with the UPDRS scoring system, before and after the therapy. UPDRS is an international scoring system that has been used to assess PD patients. The patient is considered as normal if the UPDRS scores 0 and is worst if the scores total 176 [32]. In our study, 5 patients scored HPG and 7 patients scored differently after undergoing hESC therapy by at least one score less than before for some of the affected parameters. Two patients reached HPG for walking and 5 patients showed improvement by at least one score of the UPDRS.

The clinical improvement in these patients was also reflected in their SPECT scans. Significant reduction in hypo perfusion was noted in several regions of the brain in the SPECT scan done subsequent to the hESC therapy.

Patients have been followed up and their condition is stable after undergoing hESC therapy, which remained unachievable by the presently available PD therapy. In fact, two patients have reduced their medication and four patients have reduced it by half of the dosages. Other two patients remained stable in their condition over a period of two years and one has been recently followed up and had still the same medication without a necessity to increase the dosages.

Conclusion

hESC therapy could be prospected as a future therapy for PD that could be helpful in slowing down the progression of PD. However, well designed clinical studies on patients of different age groups and with long-term follow up is needed to elucidate the benefits of initiating hESC therapy at an early stage of the disease.

Future Perspective

Human Embryonic Stem Cells (hESC) therapy has emerged as one of the potential cell based therapies for restoration of lost cell and tissue function. The pluripotent nature of the hESCs allows them provide to be used as a new therapeutic treatment option for Parkinson's disorders. It is also speculated that hESCs can be used to treat congenital diseases Therefore, hESC therapy could be a prospected future therapy for improving the quality of life of PD patients in addition to slowing down the progression of PD.

Executive Summary

- Evaluation of the efficacy of hESC therapy using the Unified Parkinson Disease Rating Scale (UPDRS)
- The study included nine PD patients aged between 52-74 years who had undergone treatment with anti-Parkinson's medications prior to initiation of hESC therapy.

- The patients were categorized as;
    - W1 = number of patients staged by symptoms that score less than the highest possible grade (HPG) at the time of admission (baseline, BL) and reached HPG at the end of therapy (ET)
    - W2 = number of patients staged by symptoms that scored less at ET by at least one grade less of UPDRS as compared to the BL scores.
- Post hESC therapy, remarkable improvement was observed in the perfusion in cerebral and cerebellar regions in the SPECT scan without any reported adverse events.

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Ethical Disclosure

N/A.

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