Five year follow-up on the first in human transplantation of undifferentiated stem cells into Parkinsonian patients reveals no adverse effects with improvement in motor function or arrest of the disease progression in five out of seven patients

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Abstract

Parkinson's disease is multifactorial progressive neurodegenerative disease with extremely complex pathogenesis. Current narrow approach of dopamine replacement therapy has failed to cure or arrest PD.

To address this problem, a novel line of undifferentiated non-tumorigenic neural progenitor stem cells (NPC) that are able to tune their proliferation and vector of differentiation to micro-environmental cues, was manufactured, fully characterized and used in this study. Using stereotactic surgery, NPC suspensions were bilaterally injected into patients' dorsal putamina. Neurological and neuropsychological evaluations and MRI were performed pre-operatively and 1, 2, 4 and 5 years post-surgery, as well as PET with Raclopride, DTBZ and F-DOPA – preoperatively and 1 and 2 year postoperatively. Seven of eight patients have completed 5-year follow-up.

One year after cell grafting, all but two of the seven patients completing the study showed various degrees of motor improvement. By the 5th year, UPDRS III scores somewhat increased compared to 1 year postoperatively, but remained better than at baseline in 4/7 patients in OFF condition and in 5/7 in ON condition, and five of them showed better response to medication. MRI showed no anatomical abnormalities. PET imaging showed a trend toward enhanced midbrain dopaminergic activity None of the patients developed dyskinesias, tumours, or any detectable immune responses to the grafted cells.

Introduction

Idiopathic Parkinson's disease (PD) is a progressive, degenerative neurological disorder of the basal ganglia of unknown origin that mainly affects pigmented dopamine-producing nerve cells in the *substantia nigra pars compacta* (SNpc)[1,2]. Depletion of ~ 60% of dopaminergic neurons of the SNpc leads to motor abnormalities such as resting tremor, muscle rigidity, bradykinesia, impaired gait and postural instability, speech, and mask-like facial expression[3].

Loss of nigral dopaminergic neurons has also been related to non-motor symptoms present in Parkinson's patients, such as dementia and cognitive impairment, depression and anxiety[4,5]. Moreover, non-motor symptoms refractory to available treatments for dopamine deficiency in PD might also be attributed to non-dopaminergic neurotransmitter deficiencies within or outside the basal ganglia that are known to occur in PD such as striatum serotonin, acetylcholine, gamma aminobutyric acid, and noradrenaline which would imply the requirement of non-dopaminergic drugs besides dopamine to treat PD[4,6,7].

We have performed a surgical procedure to treat PD involving bilateral intraputaminal grafting of undifferentiated human fetal-derived stem cells (NPC). Primary aim has been to explore the safety of the procedure and to identify possible neurological (motor function) and neuropsychological (non-motor symptoms) benefits the procedure might have induced.

Methods and Materials

Design of the study

Eight patients with moderate PD were selected for interventional, open label, longitudinal study. They underwent baseline, 6 months, 1, 2, 4, and 5 years post-surgery neurological and neuropsychological evaluations, and PET imaging (baseline, 1, 2, and 5 year [ongoing] post-operatively). MR images were performed before surgery and 24 h, 6 months, and 1, 2, 4, and 5 years post-surgery. Patients were tested for immune response to stem cells at baseline and 6 months post-surgery. Patients were monitored for any adverse effects.

Intervention: Undifferentiated NPC brain grafting for PD

MRI-guided bilateral stereotactic intraputaminal implantation of NPCs into the brains of PD patients was performed as previously described[8]. Briefly, two different needle tracks through the same burr hole were selected for each side. Target locations were determined by height and length of putaminal nuclei. Each needle track received $1X10^6$ cells in 1 mL of culture medium. Patients' immunosuppressant regimen with cyclosporine A at 15 mg/kg/day and indomethacin at 225 mg/day was started 10 days prior to surgery, and initially designed to be continued for 6 months postoperatively.

Procedures to obtain and release NPC

Generation, expansion, and characterization of the NPC cell line was performed as previously described[8]. Briefly, human fetal brain tissue was procured via routine sterile manual aspiration. Maternal blood samples (sera) were tested for transmissible diseases. Potential donors were screened according to 21 CFR 1271 Subpart C. Cells were expanded in closed bioreactor system in serum and xeno free medium formulation. Master and Working Cell Banks were established, characterized, tested for genetic abnormalities and tumorigenicity.

Immunogenicity testing

Patients' whole blood specimens were evaluated by flow cytometry for NPC specific antibodies (serological assay) and for antibody-dependent cell-mediated cytotoxicity (cytotoxicity assay) 1 month and 6 months after cell implantation as compared to baseline values obtained 1-mo prior to grafting.

Neurological evaluations

Neurological evaluations consisted of the UPDRS (ON and OFF states) as well as the modified Hoehn and Yahr for PD staging[9], and Schwab and England activities of daily living scales[10].

Neuropsychological evaluations

Neuropsychological performance was assessed using the following instruments: Brief neuropsychological (NEUROPSI)[11] and computerized test batteries, the Mini-mental Parkinson State Examination (MMPSE)[12], and Mexican adaptations of Beck anxiety[13] and depression inventories[14].

PET molecular imaging

In vivo pre- and post-synaptic state of patients' nigrostriatal dopaminergic system, was investigated by PET molecular imaging in their OFF condition at baseline (before surgery), and 1, 2, and 5 [ongoing] years post-surgery. Radiopharmaceuticals used were: RAC, FDOPA, and DTBZ.

Results

Neither the craniotomy nor the stereotactic grafting of NPC to patients' putamina resulted in any complications. No immunological abnormalities or tumors were found within 5 years post-implantation.

None of the patients developed dyskinesia as the result of the treatment. Patient 5 who suffered severe dyskinesia pre-treatment is dyskinesia free and is taking reduced medication dosage at 5 years.

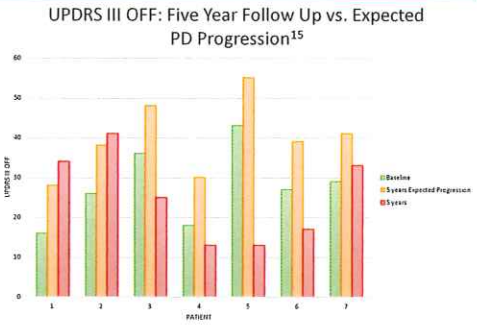

Figure 1. UPDRS III (Motor Scores) in OFF condition at the baseline and 5 years after the implantation vs. expected natural progression of disease (2.4 points per year[15]). Four out of 7 patients have lower scores at 5 years than at the baseline. Five out of 7 patients have lover scores than would be expected without treatment.

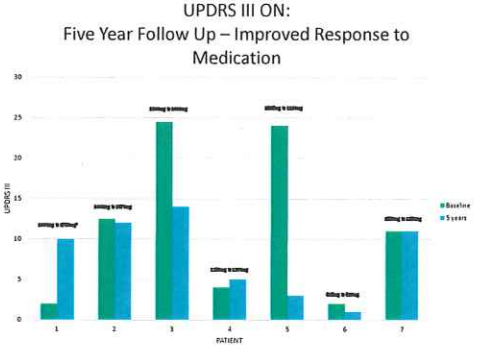

Figure 2. UPDRS III (Motor Scores) in ON condition at the baseline and 5 years after the implantation. Four out of 7 patients take decreased medication dosages at 5 years than at the baseline.
*Daily levodopa equivalent dose.

Results (cont.)

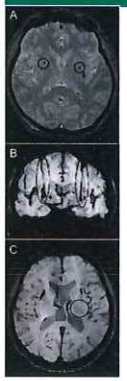

Figure 3. Brain MR images of a grafted patient. A. T2 axial image showing the 2 sites of placement of an NPC suspension in either putamen (circles) at 24 h post-surgery. B. SWAN coronal image showing both implant trajectory paths to either putamen (ellipses) at one year post-surgery. C. T2 axial image at one year post-surgery showing robust graft outgrowth in the right putamen (circle) in P4 with no topographical abnormalities of the brain structures.

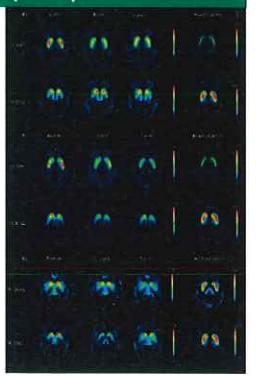

Figure 4. PET molecular imaging. Representative axial RAC, FDOPA and DTBZ PET images of healthy controls and PD patients in their OFF condition for in vivo pre- and post-synaptic assessment of the state of their nigrostriatal dopaminergic system (putamina and caudate nuclei) at baseline, and 1- and 2 years after surgery.

Conclusion

Five years post-transplantation neurological, neuropsychological and radiological evaluation showed that undifferentiated NPCs can be delivered safely by to both putamina of patients with PD without causing adverse effects. The 5-year data suggest that the NPCs are able to stop or slow down the motor deterioration one would expect to see in this timespan. These results merit further controlled clinical trials to test further safety and efficacy of intracerebrally grafted undifferentiated NPCs to treat multiple deficiencies in PD.

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