Intrastriatal Implantation of Human Retinal Pigment Epithelial Cells Attached to Microcarriers in Advanced Parkinson Disease

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Background: Human retinal pigment epithelial (RPE) cells produce levodopa and can be isolated from post-mortem human eye tissue, grown in culture, and implanted into the brain attached to microcarriers. These implants ameliorated the motor deficits in rodent and non-human primate models of Parkinson disease.

Objective: To evaluate the safety and efficacy of unilateral implantation of human RPE cells attached to gelatin microcarriers into the putamen contralateral to the more symptomatic side of patients with Parkinson disease.

Design: Open-label pilot study.

Setting: A tertiary referral center for movement disorders.

Patients: Six patients with advanced Parkinson disease.

Interventions: We performed stereotactic intrastriatal implantation of approximately 325,000 RPE cells on microcarriers.

Main Outcome Measure: Change from baseline to 12 months in the Unified Parkinson’s Disease Rating Scale motor subscore with the patients in the practically defined off state (not taking antiparkinsonian medications for at least 12 hours overnight).

Results: The implants were well tolerated. We observed an average improvement of 48% at 12 months after implantation in the Unified Parkinson’s Disease Rating Scale motor subscore with the patient in the off state, which was sustained through 24 months. Improvement was also observed in activities of daily living, quality of life, and motor fluctuations. No off-state dyskinesias were observed.

Conclusions: Implants of human RPE cells attached to gelatin microcarriers appear to be safe and well tolerated, and they improved motor symptoms in patients with Parkinson disease. On the basis of these results, a randomized, double-blind, placebo-controlled study has been initiated.

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Parkinson disease (PD) is a neurodegenerative disorder characterized by tremor, bradykinesia, rigidity, and posture instability. The neuro-pathologic correlate of this syndrome is mainly a progressive dopaminergic cell loss in the substantia nigra pars compacta and degeneration of the nigrostriatal dopaminergic pathway, although more widespread abnormalities have been observed.

Current pharmacological therapy for PD is focused primarily on ameliorating the dopamine deficiency in the brain. Most patients with PD require levodopa therapy for adequate symptomatic control of the symptoms 3 to 5 years after the diagnosis of PD. However, disease progression and long-term oral treatment with levodopa, with or without concomitant dopamine agonist therapy, may lead to the development of motor fluctuations and dyskinesias. One of the theories implicated in the development of these motor complications is the chronic intermittent dopaminergic stimulation produced by these short-acting agents. Research studies demonstrate that maintenance of continuous levels of levodopa via pumped intravenous infusion ameliorates “on/off” motor fluctuations, “wearing-off” phenomena, and dyskinesias. These studies suggest that a continuous striatal delivery of levodopa or dopamine may be closer to a physiologic state and have a better therapeutic profile than oral levodopa.

Striatal transplantation of dopamine- or levodopa-producing cells is an experimen-
cells produce levodopa. They can be isolated from post-mortem human eyes, grown easily in culture, and stored frozen for prolonged periods. These cells are reported to undergo apoptosis both in vitro and in vivo in the absence of a support matrix, but when passively attached to gelatin microcarriers, they can be transplanted into the brain and attain prolonged survival, even in the absence of immunosuppressive therapy.

Preclinical studies suggest the safety and effectiveness of RPE cells as a suitable cell type to serve as a potential durable source of levodopa for implantation into the striatum of patients with PD. Microcarrier-bound RPE cells implanted into the striatum of 6-hydroxydopamine lesioned rats showed a significant reversal of apomorphine-induced circling behavior. In a blinded sham-controlled study, which used the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) monkey model of parkinsonism, 16 hemiparkinsonian rhesus monkeys (Macaca mulatta) received either a dose of approximately 60,000 or approximately 280,000 RPE cells on gelatin microcarriers, microcarriers alone, or needle sham surgery in the striatum ipsilateral to the lesion. This study demonstrated improvement of parkinsonian symptoms in only the animals treated with high-dose RPE cells. A controlled safety study of intrastratal implants of RPE cells attached to gelatin microcarriers (Spheramine; Titan Pharmaceuticals, Inc, Somerville, NJ) conducted in 32 nonimmunosuppressed cynomolgus monkeys (Macaca fascicularis), with maximal doses up to approximately 443,350 RPE cells, showed no evidence of abnormal histologic sequelae after exposure periods of 6 and 16 months.

**PATIENTS**

We studied 6 patients from our movement disorders clinic with a mean age of 52.2 years under an investigational new drug exemption after institutional review board approval and informed consent. The mean duration of PD was 10.2 years, and the baseline Hoehn and Yahr off-state score ranged from 3 to 4. The baseline Hoehn and Yahr on-state score ranged from 2 to 3. The mean baseline Unified Parkinson’s Disease Rating Scale (UPDRS) total score was 118 during the off state.

**STUDY DESIGN**

We conducted an open-label pilot study to evaluate the safety, tolerability, and efficacy of implanted RPE cells on gelatin microcarriers in 6 patients with advanced idiopathic PD. All patients were levodopa responders and demonstrated moderate to severe PD symptoms and signs during the practically defined off state (not taking antiparkinsonian medications for at least 12 hours overnight) (Figure 1, Table 1). The patients demonstrated bilateral but asymmetric PD clinical features, motor fluctuations, dyskinesias of varying degrees, and balance problems in the off state. The patients were prescribed optimal antiparkinsonian medications, and the doses were not changed for at least 3 months before the surgery. If changes in the parkinsonian medications were considered necessary before the surgery, the procedure was postponed until they reached 3 months of stable doses. All of the patients underwent baseline evaluations 1 month before surgery.

We used magnetic resonance imaging (MRI)–guided stereotactic surgery and implanted approximately 325,000 RPE cells on gelatin microcarriers unilaterally in 3 tracts in the postcommissural putamen contralateral to each patient’s more affected side. We chose the dose based on the preclinical experience in nonhuman primates. All patients continued to follow their same baseline medical treatment regimen immediately after the implantation surgery. Immunosuppression was not used. We conducted safety visits monthly for the first year and at 15, 18, and 24 months. We performed efficacy evaluations at 1 and 3 months after surgery and then at 6, 9, 12, 15, 18, and 24 months. Yearly follow-up visits are ongoing and will continue.

**SURGERY**

We affixed a functional Cosman-Roberts-Wells stereotactic frame (Radionics, Burlington, Mass) to the skulls of the patients while they were under local anesthesia, attached a localizer, and performed brain MRI. From these images, stereotactic coordinates were generated that targeted the postcommissural putamen.

With the patient under general anesthesia and in the supine position, a coronal incision was made in the scalp anterior to the coronal suture to allow access to the skull. A burr hole was made in the calvarium. There were 5 entries into the putamen, spaced 5 mm apart to evenly distribute the RPE cells attached to gelatin microcarriers over the postcommissural region contralateral to each patient’s more affected side. Two deposits of 25 µL each were made approximately 5 to 10 mm apart in each of the 5 targets (Figure 2). The desired result was a column of cells on microcarriers that extended from the deepest point back along the needle tract to the dorsal edge of the putamen. A total of 250 µL (approximately 325,000 cells on microcarriers) was injected. The patients were taken to the recovery room and then transferred to a hospital room, and they were discharged home generally within 3 days, after a postsurgical brain MRI was performed.

**STATISTICAL ANALYSES**

All groups of data were analyzed using StatView statistical software (SAS Institute Inc, Cary, NC). The nonparametric Fried-
A man test was used to compare baseline, 12-month, and 24-month data. For post hoc comparisons, a Fisher least significant difference strategy was used, and when the Friedman test showed significance across the time points, a subsequent paired sign test was used to compare baseline with the 12-month and 24-month data. $P < .05$ was considered statistically significant.

We used the Fisher protected least significant difference method to test for our 2 planned comparisons (baseline compared with 12 months and baseline compared with 24 months). This multiple comparison procedure is often considered liberal compared with other multiple comparison procedures, so interpretation of the strength of the results may be made with this in mind.

RPE CELLS ON GELATIN MICROCARRIERS

Human RPE cells were isolated from postmortem human eyes obtained by accredited tissue banks after appropriate consent. The isolated cells were expanded and tested for sterility, mycoplasma, endotoxin, adventitious viruses, bovine viruses, human immunodeficiency virus types 1 and 2, human herpesvirus 6, hepatitis A, B, and C, and Epstein-Barr virus. The RPE cells were passively attached to steam-sterilized porcine gelatin microcarriers. The product was shipped at 2°C to 8°C to the surgical site and reduced to slurry. A final viable cell count was used to determine the volume of slurry for dosage.

RESULTS

SAFETY EVALUATIONS

Assessment of the safety and tolerability of implanted RPE cells on gelatin microcarriers, the primary objective of this phase 1 pilot study, consisted of clinical examinations, elicited adverse events, vital signs, periodic brain MRI, periodic neuropsychological evaluations, and standard laboratory studies. Postoperative brain MRI in all patients confirmed accurate placement of the implants. Follow-up brain MRI at 3, 6, 12, and 24 months in all patients showed no abnormal signal indicative of inflammation or rejection.

EFFICACY EVALUATIONS

The predefined primary outcome measure was the change from baseline to 12 months in the UPDRS motor sub-score in the practically defined off state. We observed both a clinically and statistically significant improvement from baseline in the UPDRS motor off-state score in all 6 patients (Figure 1, Table 1). This overall change in the UPDRS motor off-state score was statistically significant ($P = .006$; Friedman test), averaged 48% across all subjects at 12 months (range, 41%-61%; $P = .03$; paired sign test), and was sustained through 24 months after treatment with an average of 41% reduction of the disability score (range, 29%-58%; $P = .03$; paired sign test). Motor improvement was most robust contralateral to the implanted striatum. The UPDRS total score demonstrated significant improvement ($P = .006$; Friedman test) from baseline to 12 months after implantation ($P = .03$; paired sign test), and this was sustained through the 24-month follow-up evaluation ($P = .03$; paired sign test) (Table 1).

We found that on-state time (time when the patients feel that the medication is effective to control their symptoms and have their typical degree of benefit), mea-
We determined the frequency and severity of adverse events via patient reports with open questioning monthly during the first year and every 3 to 6 months during the second year. The patients tolerated the implantation well, and there were no serious adverse events related to the treatment. There was a small hemorrhage (4 × 7 mm) lateral to the third implant tract in 1 patient; this hemorrhage was asymptomatic and was seen on the postsurgical brain MRI, and there were no neurologic deficits or mass effect either immediately after surgery or subsequently. There was 1 serious adverse event: recurrent depression with suicidal ideation in 1 patient at 14 months after implantation. This patient was admitted to the hospital for 1 day and treated medically, and the episode resolved in 2 weeks. The adverse events possibly or probably associated with the treatment were transient mild increases in peak dose dyskinesias and the appearance of visual hallucinations. The increase in peak dose dyskinesias occurred in 4 patients during the first 2 to 12 months after implantation and resolved after reduction of the dopaminergic medications (Table 3). Mild visual hallucinations were reported in 3 patients during the first 6 months after implantation and resolved after reducing the antiparkinsonian medication in 1 patient and spontaneously in the other 2 patients. These patients did not have a history of hallucinations. One patient reported increased freezing episodes at 7 months after implantation that resolved after restarting amantadine hydrochloride therapy. The most frequent adverse effect was transient postoperative headache in the 6 patients, which resolved spontaneously within 1 to 2 weeks. There were no significant changes in laboratory results or neuropsychological assessments.

**Comment**

In this open-label pilot study we implanted approximately 325 000 RPE cells attached to gelatin microcarriers unilaterally into the putamen contralateral to the more affected side of the body in 6 young patients with advanced idiopathic PD. The implants were well tolerated without serious adverse events related to the treatment. Improvements were observed over the 24-month observation period in the UPDRS motor off-state subscore, activities of daily living subscale scores, and PDQ-39 scale and motor fluctuation scores, with increased on-state time and reduction of off-state time and on-state time with dyskinesias. Notable in this study was the persistent improvement in the percentage of on-state times, even in the presence of modest reductions in antiparkinsonian medications. The level of improvement seen in this open-label study with unilateral implantation was in the range of that seen in deep brain stimulation–treated patients. It is possible that the results may not be as good in older patients.

**Placebo effects are consistently reported in PD studies**, and may play a role in this open-label study. We observed various degrees of improvement across a range of different standardized measures. An observation of note

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**Table 2. Patient Diary Results as Mean ± SD of Percentage of Time “On State,” “Off State,” and “On State With Dyskinesias”**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>12 Months</th>
<th>24 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time on state, %</td>
<td>44.4 ± 13.7</td>
<td>54.7 ± 12.5</td>
<td>64.7 ± 14.2</td>
</tr>
<tr>
<td>Time on state with dyskinesias, %</td>
<td>14.2 ± 14.7</td>
<td>15.0 ± 15.1</td>
<td>7.0 ± 9.8</td>
</tr>
<tr>
<td>Time off state, %</td>
<td>41.4 ± 13.9</td>
<td>30.3 ± 12.8</td>
<td>28.3 ± 11.5</td>
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</table>

*Data are presented as mean ± SD. “On state” indicates time that patients feel that the medication is effective to control their symptoms and have their typical degree of benefit; “off state,” time that patients feel that the medication is not providing benefit.

**Table 3. Individual Patient and Group Mean ± SD Dopaminergic Medication Dosages at Baseline, 12 Months, and 24 Months**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Baseline</th>
<th>12 Months</th>
<th>24 Months</th>
</tr>
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<tbody>
<tr>
<td>1001</td>
<td>620</td>
<td>520</td>
<td>560</td>
</tr>
<tr>
<td>1003</td>
<td>475</td>
<td>323</td>
<td>535</td>
</tr>
<tr>
<td>1005</td>
<td>1100</td>
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<td>1006</td>
<td>456</td>
<td>306</td>
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<td>1008</td>
<td>550</td>
<td>325</td>
<td>675</td>
</tr>
<tr>
<td>1009</td>
<td>700</td>
<td>700</td>
<td>700</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>650 (238)</td>
<td>496 (214)</td>
<td>639 (214)</td>
</tr>
</tbody>
</table>

*Dosages are given in milligrams per day.
in this study with RPE cell implants is that off-state dyskinesias were not seen in any patient throughout the 24-month follow-up period.6,7 Human RPE cells do not appear to make synaptic connections, since they are nonneuronal cells and are attached to microcarriers. On the basis of the motor improvement and tolerability observed in this open-label study, a randomized, double-blind, placebo-controlled study has been initiated to more objectively test efficacy and continue to assess safety.

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Preclinical data: Subramanian.
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REFERENCES

11. Cherkesky BD, inventor; Titan Pharmaceuticals Inc, assignee. Method of increasing viability of cells which are administered to the brain or spinal cord. US patent 5 618 531. April 8, 1997.