Neurotransplantation for patients with subcortical motor stroke: a Phase 2 randomized trial

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Object. No definitive treatment exists to restore lost brain function following a stroke. Transplantation of cultured neuronal cells has been shown to be safe and effective in animal models of stroke and safe in a Phase 1 human trial. In the present study the authors tested the usefulness of human neuron transplantation followed by participation in a 2-month stroke rehabilitation program compared with rehabilitation alone in patients with substantial fixed motor deficits associated with a basal ganglia stroke.

Methods. Human neuronal cells (LBS-Neurons; Layton BioScience, Inc.) were delivered frozen and then thawed and formulated on the morning of surgery. The entry criteria in this randomized, observer-blinded trial of 18 patients included age between 18 and 75 years, completed stroke duration of 1 to 6 years, presence of a fixed motor deficit that was stable for at least 2 months, and no contraindications to stereotactic surgery. Patients were randomized at two centers to receive either 5 or 10 million implanted cells in 25 sites (seven patients per group) followed by participation in a stroke rehabilitation program, or to serve as a nonsurgical control group (rehabilitation only; four patients). The surgical techniques used were the same at both centers. All patients underwent extensive pre- and postoperative motor testing and imaging. Patients received cyclosporine A for 1 week before and 6 months after surgery. The primary efficacy measure was a change in the European Stroke Scale (ESS) motor score at 6 months. Secondary outcomes included Fugl-Meyer, Action Research Arm Test, and Stroke Impact Scale scores, as well as the results of other motor tests. Nine strokes were ischemic in origin and nine were hemorrhagic.

All 14 patients who underwent surgery (ages 40–70 years) underwent uncomplicated surgeries. Serial evaluations (maximum duration 24 months) demonstrated no cell-related adverse serological or imaging-defined effects. One patient suffered a single seizure, another had a syncopal event, and in another there was burr-hole drainage of an asymptomatic chronic subdural hematoma. Four of seven patients who received 5 million cells (mean improvement 6.9 points) and two of seven who received 10 million cells had improved ESS scores at 6 months; however, there was no significant change in the ESS motor score in patients who received cell implants (p = 0.756) compared with control or baseline values (p = 0.06). Compared with baseline, wrist movement and hand movement scores recorded on the Fugl-Meyer Stroke Assessment instrument were not improved (p = 0.06). The Action Research Arm Test gross hand-movement scores improved compared with control (p = 0.017) and baseline (p = 0.001) values. On the Stroke Impact Scale, the 6-month daily activities score changed compared with baseline (p = 0.045) but not control (p = 0.056) scores, and the Everyday Memory test score improved in comparison with baseline (p = 0.004) values.

Conclusions. Human neuronal cells can be produced in culture and implanted stereotactically into the brains of patients with motor deficits due to stroke. Although a measurable improvement was noted in some patients and this translated into improved activities of daily living in some patients as well, this study did not find evidence of a significant benefit in motor function as determined by the primary outcome measure. This experimental trial indicates the safety and feasibility of neuron transplantation for patients with motor stroke.

Key Words • stroke • transplantation • paralysis • neuron • infarction • stereotactic surgery • basal ganglion

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n the US, stroke is the third leading cause of death and the most common cause of serious adult disability. With an incidence of at least 740,000 patients, approximately 30% of patients become severely and permanently disabled. Many other survivors are left with permanent functional impairments. The economic burden for stroke is estimated to be $42 billion per year. Stroke prevention and early inter-

Abbreviations used in this paper: CI = confidence interval; CNS = central nervous system; CsA = cyclosporine A; CT = computerized tomography; DMEM = Dulbecco modified Eagle medium; EDTA = ethylenediamine tetraacetic acid; ESS = European Stroke Scale; MR = magnetic resonance; NIHSS = National Institutes of Health Stroke Scale; NT2/D1 = Ntera 2/c1.D1; OD = outer diameter; PET = positron emission tomography.
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vention to minimize the damage caused by stroke have received great attention. Rehabilitation therapy is important to maximize functional recovery in the early period after stroke. Once recovery has plateaued and the neurological deficits are fixed, however, there is no known treatment. Preclinical studies have established the potential for cultured neuronal cells derived from a teratocarcinoma cell line to be tested for safety and efficacy in the treatment of human stroke. In an animal model of stroke that caused reproducible learning and motor deficits, injection of neuronal cells resulted in a return of learning behavior retention time and motor function. Studies in the monkey, rat, and mouse lasting up to 14 months demonstrated no clinically important toxicity and no tumor formation. In experimental animals, implanted neuronal cells integrated with the host brain, sprouted axonal processes, released neurotransmitters, and demonstrated typical neuronal proteins. In a Phase 1 study our group evaluated 12 patients who received implants of neuronal cells; the safety of this procedure was acceptable and there was clinical improvement in some patients, which correlated with changes observed on PET scans. The objective of this study was to evaluate further the safety and effectiveness of neuronal cell transplantation in patients with substantial functional motor deficits following cerebral infarction by using a greater number of cells and a comparison with observational controls. The hypothesis to be tested was that implantation of neuronal cells would be safe and feasible, and would lead to improvements in motor neurological deficits resulting from basal ganglia cerebral infarction. This brain location was chosen because of its usually smaller stroke volume, ease in stereotactic targeting, and absence of cell delivery to the cerebral cortex. Such data would provide the framework for further clinical trials.

Materials and Methods

Production of Neuronal Cells

The LBS-Neurons (Layton BioScience, Inc., Gilroy, CA) were produced using antibiotic-free conditions in a Class 10,000 clean room, according to cyclic guanine monophosphate protocols at the Gilroy, California, facility. The NT2/D1 human precursor cell line from the well-characterized working cell bank (Layton BioScience, Inc.) was plated in culture. This stock culture was passaged twice per week in DMEM–F-12 growth medium. The NT2/D1 cells were scaled up for production and induced to differentiate into neurons by the addition of 10 μM retinoic acid. After 6 weeks of this treatment the cultures were harvested with trypsin–EDTA and replated at lower cell densities. These cultures were maintained in DMEM–F12 medium containing 5% fetal bovine serum and a mitotic inhibitor mixture (10 μM fluorouracil, 10 μM uridine, and 1 μM cytarabine) for 6 days. The cells were selectively harvested, purified, and extensively tested. The LBS-Neurons were cryopreserved in freezing media and stored in the vapor phase of liquid nitrogen.

On-Site Preparation of Neuronal Cells

One hour before implantation, the vials containing the cells were thawed, gently washed twice in Isolyte S (Mc-

Gaw, Inc., Irvine, CA) and centrifuged at 200 G for 7 minutes at room temperature. The cell pellet was gently resuspended in Isolyte S. The viable cell count was determined by staining a sample of the LBS-Neuron suspension with 0.4% trypan blue, and the cells were resuspended to a final concentration of 3.3 × 10^7 cells/ml in Isolyte S and aliquoted at 120 μl per sterile 1-ml vial. An aliquot was only considered acceptable if more than 50% of cells were viable. Depending on the dose to be administered, one or more vials were prepared. Vials were loaded into a closed holder and carried by hand in an upright position to the operating room for immediate use.

In the present study, doses of 5 and 10 million cells were cleared for evaluation by the US Food and Drug Administration (IND #BB-IND 7082). The trial was approved by the institutional review boards of both universities, and was reviewed by a separate data and safety monitoring board. Stopping rules were established during the initial meeting of the committee. All patients provided informed consent before entry into the study.

Clinical Trial Design

This study was an open-label trial with an observer-blinded neurological evaluation of patients with stroke who received stereotactic implants of human neuronal cells. Inclusion and exclusion criteria are listed in Table 1. Initially, nine patients were randomized to either surgery plus rehabilitation (seven patients) or rehabilitation alone (two patients). Surgery consisted of implantation of 5 million cells, which were divided into 25 implants along five trajectories (10 μl/implant). The next nine patients were randomized to receive either surgery with 10 million cells plus rehabilitation (seven patients) or rehabilitation alone (two patients). Patients were examined to determine the safety and efficacy of the cell implantation at visits occurring at Day 3 and Weeks 1, 2, 3, 4, 5, 6, 8, 12, 16, 18, 22, 26, 30, 32, and 52 following surgery. The patients continued to be seen at 1-year intervals after surgery. A course of CsA (6 mg/kg ideal body weight/day administered orally twice daily) was begun 1 week before surgery and continued for 6 months. Methylprednisolone (40 mg injected intravenously) was administered during surgery. No anticonvulsant medication was used.

Study evaluations consisted of complete neurological examinations and application of the NIHSS and the ESS performed at baseline and repeated at all follow-up visits. Additional assessments were made using the Stroke Impact Scale and the Everyday Memory Questionnaire at baseline and at Weeks 4, 8, 12, 18, 24, 26, 28, 36, and 52. Neurological function questionnaires were completed at Weeks 12, 24, and 52. All measures were completed by trained and blinded observers. Patients wore hats to prevent detection of evidence of previous surgery and they were instructed not to reveal their statuses. The Fugl-Meyer Assessment of Motor Recovery After Stroke, gait tests, Action Research Arm Test, and Grooved Pegboard testing were conduct-

ed by a physical therapist blinded to the status of each patient. Magnetic resonance images were obtained at baseline and at Weeks 4, 24, and 52 postoperatively. Additional tests included chest x-ray studies; electrocardiography performed at baseline and at postoperative Week 24 for patients who received cell implants; and analyses of com-

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TABLE 1

Inclusion and exclusion criteria*

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
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<tbody>
<tr>
<td>Neurological motor deficit (such as hemiparesis) following a completed ischemic or hemorrhagic cerebral infarction involving basal ganglia of brain</td>
<td>History of intracranial surgical procedure within 1 year of screening</td>
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<tr>
<td>Includes stroke involving one or more aspects of basal ganglia nuclei (putamen and/or globus pallidus), which surround the posterior limb of internal capsule</td>
<td>History of spinal cord injury or traumatic brain injury</td>
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<tr>
<td>Does not involve motor cortex</td>
<td>Evidence of radiculopathy on affected side</td>
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<tr>
<td>Stroke is contralateral to motor deficit</td>
<td>History of seizure disorder or current use of antiepileptic medication(s)</td>
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<tr>
<td>No substantial change in neurological deficits for ≥2 mos before enrollment per medical history</td>
<td>Exclusion of patients with a history of spasticity (except bracing and splinting)</td>
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<tr>
<td>Time interval between 1 &amp; 6 yrs from any documented stroke</td>
<td>Inability to understand or cooperate with study procedures</td>
</tr>
<tr>
<td>Age between 18 to 75 yrs</td>
<td>Prior participation in another drug, device, or biological agent trial within 30 days</td>
</tr>
<tr>
<td>No substantial adjacent cortical strokes or previous stroke involving brainstem</td>
<td>Evidence of infection on any one of the following tests:</td>
</tr>
<tr>
<td>Evidence of radiculopathy on affected side</td>
<td>Elevated white blood cell count</td>
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<tr>
<td>History of spinal cord injury or traumatic brain injury</td>
<td>Active or prior malignancy other than cutaneous basal cell carcinoma or in situ carcinoma of uterine cervix</td>
</tr>
<tr>
<td>History of intracranial surgical procedure within 1 yr of screening</td>
<td>Positive screening for occult malignancy by any one of the following:</td>
</tr>
<tr>
<td>Dimensions of motor component of stroke &gt;15 × 15 × 25 mm or max dimension of stroke &gt;40 mm along any axis</td>
<td>Cancer antigen 125, α-fetoprotein, β-human chorionic gonadotropin</td>
</tr>
<tr>
<td>Botulinum toxin injection, phenol injection, intrathecal baclofen, or any other interventional treatments for spasticity (except bracing and splinting)</td>
<td>Evidence of infection on any one of the following tests:</td>
</tr>
<tr>
<td>Modified Rankin Scale Score ≥4</td>
<td>Human immunodeficiency virus, hepatitis B, hepatitis C, rapid plasma reagin, fluorescent treponemal antibody, or purified protein derivative</td>
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<tr>
<td>Change in total ESS Score ≥3 points from screening visit to preop visit</td>
<td>Coagulopathy (platelet count &lt;100,000/μL or any prolongation of prothrombin time or partial thromboplastin time)</td>
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<tr>
<td>Residence in skilled nursing facility or other chronic care facility</td>
<td>Use of any medication with anticoagulant, antiplatelet activity within 1 wk before surgery</td>
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<tr>
<td>Gastrostomy tube or other enteral or parenteral feeding device</td>
<td>Uncontrolled hypertension, that is, systolic blood pressure &gt;180 mm Hg</td>
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<tr>
<td>History of seizure disorder or current use of antiepileptic medication(s)</td>
<td>Neurological motor deficit (such as hemiparesis) following a recovered ischemic or hemorrhagic cerebral infarction involving basal ganglia of brain</td>
</tr>
<tr>
<td>Presence of substantial cognitive deficits, aphasia, or neglect or any one of the following scores on the NIHSS or ESS:</td>
<td>Unilateral or any prolongation of prothrombin time or partial thromboplastin time</td>
</tr>
<tr>
<td>NIHSS questions:</td>
<td>Use of any medication with anticoagulant, antiplatelet activity within 1 wk before surgery</td>
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<td>Question 1a LOC &gt;2</td>
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<tr>
<td>Question 1b LOC questions &gt;1</td>
<td>Inability to understand or cooperate with study procedures</td>
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<tr>
<td>Question 1c LOC commands &gt;1</td>
<td>Inability to understand or cooperate with study procedures</td>
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<tr>
<td>Question 11 neglect = 2</td>
<td>Inability to understand or cooperate with study procedures</td>
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* Between = between; LOC = level of consciousness; pst = posterior.

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tes mellitus (five patients), hypercholesterolemia (nine patients), gastroesophageal reflux (two patients), depression (four patients), hypothyroidism (three patients), peripheral vascular migraines (one patient), cardiac valvular disease (three patients), and coronary artery disease (two patients). Eight patients were taking an oral anticoagulant drug to prevent further stroke.

Surgical Technique

One week prior to surgery, all anticoagulant medications were discontinued. On the morning of surgery, local anesthesia and mild sedation were induced in the patient and the Leksell model G stereotactic coordinate frame (Elekta Instruments, Atlanta, GA) was applied to the head. A contrast-enhanced CT scan was obtained for stereotactic targeting. Neuroimages were obtained at 5-mm intervals through the brain. Coronal and sagittal views were used to define a safe trajectory that entered a cortical gyrus and spared a sulcus. Stereotactic coordinates were obtained before placement of each instrument. We determined a point in the basal ganglia inferior to the center of the stroke region and four other targets inferior to the side of the stroke (anterior, posterior, medial, and lateral to the central target, usually spaced by 5 mm). For each of the five planned trajectories, the patient was to receive five cell implants spaced equally across a distance of 20 to 25 mm. Thus each patient received a total cell dose of 5 or 10 million cells divided into 25 implants. During surgical planning, the cells were prepared in the cellular products laboratory at each of the two study centers. After the viable cells had been prepared, they were brought to the operating room directly. After local anesthesia had been induced in the patient, a burr-hole craniostomy was created. The dura mater was opened and a 1.8-mm-OD 15-cm-long stabilizing probe was inserted to a point 4 cm proximal to the final target. A 0.9-mm-OD cannula was then inserted down to the deepest target point for the first implantation. The cannula used for cell delivery had an internal volume of 20 μl (Synergetics, St. Charles, MO). In the operating room, the cells were aspirated into a 100-μl syringe. The internal volume of the cannula was filled with the cell suspension and a 10-μl volume of cells was injected slowly at each target site over a 2-minute period. The instrument was then withdrawn to more proximal targets along each trajectory. The total time for all implantations was approximately 150 minutes. After implantation, the wound was closed and a postoperative CT scan was obtained to confirm the absence of hemorrhage. All patients were discharged home the morning after surgery. Cell preparation and surgery was performed using similar techniques at both centers.

Statistical Analyses

Statistical analyses were performed on an intent-to-treat basis. Scale scores were analyzed for changes from baseline within each patient (for example, a change in the validated ESS score of > 3). Data that appeared approximately normal in distribution were analyzed using a one-sample t-test or a paired test as necessary, whereas data not meeting this criterion were analyzed using nonparametric techniques. Sample size was determined (α = 0.05, power = 0.8) to show a significant change from baseline of five points on the ESS motor score. Two patients randomized to rehabilitation (no surgery) per dose group were not factored into the power analysis, but were selected to collect data on non-surgical outcomes. Statistical testing included a two-tailed t-test and significance was determined at the 0.05 level. The 6-month evaluation was designed a priori as the timepoint for the efficacy assessment. All statistical and data analyses were performed by an independent biostatistician.

Results

Safety of the Procedure

All patients were discharged from the hospital on the morning after surgery and resumed their preoperative medications. Computerized tomography images obtained at the conclusion of surgery in all patients did not reveal any evidence of hemorrhage. No new neurological deficits were immediately identified. One patient experienced a single seizure the day after surgery and no further seizures occurred. One patient experienced a syncopal episode 1 month after surgery. In one patient who was taking aspirin and ticlopidine after surgery, a routine MR image revealed a chronic subdural hematoma 1 month after surgery. This caused no new neurological deficits. The hematoma was drained with no new sequelae. One nonsurgical control patient died of a myocardial infarction during the follow-up period. No cell-related serious adverse events occurred. No clinically significant laboratory, neuroimaging, or electrocardiographic abnormalities that could be attributed to the neuronal-cell implantation were observed.

Functional Outcomes

European Stroke Scale scores were collected for each patient preoperatively on the day of surgery (baseline) and at predetermined intervals for 24 weeks postimplantation. On the ESS, higher scores indicated better neurological performance, and a significant change was noted to be ± three points. Between the preoperative evaluation and the day of surgery, 11 of 12 patients had no change in their ESS scores and one patient improved by one point. The mean baseline ESS total score was 68 for the 5 million cell group, 70 for the 10-million cell group, and 70 for the controls. The mean baseline ESS motor score was 28 for the 5 million cell group, 28 for the 10-million cell group, and 30 for the controls. The 6-month ESS score was calculated by averaging the scores recorded at three examinations performed at Weeks 24, 26, and 28. This was done to average out scores related to any increased or decreased patient effort obtained at any one visit. Six months after implantation of the LBS-Neurons, four of seven patients in the 5-million cell group had improved scores on the ESS (range 5.3–15 points), two patients’ scores were unchanged, and one patient’s score had decreased (4.5 points) compared with baseline scores. In the 10-million cell group, two of seven patients’ scores were improved (6.5 and 14.5 points), three patients’ scores decreased (4.5–5.5 points), and two patients’ scores remained unchanged. In the control group, one of four patients had improved scores (by 3.5 points), and the other three patients’ scores remained unchanged. The mean change in the ESS score from baseline to 6 months postoperatively for all patients who received implants was 2.7 points, whereas the mean change in the control group was 0.75 points (p = 0.148). In patients who re-
received 5 million cells, the mean change at 6 months was 4.74 points. In an evaluation in which patients served as their own controls (preoperative scores compared with postoperative scores), the mean total ESS increased from 69.3 to 74.4 at 6 months (p = 0.146). Motor elements of the ESS accounted for much of the change noted in patients treated with LBS-Neurons. A validated subscore of the ESS, the ESS motor score is a composite of the individual scores for facial movement, arm outstretched, arm raising, wrist extension, fingers, leg maintain position, leg flex, foot dorsiflexion, and gait. The mean ESS motor score at baseline for all patients who received implants was 28 and the score at 6 months was 30.7. The mean change in the ESS motor score from baseline to 6 months for all patients who received implants was 2.6 points in comparison to 1 point in the control group (p = 0.756). In patients who received 5 million cells, the mean change at 6 months was 3.74 points. In an evaluation in which patients served as their own controls (preoperative scores compared with postoperative scores), the mean ESS motor score increased from 28 to 32.2 at 6 months (mean change 4.12 [95% CI 0.3–8.5], p = 0.066). No difference was identified in NIHSS scores when control patients were compared with all patients who underwent surgery or with the separate 5- and 10-million cell groups.

The Stroke Impact Scale was used to measure the degree of disability caused by the stroke and the effects of surgery on different elements of patient performance. Patients with implanted neurons had higher daily activity scores at 6 months than control patients (p = 0.056), although a significant change was only noted when 6-month scores for patients with implants were compared with their baseline scores (p = 0.045). Compared with control scores, scores for communication (p = 0.199), feelings/mood (p = 0.413), percentage of recovery (p = 0.426), and meaningful activity (p = 0.417) did not change. Everyday Memory scores in patients with implanted neuronal cells improved compared with control scores (p = 0.012) and with the patients’ own baseline scores (mean change 13 [95% CI 4.9–21.2], p = 0.004).

The Fugl-Meyer Assessment of Motor Recovery After Stroke has been used extensively as a measure of impairment in studies focusing on functional recovery following stroke. The Fugl-Meyer instrument includes items for evaluating motor function in both upper and lower extremities, as well as items assessing balance, sensation, range of motion, and pain. The complete assessment consists of 155 items with each item rated on a three-point ordinal scale (0, cannot perform; 1, can perform partially; 2, can perform fully). The Fugl-Meyer instrument has been shown to have excellent interrater and intrarater reliability and construct validity. When patients with implanted neuronal cells were compared with control patients, no difference was identified between preoperative and 6-month postoperative scores in upper- or lower-extremity function, balance, or sensation. When scores from patients who received implants alone were compared a trend toward improvement in hand movement (mean change 1.15 points [95% CI 0.07–2.4], p = 0.06) and wrist movement (mean change 0.92 [95% CI 0.05–1.9], p = 0.06) was found.

**Action Research Arm Test.** The four subtests of grasp, grip, pinch, and gross movement were evaluated. At 6 months, patients with implanted neuronal cells had improved gross-movement scores compared with controls (mean 1.64, p = 0.017). The mean changes in score in patients who received 5 million cells was 1.57 (p = 0.043) and the mean changes in score in patients who received 10 million cells was 1.71 (p = 0.051). In a comparison of pre- and postoperative scores within the same patient, improvements were noted in gross movement (p = 0.001) and grasp (p = 0.037), but not in grip or pinch movements.

**Neuropsychological Testing**

A comprehensive neuropsychological battery was performed before and after surgery in patients treated at the University of Pittsburgh. The results of these studies are being reported separately.27

**Neuroimaging Studies**

Serial MR imaging at 6 and 12 months did not reveal any anatomical or structural changes in the brain after surgery when compared with baseline. There was no evidence of edema, contrast enhancement, mass effect, or change in the contours of the infarction. One patient experienced a chronic subdural hematoma on the side of surgery within 1 month of surgery. This was evacuated without side effects. The mean infarction size (measured in three dimensions by using calipers) at baseline was 13 mm (left–right), 22 mm (anterior–posterior), and 19 mm (superior–inferior). There was no difference in the size of the infarction between the groups and no change in size after surgery.

**Discussion**

Transplantation of human neuronal cells is one approach for ameliorating functional deficits caused by CNS disease or injury. Several investigators have evaluated the effects of fetal tissue or rat striatum transplants or cellular implants into small animal stroke models.8,23 Although transplantation of primary human fetal neurons into patients with neurodegenerative disease continues to be evaluated, the widespread clinical use of primary human tissue is likely to be limited due to the ethical and logistic difficulties inherent in obtaining large quantities of fetal neurons.80 For this reason, much effort has been devoted to developing alternate sources of human neurons for use in transplantation. One alternate source is the NT2/D1 human embryonic carcinoma-derived cell line. These cells proliferate in culture and differentiate into pure, postmitotic human neuronal cells (LBS-Neurons) on treatment with retinoic acid.124 Thus, NT2/D1 precursor cells appear to function as CNS progenitor cells with the capacity to develop diverse mature neuronal phenotypes. When transplanted, these neuronal cells survive, extend processes, express neurotransmitters, form functional synapses, and integrate with the host.36,31 During the retinoic acid induction process, the LBS-Neuronal precursor cells—which share many of the characteristics of neuroepithelial precursor cells—undergo significant changes, resulting in the loss of neuroepithelial markers and the appearance of neuronal markers. The final product is a population of human neuronal cells that is more than 95% pure and appears virtually indistinguishable from terminally differentiated postmitotic neurons.124 The cells are capable of differentiation to express a variety of neuronal markers characteristic of mature neurons, including all three neu-
Potential Mechanisms of Cell Transplantation

In addition to a humoral mechanism of action, some evidence indicates a direct action of surviving implanted neuronal cells. Animal transplantation studies of LBS-Neurons have shown graft survival, mature neuronal phenotype, and integration into the host brain in vivo.16,20,31,52 In nude mice in which LBS-Neurons were grafted in different regions of the CNS, viable cells were identified in 90% of recipients, with some grafts surviving for up to 14 months. Crafted LBS-Neurons initially remained similar to their in vitro counterparts, but then progressively acquired the phenotype of fully mature neurons in vivo.16 Transplanted neurons formed synapse-like structures and elaborated dendrites and axons, and have been tested electrophysiologically. Thus, transplanted LBS-Neurons demonstrated survival for at least 14 months postimplantation, a fully mature neuronal phenotype in vivo, and integration with the host CNS. In our first human trial, [18F]fluorodeoxyglucose PET studies revealed increased uptake at the target site, which correlated with the clinical response, and an autopsy evaluation of one graft 27 months after surgery showed surviving donor cells.19,22 The neuronal cells could improve neurological function through a number of different mechanisms. These include provision of neurotrophic support (acting as local pumps to support cell function), provision of neurotransmitters, restablishment of local interneuronal connections, cell differentiation and integration, and improvement of regional oxygen tension.8,14,27,53 Transplanted cells also may act to limit the reactive glial response and to limit retrograde degeneration, although this may not contribute to repair in a chronic injury.9,23 We believe that axonal reconnections through the grafted cells (serving as a bridge over large distances) is less likely, although this phenomenon has been observed in spinal cord injury models.21

Safety and Feasibility of the Procedure in Patients With Stroke

A major objective of this study was to demonstrate the safety and feasibility of the neuronal-cell implantation procedure. This goal was met, in that implantation was carried out successfully in all 14 patients. Although we identified no new neurological deficits due to surgery, two new neurological events occurred (seizure and a chronic subdural hematoma). A small risk of seizure should be expected given the limited cortical transgression that occurs at surgery, the loss of spinal fluid, and the accumulation of intracranial air around a brain that has some degree of atrophy. Because many patients who have suffered a stroke take antiplatelet medications or other anticoagulants, some risk for delayed intracranial hemorrhage should be expected. A second objective was to demonstrate the longer-term safety of neuronal cell implantation. This goal was also met, in that no adverse events related to the implantation occurred during 18 to 29 months of follow up in these patients or in patients from our first clinical trial (52–60 months of follow up).18 No patient sustained any permanent morbid condition related to the use of CsA, although the drug did produce variable degrees of fatigue. A review of the laboratory data revealed no consistent and clinically significant changes in hematological, chemical, or urine values.

This study was also intended to provide some information on the efficacy of neuronal cell implantation in improving stroke-related neurological deficits. Study limitations included the paucity of information known regarding optimum patient criteria (age, stroke age, size, type, or location), adequate cell number, location and number of brain implantation sites, use of immunosuppression, lack of larger control or study groups, and best way to evaluate the patient response. We did not control for any effect of CsA in the treatment groups. This study design can be criticized for not controlling for a placebo effect or any effect of CsA, but this was not a Phase 3 trial designed to address those issues.

For the ESS scores, the increases tended to be larger in the group of patients receiving 5 million cells, both in total scores and in the composite motor subscale scores. These patients had a higher incidence of ischemic rather than hemorrhagic strokes, which were more frequent among patients in the 10-million cell group. Nevertheless, the small number of patients in the 5-million and 10-million cell groups limit the ability to compare results between these groups. In addition, the ESS was developed for use in acute stroke management and may not be best suited to evaluate changes in the patient with chronic stroke. For that reason, we also includ-
ed measures of disability and more chronic deficits such as the Stroke Impact Scale and the Fugl-Meyer instrument. In a recent study of individuals admitted to inpatient rehabilitation following stroke, investigators found Fugl-Meyer motor impairment scores on admission to be a predictor of motor impairment at discharge as well as of activities of daily living and mobility functional outcome. These indications of efficacy must be tempered by the fact that signs of improvement were not consistent. Some patients had worse stroke scale and disability scores at the end of 6 months than they had at the time of implantation, although these changes were modest. In our first trial, an equal number of patients had no improvement or worsening in stroke or disability scales as had improvement. In that trial, clinical improvement correlated with changes on [18F]fluorodeoxyglucose PET imaging both at the implant site and in the contralateral cerebellum (remote diaschisis effect).

Surgical Cell Delivery

The LBS-Neurons were delivered using a stereotactic frame-based technique through a long metal cannula with an internal volume of 20 µL. Initially, a 15.5-cm-long, 1.8-mm-OD stabilization cannula was passed into the brain; the cell delivery cannula was then placed through that probe to the target. The cell delivery cannula was pushed 3.5 cm beyond the tip of the stabilization cannula. To minimize dead space within the cannula, a new instrument was created with an internal volume of 20 µL. Commercially available cannulas have an internal volume in the range of 100 microliters. The cells were delivered using a 100-µL syringe so that the cell suspension tended to layer out with gravity. If the syringe was kept in one position for several minutes, the cells would float inferiorly. Thus, during surgery the syringe and cannula were rotated to maintain a mixture of cells within the solute. Postoperative neuroimages demonstrated minimal air at the target site and no evidence of intraparenchymal hemorrhage in any patient. A total of five separate trajectories were used to obtain an implant separation of between 5 and 6 mm. This separation was chosen based on an estimated distance of processed outgrowth from the implant. Although the entire stroke volume was not surrounded in all dimensions by implants, a 25-implant approach was thought to be reasonable and one that delivered cells to a large service area of the outer portion of the stroke and regional brain, as well as to a smaller area of the internal portion of the infarction.

Identifying Suitable Patient Candidates

After completion of two clinical trials in cellular transplantation for motor stroke, can we make any suggestions regarding the optimal candidates to include in further studies? Because of the wide variety of patients and clinical factors evaluated in the first two studies (age, degree of deficit, spectrum of neurological symptoms, stroke size, stroke type [hemorrhagic or ischemic], length of immunosuppression) it is difficult to make firm comments regarding candidacy. We believe that patients with more recent strokes may have more potential to improve since their motor deficits are less likely to be fixed at the level of the distal musculature. On the other hand, both motor and cognitive improvements were measured in patients whose strokes had occurred several years earlier. We found some evidence to support the notion that ischemic stroke may be more suited to cell therapy than hemorrhagic stroke, although this difference was not significant. More patients with hemorrhagic strokes received 10 million cells and more patients with ischemic strokes received 5 million cells. Overall the 5-million cell group had higher scores. It would be difficult to infer that fewer cells led to a better result and thus we hypothesize that the 5-million cell group may have fared better because of the preponderance of ischemic strokes. A larger clinical trial would be needed to address this issue.

For a later study we propose that more recent strokes be evaluated (strokes that occur between 3 and 12 months before the patient is enrolled in the study), the need for immunosuppression be submitted to randomization, and patients with ischemic or hemorrhagic strokes be randomized. Eventually, the concept of a placebo effect would need to be tested if a reasonable and consistent level of clinical improvement is identified. Given the modest benefit observed to date in this population, further clinical research should be carefully designed to evaluate the need for immunosuppression, the value of increasing cell numbers, and improving methods of cell delivery.

Conclusions

Human neuronal cells can be produced in culture and implanted stereotactically into the brains of patients with motor deficits due to stroke. The implantation surgery caused no new deficit in any patient and appeared safe in short- and longer-term assessments. Although measurable functional improvements were noted in some patients, we did not find evidence of a significant benefit in motor function. This study was performed in a limited number of patients, usually several years after they had experienced stroke. Larger clinical studies should be performed to evaluate any differences in the effects of this procedure in patients with more recent strokes, those with ischemic stroke versus hemorrhagic stroke, patients given a greater number of neuronal cells, and patients given immunosuppression therapy.

Disclosure

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