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Neural Transplantation in Parkinson's Disease

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INTRODUCTION

Parkinson's disease (PD) is a chronic, degenerative disease characterized by a progressive loss of mesencephalic dopaminergic cells in the substantia nigra pars compacta (SNc) resulting in a loss of dopaminergic innervation to the striatum (caudate and putamen). Parkinsonian signs appear after approximately 50% of nigral cells are lost and striatal dopamine levels are reduced 80% (1). The administration of the dopamine precursor levodopa remains the cornerstone of long-term symptomatic medical management. Patients initially experience satisfactory improvement but as the disease progresses, the clinical response is frequently complicated by motor fluctuations and dyskinesias. Increased disability over time also arises in part due to nondopaminergic-responsive symptoms, including balance and cognitive dysfunction. Better treatments are needed to improve the long-term outcome of patients with PD. One approach is the transplantation of cells that might replace those that have been lost due to the disease process.

In the 1970s, Bjorklund et al. demonstrated that transplanted fetal catecholaminergic and cholinergic neurons can survive, extend processes, establish synaptic connections, and enhance the release of neurotransmitters

(2–5). Since that time, more than 300 PD patients have undergone cell transplantation under various clinical protocols. To date, insufficient clinical benefit has been demonstrated for this procedure for it to be made available as a therapeutic modality (6). New research is focusing on ways to improve the methodology of transplantation to provide meaningful clinical benefit for PD patients.

This chapter discusses the rationale for transplantation, results in animal models, results in human clinical trials, methodological issues, and prospects for the future.

RATIONALE

The basic principle underlying neural transplantation is tantalizingly simple. Functional restoration in the human brain should be achievable if lost or diseased neurons can be replaced by healthy ones (7). To be effective, transplanted cells must survive the procedure, establish lost connections, and function normally.

PD is a rational candidate for cell transplantation for several reasons:

1. PD is predominantly associated with a relatively well-defined and specific neuronal degeneration, specifically mesencephalic dopaminergic neurons.
2. The main anatomical target of degenerating neurons, the striatum, is well-defined and accessible to surgery (8).
3. Dopamine-replacement medications provide dramatic clinical benefits (9), thereby demonstrating the potential capacity of downstream response.
4. Animal models are available to test the safety, efficacy, and side effects of the procedure (10).

Commonly used animal models use 6-hydroxydopamine (6-OHDA) or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to create lesions in the dopaminergic pathways. These models have been proven to have good predictive value regarding the efficacy of potential new therapies (see Chapter 12).

6-OHDA is a specific neurotoxin for catecholaminergic neurons. In 1970, Ungerstedt and Arbuthnott showed that the dopamine agonist apomorphine induces contralateral turning and amphetamine induces ipsilateral turning in the unilateral 6-OHDA rat model (11). Denervation by 6-OHDA renders the lesioned side "supersensitive" to dopamine agonists, and the number of turns in a given time provides a quantitative assessment of the severity of the denervation. The ability of grafts transplanted into the lesioned side to reduce rotations in response to

apomorphine or amphetamine reflects normalization of dopamine innervation.

MPTP was discovered when several drug abusers accidentally injected themselves with it and subsequently developed parkinsonian symptoms (12). MPTP administration has been shown to be toxic to dopamine neurons and produce parkinsonian signs in rodents and primates. Monkeys given MPTP unilaterally in the carotid artery or after systemic treatment show signs analagous to PD, including limb and head tremor, delayed initiation of movements, difficulty eating, and freezing (13,14). Improvement in parkinsonian signs can be used to evaluate the efficacy of transplantation in this model.

The discovery of animal models that mimic the cardinal features of PD allowed more rigorous preclinical evaluation of neural transplantation. However, these are static models that do not mirror the progression of PD or its pathogenic mechanisms. It is hoped that newer transgene models of PD will more accurately reflect both the pathogenic mechanisms and progressive nature of the human disease.

RESULTS IN ANIMAL MODELS

Fetal Mesencephalic Cells

Using 6-OHDA–lesioned rats, Perlow et al. (15) demonstrated in 1979 that rat fetal mesencephalic substantia nigra (SN) dopaminergic grafts implanted into the lateral ventricle adjacent to the caudate could establish appropriate functional input to the denervated adult caudate. The reduction in turning was significantly greater for rats transplanted with SN grafts compared to those transplanted with sciatic nerve grafts (controls). Histochemical studies revealed survival, growth, and proliferation of the fetal SN grafts, while control grafts degenerated. All but one SN graft survived without rejection for at least 2 months.

A few months later, Bjorklund and Stenevi (16) used the same model to demonstrate that transplantation of rat fetal SN into the dorsal surface of denervated striatum in adult rats resulted in a reduction of amphetamine-induced turning. Long-term cell survival (up to 7 months) was good, and there was growth of dopamine fibers into the striatum from the transplant. The number of fibers formed was proportional to the number of surviving transplanted neurons. In the case with the largest number of surviving transplanted neurons and the most extensive ingrowth of fibers to the striatum, there was gradual reversal and then complete elimination of amphetamine-induced turning.

Additional studies confirmed that rat embryonic SN implanted into denervated rat striatum can result in a substantial or complete recovery of ampetamine- and apomorphine-induced turning (17–20), and biochemical and histochemical studies demonstrated that the degree of recovery was proportional to the extent of dopamine restoration and nigrostriatal reinnervation (18–20). Similar results were obtained transplanting embryonic monkey SN grafts into MPTP-lesioned monkeys, as parkinsonian signs were ameliorated and graft survival, fiber outgrowth and graft-derived dopamine production were demonstrated (21–24).

Adrenal Medulla

The chromaffin cells of the adrenal medulla normally produce epinephrine and norepinephrine, and a small amount of dopamine. However, when separated from the overlying adrenal cortex and placed under the influence of corticosteroids, their metabolism is altered so that they produce increased amounts of dopamine (9).

When grafted to the lateral ventricle or into the striatum of 6-OHDA–lesioned rats, adrenal chromaffin cells attenuated apomorphine-induced turning but not contralateral sensorimotor inattention (25–27). The behavioral effects were limited and not as great in magnitude or duration as those observed with fetal SN grafts (28).

RESULTS IN HUMAN TRIALS

Adrenal Medulla

Ethical and immunological issues regarding the use of human fetal allografts resulted in a quest for alternative cells. Although the behavioral benefits of adrenal medullary tissue transplantation in animals were modest, early human investigations focused on transplantation of adrenal medulla cells.

Direct stereotactic implantation of autologous adrenal medullary tissue into the caudate (29) and putamen (30) failed to show long-term changes. Revising the surgical procedure by placing the adrenal grafts into the intraventricular surface of the right caudate, Madrazo et al. (31) in 1987 observed impressive, sustained improvements in two patients. Preoperatively, Patient 1 was wheelchair-bound and had bilateral rigidity, bradykinesia, resting tremor, and speech impairment. At 5 months postsurgery, he was reported to be speaking more clearly, ambulating and performing routine activities independently, and had less tremor and virtually no rigidity or akinesia on either side. Improvement persisted, and at 10 months, the patient visited the clinic independently, was playing soccer

with his son, and was considering returning to work. Likewise, Patient 2, who was severely disabled prior to transplantation, exhibited impressive improvement at 3 months postsurgery, as he had no tremor, was ambulating independently, and was speaking clearly with almost normal facial expression (31). Both patients were able to discontinue antiparkinsonian medications postoperatively. Unfortunately, these results were not replicated by subsequent studies using the same techniques (32–34).

Goetz et al. (35) performed a multicenter trial utilizing the same procedure wherein 18 patients received unilateral adrenal medullary grafts into the right caudate. Evaluation at 6 months postsurgery revealed that the mean duration of on time increased from 48 to 75%, on time without dyskinesias increased from 27 to 59%, and off time decreased from 53 to 25%. Off Unified Parkinson's Disease Rating Scale (UPDRS) Activities of Daily Living (ADL) and Schwab and England scores showed significant improvement during off time. Off UPDRS motor subscale scores showed a trend toward improvement, while off Hoehn and Yahr scores did not change. Overall, the benefits observed in this study were quite modest compared to those of Madrazo et al. (31). Long-term evaluations found that benefits were maximal at 6 months and progressively and gradually declined thereafter with deterioration in most parameters by 18 months. Nonetheless, off UPDRS motor and ADL and Hoehn-Yahr scores were still statistically improved compared with baseline (36). Another study noted no benefits that could be ascribed to bilateral adrenal medulla graft placement (37).

Autopsy results from one patient whose performance level improved at 4 months postsurgery revealed necrotic adrenal tissue and no definite viable cells (38). Autopsy of another patient (who experienced marked and persistent benefit for 18 months) at 30 months postsurgery revealed that within the graft site there was a paucity of tyrosine hydroxylase (TH) immunoreactive (IR) cells, which lacked neurite extension into the host striatum (39). However, located lateral and ventral to the few surviving grafts was an enhanced fiber network of TH-IR terminals and processes, thought to represent sprouting by residual host dopaminergic neurons mediated by the host striatal response to injury (39). Similar observations have been noted in both rat (40) and monkey models (41–45). The poor survival of adrenal medullary grafts following transplantation suggests other factors are responsible for the clinical benefits observed. It has been hypothesized that the secretion of trophic factors from the graft or reactive host cells may be responsible for transplant-related functional improvement (39). However, these were uncontrolled studies, and some or all of the observed benefits could have been due to placebo effects or examiner or patient bias.

The use of adrenal autografts has been abandoned as only modest improvement was observed. Significant morbidity was associated with the surgery, including procedure-related deaths and medical and neuropsychiatric complications. The failure of adrenal cells to produce significant benefit caused investigators to turn again to fetal mesencephalic cells, as these had produced greater benefit in animal models.

Human Fetal Mesencephalic Cells

Lindvall et al. published a series of reports describing results in PD patients who received fetal mesencephalic cell transplants (46). The first report described two patients who received fetal grafts aged 7–9 weeks postconception (PC) unilaterally in the caudate and putamen. Patients received immunosuppression with cyclosporine, azothioprine, and steroids. Evaluation 6 months after surgery revealed no major therapeutic benefit in most outcome measures, but a small yet significant improvement in motor performance during off time, specifically in movement speed for pronation-supination, fist clenching, and foot lifting. There was no increase in the duration of levodopa benefit, and there was also no significant increase in fluorodopa (FD) uptake by positron emission tomography (PET) at the graft site (46).

Due to minimal benefit from the initial procedures, the same team performed subsequent transplantation studies under a modified protocol (implantation cannula was thinner, storage medium was a balanced PH-stable solution and not saline, time of storage was shorter, transplantation was solely in the putamen). In subsequently transplanted patients, there was a significant reduction in rigidity and bradykinesia, a significant decrease in off time and a reduction in the number of daily off periods (47–49). These benefits were maximal at 3–5 months (47,48) and were maintained through the first (48) and third year (49) postsurgery. Other investigations of unilateral intrastriatal fetal implantation with (50–52) or without (50,53,54) immunosuppression demonstrated similar effects on reducing disability in PD (50–55), with evidence of sustained clinical improvement as long as 46 months postsurgery (51). FD-PET assessments showed that grafts restored dopamine synthesis and storage in the grafted area (47,49–53,55), with evidence of survival even after 3 years (49). Unilateral transplantation provided benefit that was more pronounced on the side contralateral to transplantation, and thus investigations of bilateral transplantation were undertaken in an effort to increase clinical benefit.

Freeman et al. (56) noted significant improvement at 6 months postsurgery in patients who received bilateral grafts of tissue from embryos aged 6.5–9 weeks PC implanted into the posterior postcommisural putamen.

Improvements were seen in total UPDRS score during off time, in the Schwab and England disability score during off time, and in the percentage of on time with and without dyskinesias. FD-PET uptake increased bilaterally, with some patients attaining normal striatal FD uptake (56,57). Hauser et al. (58) reported results in six patients [including the four reported by Freeman et al. (56)], noting long-term benefits at clinical evaluations 12–24 months following surgery, including a significant reduction in off time, improved function in the off state, and increased on time without dyskinesias. All patients received immunosuppression, and FD uptake was significantly increased at 6 (48%) and 12 months (61%) (58). In other studies, bilateral implantation without immunosuppression also provided clinical benefit (51). In addition, sequential bilateral grafting demonstrated moderate to marked improvement after the second procedure and did not compromise the survival and function of either the first or second graft as assessed by FD-PET (59).

A retrospective review by Hagell et al. regarding bilateral putamenal transplantation studies (58–63) reported that FD-PET uptake increased from 55 to 107% at 10–23 months postsurgery for patients receiving tissue from three to five donors per putamen. These patients experienced a 30–40% overall improvement in off UPDRS motor scores and a 43–59% decrease in off time. A majority of patients also had a reduced need for antiparkinsonian medication (64). Patients with MPTP-induced parkinsonism also demonstrated substantial and sustained clinical improvement after bilateral graft implantation (65).

Freed et al. (66) performed the first double-blind, placebo-controlled trial using embryonic grafts aged 7–8 weeks PC, transplanted bilaterally into the putamen without immunosuppression. Evaluation at one year revealed significant improvement in off UPDRS motor and Schwab and England scores in subjects 60 years and younger, while the older group did not show any significant improvement as compared to the sham-surgery (control) group. At 5.5 years postsurgery, patients who demonstrated a good response to levodopa preoperatively also experienced significant improvement during off time postoperatively, regardless of age. The maximum postoperative benefit correlated with the preoperative ''best on'' levodopa response (66). Increased FD-PET uptake was detected after one year with no laterality (63) and was sustained for as long as 4 years after transplantation (67). Dystonia and dyskinesias occurred in 5 out of 33 patients who ultimately received transplants, even after levodopa was decreased or eliminated (63). Three of these five patients received deep brain stimulation of the globus pallidus interna (GPi) combined with medical treatment with a TH inhibitor and carbidopa/levodopa, while the other two received medication alone (66). Autopsy results from a patient who died 3

years after transplantation revealed surviving dopamine neurons in all transplant sites that contained neuromelanin granules that became more dense by 8 years after transplant (66). Each transplant site had dopamine-neuron outgrowth throughout the putamen (63). Another double-blind, controlled study is underway (68). Although some ethicists still challenge the idea of sham surgery (69,70), it seems clear that to expose a small number of patients to sham surgery in order to accurately assess safety and efficacy is preferable to exposing a large number of patients to a surgical treatment in which the safety and efficacy are largely unknown.

In summary, human clinical trials have shown that implanted embryonic dopaminergic neurons can exhibit short- and long-term survival as evidenced by increased FD-PET uptake. In most cases, symptomatic improvement has been observed during off periods, and the percentage of off time during the day decreased. Improved health-related quality of life and ability to resume full-time work has also been observed (71). Nonetheless, in spite of the significant symptomatic benefit that has been observed, the improvement is incomplete, both in the degree and pattern of functional recovery (6). Some patients have developed severe dyskinesias postoperatively.

ISSUES

Experience with fetal cell transplantation has suggested that many factors can affect the functional benefit derived from transplantation.

Maximizing Survival and Reinnervation

The Donor

Age. TH-IR neurons first appear in the subventricular layer at 5.5–6.5 weeks PC, while neuritic processes are first identified at 8 weeks PC and reach the striatum at 9 weeks (8,9). The optimal time for grafting is between the time when dopamine-containing cells first appear and prior to their extension of neuritic process. This time is between 5.5 and 8 weeks postconception for suspension grafts, and 6.5–9 weeks postconception for solid grafts (8,9).

Spontaneous vs. Elective Abortion. Fetal nigral tissue from elective abortions is preferable to tissue from spontaneous abortions because tissue from spontaneous abortions may contain genetic or central nervous system (CNS) defects, infections, nonviable cells, and disrupted structure, thereby providing low-quality tissue and making staging and dissection difficult (8). Relatively few spontaneous abortions occur during the optimal time for tissue transplantation.

Volumetric Issues

The amount of transplanted tissue has been variable at each center, and outcomes from clinical trials and autopsy studies have shown that to produce a clinical effect, a minimum number of neurons is needed to survive grafting. Approximately 100,000 surviving grafted dopamine neurons per putamen may be sufficient to produce clinical benefit (64). The survival of embryonic neurons after grafting is only 5–20% in both animal experiments and clinical trials (5). This makes it difficult to achieve a large number of surviving transplanted neurons and is a limiting factor in neural transplantation.

It has been estimated that mesencephalic tissue from at least three to four human embryos per side are needed to induce a therapeutically significant improvement (5). Nguyen et al. (62) noted a difference in clinical outcome after 2 years comparing patients receiving bilateral implants from 2–3 embryos (1–1.5 per putamen) to those patients receiving 6 embryos (3 per putamen). Those who received 2–3 donors showed only mild benefit, with a 6% improvement in off UPDRS motor scores and a 15% increase in off time. Those who received 6 embryos exhibited a 33% improvement in off UPDRS motor scores and a 66% decrease in off time (62). Overall results suggest that enhanced functional recovery can be better achieved by a larger number of transplanted cells.

Transplantation Technique

The choice of medium for tissue dissection and separation is potentially important, and special media have now been proposed for storage instead of the glucose-saline solution used in the past (71).

In human trials, both solid (50,51,53,56,58) and suspension (46–49) grafts have been used with apparent functional benefit. Clarkson and Freed (72) conducted a retrospective analysis of 35 patients and characterized the clinical benefits as none, mild, or moderate. They concluded that recipients of solid grafts experienced greater improvement in motor function and were able to reduce their levodopa dose more than the cell suspension groups (38% vs. 8%) (72), suggesting that solid grafts produce better outcome than suspension grafts. Forceful titrations through a pipette tip until a single cell suspension is obtained may cause mechanical injury that can result in irreversible damage to embryonic cells (73).

A delay between cannula insertion and the injection of cells into the striatum may maximize the number of surviving neurons; a 1- or 3-hour delay resulted in two to three times the number of surviving cells, while a 20-minute delay had no effect (74).

Autopsy results have shown that the territory of reinnervation surrounding graft deposits is between 2.5 and 7 mm (75,76). This suggests it is necessary to transplant cells at a 5 mm interval in three-dimensional space.

Cytoprotection

Animal studies have demonstrated that a majority of grafted neurons die within the first week (77–80) after transplantation, and neuronal death occurs as early as within 24 hours (81) to as late as the second week after transplantation (82). Apoptosis or programmed cell death (PCD) is a process wherein a cell dies through activation of genetically determined processes. Apoptosis appears to be the predominant mechanism of cell death in transplanted neurons. Activation of caspases initiates a cascade of events that lead to apoptosis. Conversely, growth factors have a protective effect on neurons (83). Pretreatment of neural grafts with caspase inhibitors and growth factors may reduce apoptosis and enhance survival; the combination may also act synergistically against PCD (83).

Oxidative stress and free radical formation also contribute to PCD. Graft treatment with antioxidants (84) and with lazaroids, compounds that inhibit the radical-mediated process of lipid peroxidation, have also been noted to improve survival (85). Neuronal injury is commonly associated with sustained elevation of intracellular calcium, and the addition of flunarizine, a calcium channel antagonist, has been shown to be protective against oxidative stress and lipid peroxidation in vitro (86).

Immunosuppression

The brain has been considered an immunologically privileged site due to the presence of the blood-brain barrier and poorly developed lymphatic system (87,88). However, some investigators have noted that the CNS is relatively immunologically responsive, and this may significantly threaten intracerebral graft survival (89–93). The presence of immune markers for microglia, macrophages, and B and T cells within the grafted region 18 months postsurgery has been reported, but the significance of this immunological response is unknown (58). In human trials, both immunosuppressed and nonimmunosuppressed patients have shown clinical benefit after transplantation. Continued benefit and increased FD uptake on PET have been observed from 6 to 12 months after withdrawal of immunosuppressive drugs (58) and up to 4 years without immunosuppression (67). The use of encapsulated cells for transplantation can provide an immunoprotective barrier (94) but allow nutrients to pass. Microcarrier beads cotransplanted with cells may provide protection by establishing a matrix for the cells to attach to and grow in a cell culture (95).

Location of Graft

Caudate vs. Putamen. Rat studies have demonstrated that dopamine in the striatal complex and nucleus accumbens subserves various types of behavior, and nigral transplants placed in various parts of the forebrain have a strong regional specificity of function (96,97). In humans, the putamen is most important for motor function (98,99) and is associated with the most extensive dopamine depletion in PD (100). The posterior or postcommissural putamen is the most crucial striatal region for nigral grafting (101). There is no clear FD-PET evidence for survival of dopaminergic grafts in the caudate, as the conditions for survival may be more favorable in the putamen (6).

Unilateral vs. Bilateral. Most studies have noted more clinical benefit with bilateral grafts, correlating with increased FD-PET uptake on both sides.

Alternative Target Areas. Grafting solely in the striatum, whether unilateral or bilateral, does not reinnervate the other structures such as the substantia nigra or subthlamic nucleus (STN). Therefore, intrastriatal transplantation fails to reconstruct the basal ganglia circuitry. One study of intrastriatal and intranigral graft implantation (double grafts) resulted in numerous TH-IR axons from the SN grafts, which reinnervated the ipsilateral striatum. This resulted in not only a greater striatal innervation, but also a faster and more complete rotational recovery to an amphetamine challenge compared to standard intrastriatal grafts (102). Similarly, one study demonstrated that intrastriatal, intranigral, and intrasubthalamic nucleus dopaminergic transplants resulted in improvement of complex sensorimotor behavior in hemiparkinsonian rats with evidence of dense TH-IR cells and neuritic outgrowth from all three grafted regions (103).

Age of Recipient

In aged rats, implanted grafts have been noted to be smaller and less effective, and neuronal survival significantly diminished (104,105) as compared to transplantation in younger rats. Symptomatic benefit was more delayed following surgery and no significant improvement in off UPDRS motor and Schwab and England scores was seen in older subjects compared to younger subjects in the double-blind controlled study after one year postsurgery (63). In contrast, some investigators found that clinical benefit did not correlate with age (72).

Alternative Cells

Human fetal neurons have been used to test "proof of principle" that neural transplantation is feasible and can provide clinical benefit. New cells are being considered for possible testing and others are in development. Greater clinical benefit is the primary goal.

Carotid Cell Bodies

Carotid body cells are derived from the neural crest and have the highest dopamine content in the body. Intrastriatal grafting of these cells in 6-OHDA lesioned rats resulted in complete disappearance of motor asymmetries and deficits in sensorimotor orientation (106). This functional recovery started within a few days postsurgery and progressively increased during the 3-month study. The behavioral effects were correlated with long-term survival of the glomus cells in the host tissue, where they retained their ability to secrete dopamine and were organized in clusters containing fibers extending outside the graft (106). There are no trials to date of carotid cell bodies transplantation in humans.

Sertoli Cells and Teratocarcinoma

Sertoli cells secrete a wide variety of nutritive, trophic, regulatory, and immunosuppressive factors. Transplantation of rat and porcine Sertoli cells survived in a normal rat brain without immunosuppression (107). Cotransplantation of these cells with dopamine fetal neural cells in parkinsonian rats improved parkinsonian features significantly and enhanced the survival and fiber outgrowth of the transplanted neurons (108).

Teratocarcinoma is a malignant tumor that contains a variety of parenchymal cell types that arise from totipotential cells and are principally found in the gonads. Neurons from human teratocarcinoma (hNT) were implanted alone or in combination with rat Sertoli cells; hNT cells cotransplanted with Sertoli cells showed increased graft survival and were associated with an increase in graft size and fewer microglia, suggesting persistent immunosuppressive effects of Sertoli cells (109). Neural transplantation of hNT into ischemic rats has been shown to produce amelioration of behavioral symptoms (110), but intrastriatal and intranigral transplantation of hNT neurons in 6-OHDA rats showed a low number of TH-IR neurons, which was not sufficient to produce significant functional recovery (111).

Porcine Cells

Intrastriatal implantation of embryonic porcine mesencephalic grafts in immunosuppressed 6-OHDA–lesioned rats resulted in a significant, sustained reversal of amphetamine-induced turning (113). Histological analyses showed graft survival and fiber outgrowth with immunosuppression (113,114).

An initial open-label study of 10 patients who received unilateral intrastriatal transplantation demonstrated no major complications. Off UPDRS scores at 12 months improved 19% and in several patients improved more than 30% (115). A double-blind, randomized, controlled study of bilateral transplantation into the caudate and putamen (with immunosuppression) demonstrated a 25% and a 22% mean improvement of off total UPDRS score in the transplant and control groups, respectively, at 18 months postsurgery ($p = 0.599$). There were no differences seen in change in off time. FD-PET showed no changes 12 months postsurgery. Based on this study, there is no evidence that porcine cell transplantation has clinical efficacy in PD patients (116). Autopsy results of one patient from the initial open-label study at 7 months demonstrated survival of 642 surviving porcine TH neurons, with extensive growth of porcine axons within the grafts and a large number of porcine axons extending from the graft sites into the host striatum (117). Immunological concerns and risk of viral transfer still needs to clarified.

Retinal Cells

Human retinal pigment cells (hRPE) are derived from the inner layer of the neural retina located between the photoreceptors and choriocapillaries (118). They also synthesize and secrete dopamine (119) and secrete several trophic factors (120). Animal studies have shown that intrastriatal implantation of these cells on microcarriers into 6-OHDA rats reduced apomorphine-induced turning (119,121) and behavioral deficits were reversed in MPTP-lesioned monkeys (122,123) with minimal host immune response (124). An open-label study of the unilateral intrastriatal transplantation of hRPE on microcarriers (Spheramine) in 6 patients without immunosuppression showed that off UPDRS motor scores improved 33% at 6 months, 42% at 9 months, and 44% at 12 months postsurgery. Follow-up at 15 months showed continued clinical efficacy with a 44% improvement in off UPDRS motor scores. Bilateral improvement was seen, with greater effect on the contralateral side. There was a 37–53% reduction in off time, and half of the patients had lower Dyskinesia Rating Scale scores than at baseline (124). Double-blind studies are underway.

Stem Cells

Stem cells (SCs) are multipotential precursor cells that have the capability to self-replicate under environmental stimulation. Various stem cell types have been isolated from mice, including adult and fetal neural SCs, lineage-restricted precursor cells, neural crest SCs, embryonic cells, embryonic carcinoma cells, and immortalized multipotent cell lines (126). Human fetal and adult neural SCs, lineage-restricted precursor cells, and embryonic cells have also been isolated (126). In vitro methods have recently been developed that allow neuronal growth and differentiation from SCs. Transplantation of these cells in rats has demonstrated that they can migrate and integrate into the neural networks and reconstitute the three neural lineages, namely neurons, astrocytes, and oligodendrocytes.

Proposed therapeutic strategies for cell replacement in PD include the use of embryonic mesencephalic progenitors (127,128), neural SCs (129–131), and engineered neural SCs ready to differentiate into dopaminergic neurons (132) and embryonic SCs (133,134) that can produce growth factors (135). Implementation and testing of these proposed strategies is limited by the poor survival of dopaminergic neurons (136). The oncogenic potential or "tumorigenesis" of SCs needs to be addressed further.

THE FUTURE

PD is a chronic, degenerative disease characterized mainly by dopamine depletion in the nigrostriatal system. Cell transplantation has the potential of restoring function in PD patients by replacing lost neurons. After two decades of research, there is much hope, but no transplantation strategy has yet been proven to provide PD patients consistent and meaningful benefit. However, the obstacles to achieving this goal have become more clearly defined. New cells are being developed and tested in animal models. Some of these are genetically modified to increase their own survival or to help protect host neurons. There is great hope that stem cells may be able to migrate to areas of injury or degeneration, transform into multiple lost cell types, and restore normal neuronal function. Transgene animal models may be helpful to predict long-term outcome following transplantation. Double-blind clinical trials have now become accepted as a means of clearly defining the safety and efficacy of transplantation.

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