

State-of-the-Art Review

The Future of Cell-Based Transplantation Therapies for Neurodegenerative Disorders

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ABSTRACT

Parkinson's disease is a common neurodegenerative disease with a lifetime incidence of 2.5% and a prevalence of at least 2% in individuals over 70 years old. Patients can be effectively treated with drugs that target the dopaminergic nigro-striatal pathway, but over time the efficacy of these medications is limited by the development of profound motor fluctuations and dyskinesias. This has prompted the search for alternative treatments, including the use of cell replacement therapies. Over the last decade, human fetal nigral transplants have demonstrated that dopaminergic neurons can survive and provide clinical benefit for patients with Parkinson's disease. However, there are clearly ethical concerns and a limit to the supply of this tissue as well as more recently anxieties over side effects. As a result, alternative sources of tissue have been investigated, and one such source are stem cells, which provide an attractive renewable tissue supply. In this review, we will discuss the current state-of-the-art and the characteristics of Parkinson's disease that increase its attraction as a target of stem cell therapy against results of current clinical trials using fetal neural grafts. Then we will discuss the various types and sources of stem cells, and some early transplantation results in animal models of Parkinson's disease. Finally we will discuss the prospect of using stem cells to deliver drugs and neurotrophic factors involved in neuroprotective and neuroreparative strategies in Parkinson's disease and other neurodegenerative conditions.

INTRODUCTION

STEM CELL-BASED THERAPIES for neurological disorders such as Parkinson's disease (PD) promise to provide potentially curative treatments for this and other progressive and debilitating conditions. PD patients can be effectively treated symptomatically with drugs, but over time the efficacy of these medications is limited by the development of a number of complications, including motor fluctuations and dyskinesias. At this stage of disease, other therapies are often required, including deep brain stimulation (DBS). However, all of these treatments are only symptomatic and do little to halt or reverse dis-

ease progression, although this has recently been challenged with dopamine agonists in early disease (1,2). Therapies that actually cure the patients of PD are still not available, but the use of neurotrophic factors such as glial cell-line derived neurotrophic factor (GDNF) (3) and cell-based therapies offer exciting possibilities. It is this latter area of therapy that we will explore in this review.

Over the last 10 to 15 years human fetal nigral transplants in patients with PD have demonstrated that dopaminergic neurons can survive and provide clinical benefit to some patients (4). However, there are ethical concerns and a limit to the supply of this tissue, as well

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as more recent concerns over side effects (5). As a result, alternative sources of tissue have been investigated, of which one is stem cells, cells that are capable of division and differentiation.

RATIONALE AND RESULTS OF NEURAL TRANSPLANTATION IN PD

PD is a common neurodegenerative condition that tends to present late in life and is characterized by the presence of a resting tremor, rigidity, and bradykinesia with varying degrees of cognitive, autonomic, and psychiatric abnormalities.

The cells that predominately degenerate in PD are the dopaminergic neurons, which have their cell body located in the substantia nigra pars compacta (SNpc) and send axons to the caudate and putamen (collectively known as the striatum). The progressive loss of these cells results in a gradual decrease over time of striatal dopamine levels, which in turn produces a decrease in striatal output to the thalamus and thus in cortical motor output. This can account for some of the observed motor symptoms, especially bradykinesia and rigidity, but other features such as tremor probably have a largely nondopaminergic component (6). Nevertheless, successful drug therapies have been shown to activate the dopaminergic nigrostriatal network and thus the aim of current neurotransplantation approaches is to implant dopaminergic cells into the striatum where they can restore dopamine levels back to normal and hopefully alleviate symptoms.

However, the dopaminergic cells in the SNpc that project mainly to the striatum also send axons to the olfactory bulb, medial olfactory nuclei, amygdala, hippocampus, subthalamic nucleus, locus coeruleus, and pyriform cortex (7). In addition, pathology is seen elsewhere in the CNS such as the olfactory bulb, dorsal IX/X motor nucleus, raphe nucleus, and locus coeruleus prior to significant cell loss in the substantia nigra (8). In later stages of the disease, the basal nucleus of Meynert, CA2 region of the hippocampus, and the cortex can be affected (8) as well as areas outside of the central nervous system (9). Therefore, both the loss of dopamine in other regions of the brain and pathology in structures other than the SNpc may ultimately limit the therapeutic efficacy of solely transplanting dopaminergic cells into the striatum.

Nevertheless, this has been the approach adopted to date with most of the cell-based clinical trials using dopaminergic neurons derived from the mesencephalon of 6- to 8-week-old human embryos that are ectopically transplanted into the patients' striatum (4,10). A recent meta-analysis of 11 studies reported that high levels of recovery were identified on most outcome measures (11), although it should be stressed that many of the measures were subjective clinical evaluations in open-label studies

where the examiner and patients were not blind to the condition and where many sources of bias can be introduced (see Ref. 12). Therefore some improvement in scores might be attributed to unintentional experimenter bias, placebo effect, and demand characteristics. This is an important point and should be emphasized, because evidence of a strong placebo response in PD exists and is as high as 59% in some drug studies (reviewed in Ref. 13). In addition, even "objective" measures of striatal dopamine levels using positron emission tomography (PET) scans show an increase in DA levels (up to 28% in one PD patient) due to a placebo effect (14), although such changes are only seen acutely whereas successful grafts have shown increased dopamine (^{18}F -fluorodopa) over 10 years after implantation. Therefore, while the results of some open-label trials have been dramatic (15), the beneficial changes observed from pre- to post-transplantation cannot strictly be said to be attributable to the effect of the transplant. As a result, double-blind placebo-controlled trials have been undertaken (5,16). The results of the first of these studies were less dramatic in terms of demonstrating graft efficacy, but there were significant methodological differences between this study and the previous open-label ones, which makes comparisons difficult. For example, less fetal tissue was grafted, there was prolonged storage time from harvest to implantation, and no immunosuppression was administered in the grafted patients. The authors reported no improvement in the transplant group from baseline in their primary outcome measure, which was a subjective global rating of improvement or deterioration at 1 year post-grafting; nor was there a significant difference in the total Unified Parkinson's Disease Rating Scale (UPDRS) score ("off" medication) between the transplanted group and sham-operated controls. However, there was a significant difference between groups in UPDRS motor score ("off" medication) with the transplant group showing a 15% decrease, which was smaller than the improvement seen in other open-label studies. This may reflect the grafting of less dopaminergic cells as the postmortem analysis would suggest, in agreement with the fluorodopa PET studies.

The authors then divided the participants into older (>60) and younger (≤ 60) groups for subsequent analysis and showed that the younger transplanted patients had greater improvements on a number of variables than the younger sham-operated controls. It has been suggested that younger patients may therefore benefit more from such therapies (10,16), although it should be noted that, despite having similar baseline scores on the UPDRS while 'off' medication, the younger patients had a much greater improvement on the UPDRS while taking levodopa. Therefore, it might not be younger individuals per se that show greater improvement, but, rather, those that respond better to pharmacological replacement of striatal dopamine levels may also respond better to surgical re-

placement with dopaminergic cell transplants. Furthermore, only in younger patients did changes in striatal dopamine levels (as determined by a PET scan using ^{18}F -fluorodopa) show a correlation with improved UPDRS motor score (16). There was no correlation ($r = 0.01$, $p = 0.9$) in the older group, suggesting that increasing striatal dopamine levels via neural transplantation is not sufficient to improve recovery in those individuals who have a smaller response to standard levodopa therapy. The reason for these differences in responsiveness with age is not clear, but they do reflect to an extent the earlier studies using adrenal medullary grafts (17). Of more concern in this study was the development of side effects in the form of dyskinesias “off” medication (so-called “runaway dyskinesia”). About 15% of patients developed such a problem, which in a couple of cases led to further neurosurgical interventions because of their severity (18). The cause of these adverse effects is not known, but may relate to the placement of grafts into the ventral striatum as well as the selection of patients with advanced PD and pre-existing dyskinesia “on” medication. This latter suggestion gains support from the other double-blind neural transplant trial in advanced PD that has recently been published (19). This showed that significant numbers of patients also developed dyskinesias post-grafting, but again, the patients selected for this study all had advanced PD. However, this study was also disappointing in that it failed to show improvements in primary clinical outcome variables at 24 months post-transplantation. This may have been related to immune rejection of the transplant because the patients seem to deteriorate significantly once the immunosuppressive therapy was stopped at 9 months.

Another double-blind placebo-controlled study using fetal porcine ventral mesencephalic tissue not included in the above meta-analysis has been reported in abstract form (20). The primary outcome variable was UPDRS motor score in the ‘off’ state 18 months after surgery and the transplant group showed a 24.6% improvement from baseline, which was similar in magnitude to other open-label studies. However, the sham-operated controls also showed a 21.6% improvement, indicating a large placebo effect in this study. Furthermore, there were no significant differences between groups on other variables such as clinical evaluation of motor skills, investigator global evaluations, and percentage of waking hours spent in the ‘off’ state. This study was based on a previous open-label clinical trial using porcine tissue that showed a 30% improvement in total UPDRS scores from baseline in 3 individuals (21). However, there was no indication of increased dopaminergic activity in the graft as determined by PET scanning, which is not surprising given the difficulties of rejection encountered experimentally (22). The explanation for the large effects in the control and grafted groups is not known, but given the experimental status of

neural xenografts, a negative study outcome would be entirely predicted and highlights the difficulties and risks of undertaking such premature clinical trials.

To summarize, the results of a recent meta-analysis showed consistent improvements on a number of clinical outcomes using fetal dopaminergic allografts in patients with advanced PD. However, the extent to which the results truly reflect changes due to the efficacy of the treatment cannot be absolutely determined because all but one study were open-label, and a recent double-blind placebo controlled trial has further complicated the situation.

Practical and ethical concerns with using human fetal tissue, compounded recently by the development of side effects, has led to a re-evaluation of this approach and has catalyzed the search for alternative sources of cells. One option is the use of xenografts, cells transplanted from another species, of which the best characterized and favored is the pig (22, and see above). But perhaps a more attractive alternative as a means of developing a widely available cell based therapy for neurological disorders is the stem cell.

WHAT IS A STEM CELL?

Stem cells are capable of self-renewal and differentiation (23,24) and are typically defined by the tissue from which they were derived; for example, neural stem cells from the brain, hematopoietic stem cells from bone marrow, and embryonic stem cells from the inner cell mass of developing embryos, and so on. Despite having different sources, all stem cells nevertheless share these common features of self-renewal and differentiation and thus are attractive for transplantation therapies.

The ability of an undifferentiated cell to form different cell types becomes more restricted as development proceeds. A single fertilized zygote is able to divide and form all the cells of the adult organism as well as part of the placenta (trophoblast) and is referred to as being totipotent. As development continues, cells from the inner cell mass—ES cells—will eventually form all the cells in the adult organism but do not contribute to the formation of the trophoblast and are referred to as pluripotent (Fig. 1). Later in development, cells become more restricted in their fate potential and their ability to divide. They are referred to as multipotent stem cells (25) and give rise to tissue-specific progeny, which, until recently, were thought to give rise to only cells from that tissue. Progenitor (26) or transit amplifying cells (27) are those that give rise to specific cell types within a particular tissue, and in the brain, for example, this refers to neuronal progenitors and glial progenitors that give rise to neurons and glial cells, respectively. The term ‘precursor’ refers to a cell that is earlier in a development

pathway than another (26), but may not necessarily be a stem cell (and hence has been preferred to the term stem cell in some studies).

The behavior of stem cells has been described from a number of species and human stem/progenitor cells can be derived from a variety of sources such as fetuses, embryos, adult bone marrow (hematopoietic or mesenchymal stem cells; 28), the adult brain (from the subventricular zone and hippocampus; 29), the dental pulp of deciduous teeth (30), and cells derived from the umbilical cord (31). In all cases, the cells should ideally have the capacity to be generated in large enough numbers to be grafted. Once transplanted, they should not be rejected by the host and should differentiate into the desired cell type at the correct location, form axons and synapses, and integrate into the existing circuitry and by so doing provide functional benefits. They should not introduce any infectious agents or form tumors. Whereas cell replacement has been the primary objective for stem cell therapy, these cells could also be used in other ways to treat neurological disease (e.g., neurotrophic factor delivery; see below) as well as to provide a more effective method of delivering chemotherapy for gliomas.

TRANSPLANTATION OF HUMAN STEM CELLS IN ANIMAL MODELS

Embryonic stem cells

Human embryonic stem (ES) cells, when transplanted directly into the brain, can form teratomas, which are solid tumors made up of cells from all three germ layers (32). To avoid this, ES cells can be first manipulated in vitro to direct them down a neuronal lineage or a subset of cells can be selected using fluorescence-activated cell sorting (FACS). Using these approaches, neural precursor cells (NPCs) derived from ES cells have been transplanted into the lateral ventricles of mice and rats and have been shown to incorporate into many areas of the brain and differentiate into neurons, astrocytes, and oligodendrocytes (33,34). The transplanted cells migrate from the subventricular zone to the olfactory bulb along the rostral migratory stream, indicating that these cells can respond to cues in the host environment and travel along established migratory pathways in the neonatal or fetal brain, whereas mouse ES cells can be made to differentiate into dopaminergic neurons using a cocktail of developmentally relevant factors; however, this has not been demonstrated yet with human ES cells. (35) Encouragingly, these ES-derived dopaminergic cells had electrophysiological properties of dopaminergic neurons in vivo and mediated functional behavioral recovery in an animal model of PD. Furthermore, they did not form teratomas, in contrast to other studies that have used these same types of cells (36).

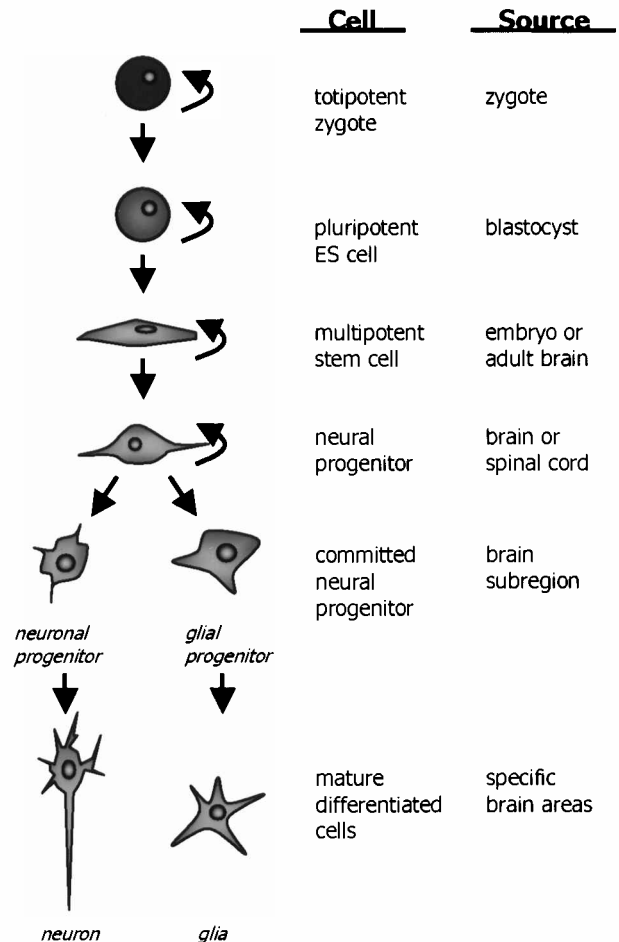


FIG. 1. Development, potential, and hierarchical organization of stem cells in the nervous system. Reprinted with permission from Gage FH. (2000). Mammalian neural stem cells. *Science* 287: 1433–1438 (ref. 68). Copyright ©2000 American Association for the Advancement of Science.

Neural stem cells

Neural stem cells (NSCs) can be derived from embryos as well as from the human adult brain after death, but in the latter case do not appear to give rise to many neurons (37) and have not yet been transplanted in animal models. NSCs have also been obtained from human fetal brains or late-stage embryos, and when transplanted into the permissive neonatal rodents' brain are able to engraft, migrate, and differentiate into neurons (38–42), although similar but less dramatic results are seen in adult rat brains (43). Human NSCs can also differentiate into tyrosine hydroxylase-expressing neurons when transplanted into MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-lesioned mice, but these cells were few in number and there was evidence of ongoing graft rejection (44). In addition, when transplanted in to a rat model of Huntington's disease, human NSCs can differentiate to express markers of

mature striatal neurons (DARPP-32) and project diffusely throughout the host brain (45).

Taken together, the above animal studies demonstrate that embryonic human neural stem cells can be expanded in culture, selected for specific markers, survive transplantation, migrate throughout the host brain, and differentiate into region-specific cell types.

Other sources of cells

Cells of a nonneuronal origin (excluding ES cells) have been transplanted in the expectation that they may transdifferentiate into neurons. However, although some success has been shown using a variety of cell sources, most notably bone marrow stromal cells (46), the issue of whether this represents true transdifferentiation or simply cell fusion is unresolved (47,48). Furthermore, the number of cells capable of this transformation is small and thus its therapeutic value questioned.

More recently, we have used a variety of porcine NPCs derived from different regions of the developing brain including the ventral mesencephalon (VM) to try and promote functional recovery though dopaminergic differentiation in rat models of PD. Although showing graft survival and differentiation into neurons and astrocytes with cell migration and axonal outgrowth, these studies have failed to show significant dopaminergic differentiation (49,50).

Many of these therapies remain a long way from the clinic, however early attempts with autologous neural precursor cells have been undertaken in some centers (51), although in the opinion of these authors such clinical studies are premature.

OTHER THERAPEUTIC USES FOR STEM CELLS

As well as replacing cells lost to a degenerative process, stem cells and, in particular, neural stem/progenitor cells could potentially be used to deliver therapeutic substances such as neurotrophic factors, in the case of neurodegenerative disorders. The aim is to modify the cells to express the protein of interest using *ex vivo* gene therapy and then transplant them into the desired location where they would produce the protein or peptide of interest in a regulated fashion.

In terms of neurotrophic factors, it is well recognized that neurons require adequate tropic support for their growth, development, and maintenance. Substances such as glial cell line-derived neurotrophic factor (GDNF) have been shown to reduce cell death in dopaminergic neurons *in vitro* (52), to promote graft survival *in vivo* (53), to be reduced in the substantia nigra in patients with PD (54), infusion of GDNF directly into the brain parenchyma via a minipump promoted structural and func-

tional recovery in a primate model of PD (55), and more recently in patients with PD (3). Brain-derived neurotrophic factor (BDNF) prevents striatal cell death in excitotoxic models of Huntington's disease (HD) (56,57), and individuals with HD have decreased striatal levels of BDNF compared to age-matched controls (58). Similar findings are seen with ciliary neurotrophic factor (CNTF) in primate models of HD (59), which has lead to a pilot study involving the use of CNTF delivered via an encapsulated polymer system in patients with mild to moderate HD (60). The use of stem cells engineered to release such factors may prove beneficial for the treatment of these neurological disorders.

Stem cells have also been considered for a range of other neurological problems, for example, to deliver chemotherapeutic agents to brain tumors. Aboody et al. demonstrated that transplanted neural stem cells preferentially migrate toward experimentally induced gliomas *in vivo*, and that when these cells are transfected with the cytosine deaminase gene, which has antimetabolic activity, they decreased the size of the tumor (61). Another study transfected mouse neural progenitor cells with interleukin (IL)-4, injected them into gliomas of mice and rats, and found that a significant proportion of injected animals survived at 90 days compared to noninjected controls (62). However, injecting the control, nontransfected progenitor cells also caused a smaller but significant increase in survival at 90 days.

In addition, certain neurological disorders caused by single gene deficits such as Tay-Sachs and mucopolysaccharidosis type VII (MPS VII) might be amenable to replacement by cell-based approaches. Both are lysosomal storage disorders and caused by a deficiency in the β -hexosaminidase α -subunit gene and the β -glucuronidase gene, respectively. Neural cells lines constructed to produce the human β -hexosaminidase α -subunit and transplanted into the brains of newborn mice were shown to produce therapeutic levels of the protein throughout the brain (63). In addition, neural progenitors carrying the β -glucuronidase gene transplanted into the lateral ventricle of newborn mice were engrafted along the neuraxis, expressed the protein, and corrected lysosomal storage in neurons and glia of affected mice (64). Finally, stem cells have been used in the treatment of stroke (65), and have been considered as treatments for multiple sclerosis (66), and spinal trauma (67), as well as a range of other neurological conditions.

CONCLUSION

Over the last 20 years, cell-based therapies for the treatment of neurological disorders have moved from the lab to the clinic. In particular, PD has been used as the prototypical disorder given its core pathological deficit of dopaminergic cell loss. Therefore, this disease has

been subject to a range of transplant strategies, of which the most successful to date has involved the transplantation of human fetal dopaminergic VM tissue into the striatum. However, this approach is fraught with practical and ethical difficulties as well as the development of side-effects in some studies. As a result, alternative sources of cells have been sought, including stem cells that offer great hope. Indeed, stem cells have the potential to provide treatments for many neurological conditions through a variety of different mechanisms, but care must be taken in translating lab-based experimental results into the clinic because premature human trials could be damaging to both the patients and the field of cell-based therapies in general.

ACKNOWLEDGMENTS

This work was supported by the Natural Sciences and Engineering Research Council of Canada and ORS Award to S.E.L. The authors' own work was supported by MRC, PDS, and the Wellcome Trust.

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Received September 8, 2003; accepted September 23, 2003.