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Neural transplantation for treatment of Parkinson's disease

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Neural transplantation has emerged as an efficacious experimental treatment for CNS disorders, especially Parkinson's disease. However, logistical and ethical issues impede large-scale clinical trials. To this end, alternatives to human fetal cells as donor cell grafts have been examined, including xenografts, stem cells, genetically engineered cells, immortalized cell lines, or paraneural cells that secrete specific neurotrophic or growth factors. Accumulating evidence also suggests that exogenous treatment with neurotrophic or growth factors, immunosuppressants, free radical scavengers, and anti-apoptotic agents can enhance survival and functional effects of the grafts. This article will review recent studies demonstrating the potential of these alternative cell graft sources and novel drugs for treating Parkinson's disease.

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▼ Neurological disorders characterized by degeneration of the basal ganglia, such as Parkinson's disease (PD), have attracted extensive research interest. Although PD largely results from nigrostriatal dopamine deficiency and is primarily characterized by motor symptoms (rigidity, bradykinesia and resting tremor), secondary brain degenerations (e.g. deterioration of motor cortical area) and cognitive dysfunctions are also hallmarks of the disease. Because of the pathophysiological features of PD, the responsiveness of the symptoms to dopamine treatment and the anatomy of basal ganglia, positive outcomes from laboratory studies of embryonic dopamine cell grafts in parkinsonian animals have become the cornerstone for proceeding with clinical trials of neural transplantation therapy.

Cellular transplantation was introduced in the clinic 15 years ago. To date, >300 PD patients around the world have received neural transplantation of embryonic ventral mesencephalic (VM) tissue. Clinical improvement and increased F-DOPA-PET [6-(18F)fluoro-L-dopa positron emission tomography] correlated with graft survival and host reinnervation in two bilaterally transplanted PD patients [1]. *In vivo* dopamine release was visualized (using [^{11}C]-raclopride PET) from nigral transplants in a PD patient that received the grafts ten years earlier [2].

The most recent report on transplanted PD patients involved using a double-blind, placebo control group [3]. The authors report that human fetal dopaminergic transplants produced age-dependent clinical improvement. Notably, some transplanted patients displayed dyskinesias and facial dystonias. The occurrence of dyskinesias in these patients was initially attributed to transplantation of too many dopamine cells. However, comparisons of Freed's study with the data from other clinical trials (Thomas B. Freeman, University of South Florida, Tampa, Florida, USA; Looe Lindrall, University of Lund, Lund, Sweden) suggest that other factors might have precipitated such dyskinesias (Table 1). Data on another double-blind study (University of South Florida) are forthcoming and should provide definitive measures of efficacy of neural transplantation therapy in PD.

The need for highly viable donor cells and grafts

Fetal cells remain the most widely studied graft source for transplantation. Unfortunately, ethical and logistical issues hinder the use of primary fetal cells in the clinic. Notwithstanding these obstacles, transplanted PD patients have been observed to display modest-to-good clinical improvement. Such variable improvements are partly a result of the variable viability of human fetal tissue. Although improving technical procedures (i.e. collection, dissection, storage and preparation of the fetal tissue) can help to assure successful clinical outcome, the logistics (e.g.

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ample supply of disease-free and homogenous dopaminergic cells) remain suboptimal for the clinical use of human fetal tissue. Thus, a search for non-primary fetal graft sources has become a major research endeavor in neural transplantation therapy.

In demonstrating the potential of alternative donor cells for transplantation, the safety and efficacy of these novel cells should be critically evaluated. For safety issues, the primary concern is the tumorigenicity of the grafted cells. In the laboratory, tumor formation has not been observed in transplanted animals; however, long-term characterization of the graft should be carried out to fully verify whether these grafted cells have indeed attained a 'post-mitotic' feature (i.e. a neuronal phenotype). For efficacy issues, the evaluation of long-term functional recovery should be demonstrated with accompanying good cell survival. When both safety and efficacy criteria have been achieved, only then should such novel cells be explored for clinical applications.

Table 1. Colorado University and University of South Florida transplant procedures

Variable	Colorado	Florida
Mean grafted dopaminergic cells	40,000–60,000	80,000–135,000
Mean neurite outgrowth	2 mm	2.5–7.0 mm
Cell storage time pre-transplant	One week or more	24–48 h
Immunosuppression	None	Short- to long-term
Stereotactic approach	Frontal pass	Dorsoventral pass
Notable observation	Increased dyskinesia	Decreased dyskinesia

Alternatives to fetal cell grafts

Porcine xenografts

Porcine brain tissue is a major source of xenografted material for neural transplantation because of the pig's brain cell size compared with that of humans, and the large litter size provides an ample supply of donor cells. In addition, the ability to rear pigs in captivity under controlled conditions greatly reduces the risk of infectious diseases. However, a major obstacle with using pig neurons as a graft source is with minimizing xenograft rejection. Indeed, even with systemic immunosuppression treatment, many xenografted porcine brain cells are rejected in rodents [4].

Manipulating the cellular components of the porcine xenografts can decrease their immunogenicity. Neural stem-cell mitogens can be used to expand precursor cells and reduce the immunogenicity of porcine xenografts [5]. Alternatively, pretreatment with antibodies against the α-galactosyl epitope and complement can remove microglial cells, therefore, reducing the immunogenicity of porcine tissues [6]. The transplantation of these expanded neural precursor cells or antibody-pretreated porcine cells into hemiparkinsonian rats enhanced graft survival and functional recovery [5,6].

In the clinic (Phase I), 12 million porcine fetal VM cells and 24 million porcine fetal striatal cells have been transplanted in PD and Huntington's disease (HD) patients, respectively [7]. Of note, ten transplanted PD patients displayed clinical improvement (>30%) when evaluated at 12-months post-transplantation. Examination of the brain from a patient who died seven months after transplantation revealed some surviving porcine fetal VM cells. In the 12 transplanted HD patients, although there was no change in total functional capacity score at one-year post-transplantation, no adverse effects were observed.

The possibility exists that porcine endogenous retroviruses can promote *in vivo* infection following transplantation. Although there is no documented evidence of *in vivo* infection in xenotransplanted patients, long-term monitoring for infection and/or toxicity needs to be performed to address this safety concern. Thus, to advance the therapeutic use of porcine xenografts in clinical neural transplantation, studies should address minimizing the host-immune-mediated rejection and managing the risk of retroviral infections (Table 2).

Autologous grafts

Adrenal cells Adrenal medulla tissues have the capacity to secrete dopamine and this feature justified their use as an alternative graft source to fetal dopaminergic cells. The first clinical trials of neural transplantation, performed by Swedish neurosurgeons in 1982, used adrenal medullary tissues as a graft source. Although significant clinical benefit in PD patients that received adrenal medullary grafts was reported by a Mexican group [8], such a procedure could not be replicated and was abruptly abandoned. The failure to replicate this study is primarily because of the low survival rate of adrenal tissues following transplantation. Recent methods have been developed to enhance survival of adrenal chromaffin cells, such as treatment with nerve growth factor [9], pre-seeding onto microcarrier beads [10] and using a 'ferromagnetic' technique to remove non-chromaffin endothelial cells [11]. In the clinic, Date and colleagues [9] have demonstrated that adrenal medullary tissues displayed an enhanced survival when co-grafted with peripheral nerve tissues in PD patients.

Table 2. Non-primary fetal cell graft sources

Alternative donor	Clinical trials?	Additional research
Porcine xenografts	Yes, for PD and HD	Reducing immunogenicity and managing viral infections
Adrenal cells	Yes, for PD	Enhancing long-term graft survival validating clinical data
Kidney cells	No, only rodent data	Establishing clinical source and examining regenerative effects
Sertoli cells	Yes, for diabetes mellitus	Establishing clinical source
Carotid cells	No, only rodent and monkey data	Monitoring long-term survival and functional effects
Encapsulated cells	Yes, for PD, cancer pain and ALS	Validating clinical data
Cell lines, engineered or immortalized cells	Yes, NT2N cells for stroke	Validating clinical data (tumorigenicity), optimizing timing and route of delivery
Stem cells	No	Regulating multipotentiality and plasticity of cells

Abbreviations: ALS, amyotrophic lateral sclerosis; HD, Huntington's disease; PD, Parkinson's disease.

The major challenge in the clinical application of adrenal medulla intracerebral transplantation is the accurate assessment of the clinical outcome of such transplantation therapy. To date, Mexican and Japanese neurosurgeons have been the only major groups aggressively pursuing such adrenal medullary transplantation for PD patients, with better clinical outcomes reported. In addition, there is still debate on the long-term survival of the adrenal medulla in the brain; new methods such as the ones noted above might enhance the survival, as well as functional effects, of these adrenal medullary grafts.

Kidney cells The exogenous application of the transforming growth factor-β (TGF-β) family of proteins, in particular glial-cell-line derived neurotrophic factor (GDNF), is neuroprotective in many models of neurological disorders. Because these proteins do not readily cross the blood–brain barrier (BBB), transplanting a tissue source that already contains a variety of these proteins might provide more beneficial effects. Fetal kidney tissues express high levels of GDNF, as well as other TGF-β neurotrophic factors [12]. Co-transplantation of fetal kidney tissues and fetal VM neurons produced enhanced fiber outgrowth from VM grafts to the lesioned striatum, and promoted more robust behavioral recovery compared with transplantation of the fetal dopaminergic neurons alone [13]. Transplantation of fetal kidney tissues alone, even without the co-transplanted dopaminergic neurons, is also neuroprotective in parkinsonian rats [14]. Similarly, transplantation of fetal kidney tissues protects against stroke-induced neurological deficits in rats [15]. The secretion of trophic factors by the transplanted kidney cells, most probably GDNF, is suggested as the underlying mechanism for such neuroprotection.

The use of fetal kidneys as the graft source might encounter the same controversy that now surrounds the use of fetal tissue in the clinic. One way to minimize the difficulties associated with the use of fetal kidney tissue is to establish kidney cell lines that possess the same beneficial characteristics as the primary cells. Alternatively, xenografts of porcine fetal kidneys should be explored. In addition, although the trophic factor-mediated neuroprotective mechanism seems likely, a more in-depth examination is needed (e.g. exposing the fetal kidneys to antibodies against trophic factors). Finally, the functional effects of fetal kidney grafts in PD and stroke animal models were evaluated in a 'neuroprotective' paradigm, indicating the need for 'neuroregenerative' assessment to better demonstrate the clinical relevance of such treatment.

Sertoli cells Testis-derived Sertoli cells have been used to provide localized immunosuppression in transplantation of islet cells for type I diabetes mellitus [16]; preliminary clinical trials are under way in Mexico and Canada. Sertoli cells also possess trophic factor properties [17]. The transplantation of Sertoli cells alone or combined with bovine adrenal chromaffin cells has been shown to ameliorate parkinsonian symptoms in rats [18,19]. The degree of dopamine depletion influences such beneficial effects of Sertoli cells, in that only lesioned animals that displayed 70% or less reduction of tyrosine hydroxylase (TH, the rate limiting enzyme for dopamine synthesis) in the nigra were responsive to Sertoli cell grafts [20].

The clinical source of Sertoli cells remains to be established. Because the procurement of prepubertial human Sertoli cells could encounter problems similar to the use of fetal human tissue, alternative sources should be explored such as porcine xenografts or immortalized Sertoli cells, or autologous banking of prepubertal Sertoli cells. Additional laboratory studies should demonstrate Sertoli cell-mediated immunosuppression and trophic-factor support to the graft.

Carotid glands Glomus cells from the carotid-body function as chemosensors that monitor changes in the level of

oxygen in the blood and stimulate the medulla to elicit an adjustment in breathing rate. Because of these novel features of glomus cells, they have the ability to survive and secrete dopamine in hypoxic conditions. Accordingly, glomus cells might survive better than fetal cells when transplanted into a lesioned brain. Indeed, >60% of grafted rat glomus cells survive in the lesioned rat brain for up to three-months post-transplantation [21]. Interestingly, the dopaminergic population in the glomus cells can be expanded *ex vivo* by treating the cells with basic fibroblast growth factor (bFGF) [22]. Because an increased number of dopaminergic cells might correlate with functional recovery in PD, the high number of grafted dopaminergic cells could be a crucial factor for the success of transplantation. Of note, the robust survival of TH-positive grafted autologous glomus cells appears to mediate significant improvement in fine motor abilities of transplanted parkinsonian monkeys [23].

The use of carotid-body cell aggregates needs replication from other research teams because both preclinical (rodent and primate) studies came from the same research team [21,23]. A scenario similar to the initial use of adrenal cells can be avoided by participation from other transplant groups in the clinical trials. The long-term characterization of cell survival and functional effects should also be monitored in subsequent studies.

Encapsulated cells The encapsulation of cells within polymer membranes before transplantation provides a means of achieving continuous, site-specific delivery of therapeutic molecules to the CNS [24]. Dopamine-secreting cells, such as adrenal medulla tissues, can be encapsulated and subsequently transplanted directly into the brain. Because the cells are not directly exposed to the host tissue and the polymers used are often biocompatible, they elicit minimal host immune response thereby improving cell survival. Transplanted encapsulated PC12 cells (derived from rat pheochromocytoma) have been shown to survive for up to six months, and these released dopamine into the surrounding host striatum, and improved behavioral function in parkinsonian rodents and primates [25].

Some disadvantages accompany the encapsulation method, such as the physical barrier preventing cells from integrating into host tissue, the extent of dopamine diffusion from the polymer membranes, and the duration and consistency of cell viability and device potency. Nonetheless, the use of encapsulated cells for neural transplantation has undergone extensive testing for safety and efficacy. The technology is ready for limited clinical trials, but because it is company-owned, it would be best to get an unbiased transplant team to conduct the clinical trials. Preliminary trials of encapsulated dopamine-secreting cells for PD patients are now being undertaken in Japan. Phase I clinical trials were reported for the treatment of amyotrophic lateral sclerosis and chronic cancer pain [26,27].

All of the autologous graft sources discussed here share the burden of proving that dopamine replacement therapy is possible without striatal innervation. Indeed, with appropriate adjustments of drug regimen, a large number of PD patients can be maintained on systemic L-DOPA for extremely long periods of time [28]. This would suggest that a means of continuous site-specific delivery of dopamine might be sufficient enough even in the absence of proper striatal innervation. Autologous grafts might function as such; however, this hypothesis remains untested and needs to be examined in the laboratory.

Cell lines, genetically modified or engineered cells and immortalized cells

Cell transplantation, neurotrophic factor treatment and gene therapy appear to complement each other by optimizing each other's therapeutic benefits. For example, to achieve a sustained therapeutic effect from the application of a gene product to affected regions of the CNS, a continuous secretion of the gene product might be necessary and this could be accomplished by the transplantation of cells that are genetically engineered to express the therapeutic protein (i.e. trophic factor) of interest.

Cell lines

Although such a prolonged release of proteins that promote neuron survival could be achieved by transplantation of primary fetal cells, the use of cell lines would be associated with less controversy. This is because the cell line is always readily available; the cells can be maintained in culture indefinitely, and because of their clonal nature, are uniform and well-defined. Accordingly, there is considerable interest in establishing and characterizing neuronal cell lines, and especially in identifying a human neuronal cell line for clinical application.

One human cell line, called NT2N, is derived from embryonal teratocarcinoma [29]. The exposure of parent carcinoma cells to retinoic acid and mitotic inhibitors results in almost pure populations of NT2N neurons that are terminally differentiated [29]. Mature NT2N neurons are virtually indistinguishable from terminally differentiated post-mitotic, embryonic neurons.

NT2N cells have been transplanted in animal models of PD and HD. In NT2N-transplanted PD rats, TH immunoreactivity was observed in grafted neurons, but these animals displayed no significant functional recovery [30]. It was suggested that the low number of TH-positive neurons was not sufficient to produce behavioral effects. By contrast,

partial functional recovery was noted in transplanted HD rats [31]. Additional studies are needed to optimize graft survival and the behavioral effects of NT2N cells.

Phase I clinical trials of NT2N cells have been performed in patients with stable basal ganglia stroke [32]. Evaluations (12–18 months) of the patients revealed no adverse cell-related serologic or PET imaging effects, suggesting that transplantation of NT2N cells is feasible in patients with motor infarction. However, the efficacy of NT2N cells remains to be fully evaluated. In addition to the trial being open-labeled, most of the stroke assessment parameters used in the study revealed that the transplanted patients were not statistically improved compared with baseline data. Pending the results of long-term monitoring of these patients, which should reveal any toxic effects of NT2N transplants, additional trials should be cautiously planned.

Immortalized cells

Dopamine neurons can be immortalized by inserting the large T-antigen gene of the SV40 virus into the cells [33]. Characterization of a clone of rat fetal VM neurons, called 1RB3AN27, revealed that they contained TH and dopamine transporter proteins. Transplanted 1RB3AN27 cells into PD rats produced functional recovery with differentiated immortalized neurons being more effective than undifferentiated ones. Although these cells were reported to be non-tumorigenic and non-immunogenic, the mechanism for regulating immortalization of these cells needs further examination.

A more promising type of immortalized cells are the conditionally immortalized neuroepithelial stem cell lines, called MHP36, in which the immortalizing gene is down-regulated upon transplantation into a host brain [34]. The 'immortal' property of these cells is thus autoregulated once transplanted, assuring that there is no aberrant cell division that might lead to tumor formation. Interestingly, the eventual neuronal phenotype of the transplanted MHP36 cell line appears to be dictated largely by the damaged host microenvironment. For clinical application, a few laboratories have initiated the immortalization of human-derived cells. Additional studies should design approaches that can fully control immortalization and subsequent neuronal differentiation of grafted immortalized cells.

Gene therapy

Gene therapy for the treatment of PD involves vectors that are engineered with the GDNF gene and subsequently injected into the brain. The specific brain location of gene delivery in PD animal models shows that both intranigral [35] and intrastriatal [36] gene delivery techniques are effective in protecting dopaminergic neurons. Alternatively, instead of direct gene infusion, intracerebral transplantation of genetically modified cells engineered to secrete GDNF has been shown to ameliorate parkinsonian deficits [37]. Because these grafted cells can survive over a prolonged period of time, continuous GDNF secretion in the brain can be accomplished without the need for multiple infusions. Gene therapy or transplantation of genetically engineered cells to secrete GDNF thus shows promise in rodent models of PD.

In primate models of PD, gene therapy has also been demonstrated to promote protection of dopaminergic neurons. Lentiviral delivery of GDNF into the striatum and substantia nigra of parkinsonian monkeys produced behavioral normalization and rescue of nigrostriatal dopaminergic pathways [38]. Because the treatment intervention in this study was initiated during the early onset of dopaminergic depletion, the clinical application of this study might be limited. The next step is to examine the efficacy of GDNF gene therapy in monkeys with stable parkinsonian symptoms.

Stem cells

Neural stem cells are the most recent addition to the list of novel cells that possess potential clinical use for transplantation. These alternative graft sources appear to avoid host immune responses and their ready availability and 'multipotentiality' are just a few of their potential advantages over primary fetal tissues. Transplanted neural stem cells isolated from the fetal rat VM produced functional recovery in PD rats [39,40]. Human neural stem cell lines are currently under investigation, with novel sources including bone marrow and umbilical cord blood. Accumulating evidence shows that dopamine neurons can be generated from such cells; however, so far the generated neurons have survived poorly after transplantation in animals [41].

In establishing stem cells as an alternative graft source, logistical, ethical and, recently, political issues need to be resolved. There is disagreement over the feasibility of 'adult' stem cells compared with embryonic stem (ES) cells. Adult stem cells might be capable of developing into only a limited number of cell types, whereas ES cells have the ability to form any fully differentiated cell of the body and exhibit remarkable long-term proliferative potential, providing the possibility for unlimited expansion in culture. Because of these novel properties of ES cells, they could potentially provide an infinite source of different tissue types for many neurological disorders. However, ES cells could retain their mitotic ability after transplantation, which could give rise to tumors. Accordingly, the limited plasticity of adult stem cells might be advantageous in

Table 3. Graft-survival enhancing drugs

Pharmacologic agent	Clinical trials?	Additional research
Neurotrophic factors	Yes, for PD	Enhancing timing and route of delivery
Immunosuppressants	Yes, for PD and MS	Enhancing drug delivery and minimizing side effects
Free radical scavengers	Yes, for PD	Validating clinical data
Anti-apoptotic drugs	Yes, for PD	Validating clinical data

Abbreviations: MS, multiple sclerosis; PD, Parkinson's disease.

terms of controlling their mitotic ability after transplantation. Furthermore, adult stem cells will not be subject to the ethical concerns that surround the use of fetal tissues, including the ES cells. Thus, safety and efficacy issues on the use of stem cells include the following questions: Do they maintain long-term stable neuronal phenotypes following transplantation? Are these neuronal phenotypes crucial for rescuing the degenerating brain? Are transplanted stem cells functional and thus able to provide beneficial effects?

The novel feature of stem cells (i.e. their multipotentiality) can be considered a double-edged sword: providing an unlimited supply of cells, but with a risk of tumorigenicity after transplantation. This multipotential property of stem cells needs to be fully investigated in the laboratory before embarking on any clinical applications.

Graft survival-enhancing drugs

The rationale for using drugs that will improve the viability of donor cells and/or transplanted cells is that such pharmacological treatment will result in a better functional outcome. The drugs presented below are potential therapeutic agents that could enhance both pre-transplant and post-transplant viability, as well as functional effects of the grafts. Either a multiple drug treatment regimen or finding the most effective single drug treatment could improve the functional outcome of neural transplantation therapy (Table 3).

Neurotrophic factors

Intracerebral infusion of GDNF protects against, and rescues the nigrostriatal dopaminergic pathway from, 6-hydroxydopamine- (6-OHDA) or 1-methyl-4-phenylpyridinium (MPTP)-induced neurotoxicity [42,43]. These neuroprotective effects of GDNF are accompanied by attenuation of hemiparkinsonian behavioral symptoms [42,43]. This preclinical demonstration of the efficacy of GDNF in animal models of PD has prompted small clinical trials of such therapy in patients.

However, the use of trophic factors to increase the viability of donor cells appears to be limited to pretreating the cells before transplantation because these molecules do not easily cross the BBB. A limited post-transplantation window might exist, however, for effective systemic delivery of these trophic factors into the brain, because the BBB remains permeable for a period of 8–12 days after transplantation. The same logistical issue accompanies the other drugs mentioned hereafter. For enhancing post-transplant survival and functional effects, a method to transiently permeate the BBB to deliver such drugs needs to be developed.

In a PD patient who received monthly intraventricular injections of GDNF, parkinsonian symptoms continued to worsen [44]. These observations support the contention that GDNF might only be efficacious when initiated in early stage PD. Moreover, in this GDNF-treated patient, there was no evidence of restoration of nigrostriatal neurons and no indication of intraparenchymal diffusion of the GDNF to relevant brain regions. These findings suggest that the route of delivery (i.e. intraventricular) of GDNF is not an effective approach. Future studies should address the timing and route of GDNF delivery in relation to PD progression.

Immunosuppressants

The most widely used immunosuppressant drug therapy in neural transplantation is cyclosporin-A (CsA), which is a fungal metabolite that inhibits T-cell proliferation. Immunosuppression of transplant recipients has greatly improved graft survival. Xenografting of human fetal VM cells to parkinsonian rats combined with long-term adjunct immunosuppressive therapy results in the survival of sixfold more dopaminergic neurons in the graft compared with non-immunosuppressed rats [45]. In recent years, immunosuppressants have been found to have neurotrophic and neuroprotective properties in addition to their immunosuppressive effects. Indeed, the administration of CsA alone enhanced TH immunoreactivity in normal rats, as well as in parkinsonian rats [46,47]. To cross the BBB, a high and chronic dosage of CsA might be needed to promote the neuroprotective effects. Unfortunately, high dosage and chronic CsA injection produces negative side effects such as nephrotoxicity and hallucination. To avoid immunosuppressive side effects, analogs called neuroimmunophilins (which are devoid of immunosuppressive property) have been developed, and showed positive

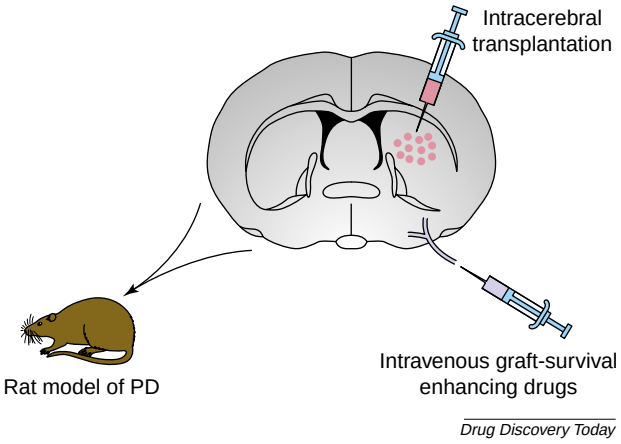

Figure 1. Combined intracerebral transplantation of non-primary fetal cells and intravenous injection of graft-survival enhancing drugs.

results in PD animal models [48,49]. Disagreement exists, however, on whether totally eliminating this immunosuppressive feature actually diminishes the neuroprotective effects of immunosuppressants. Because immunosuppressants have been used in the clinic for many years now, their additional use as neuroprotective agents can be tested expeditiously, with careful monitoring of potential side effects.

Free-radical scavengers

Oxidative stress, characterized by increased free-radical damage, has been documented in post-mortem PD brains [50], suggesting a potential therapeutic benefit from treatment with free-radical scavengers (e.g. deprenyl, 7-nitroindazole, iron chelator and vitamin E). Laboratory data suggest that oxidative stress influences graft survival. For example, PD animals that received fetal dopaminergic cells harvested from transgenic animals that over-expressed copper and zinc superoxide dismutase (a powerful catalytic enzyme for antioxidation), exhibited robust graft survival and functional recovery compared with those that received donor cells from wildtypes [51]. Similarly, a free-radical scavenger has been demonstrated to enhance fetal dopamine graft survival and behavioral effects [52].

Clinical data on the use of free radical scavengers are not conclusive. The advent of new and more potent antioxidants might reveal enhanced benefits for PD. Careful consideration should be given to effective brain delivery of the drugs, as well as toxicity monitoring. In addition, identifying the onset of free radical accumulation during the disease process could provide clues on when to initiate free radical scavenger treatment.

Anti-apoptotic agents

Apoptosis or programmed cell death has been implicated in PD etiology [53]. The occurrence of apoptosis in PD appears to be disease stage-dependent because apoptosis-positive nuclei were consistently found in brain samples from late-onset PD, but not in samples from young-onset patients [54]. The potential of anti-apoptotic agents to protect against dopaminergic depletion has been studied in animal models of PD. Transgenic mice with a specific gene modified to enhance anti-apoptotic function are resistant to dopaminergic lesions [55,56]. Furthermore, inhibitors of the pro-apoptotic caspase have been shown to reduce neurotoxin-induced dopamine depletion [57] and to increase the survival of grafted dopaminergic neurons [58–60].

Apoptosis accompanies the normal process of aging. In the clinic, therefore, interfering with apoptosis can represent an intervention that could retard not only specific disease progression, but also the aging process. The timing of aberrant apoptosis in PD should be determined to optimize the initiation of anti-apoptotic treatment. At present, it might be safe to use such anti-apoptotic drugs for pre-treating donor cells to increase grafted cell viability. However, whether this translates to improved functional outcome should be investigated.

At this time, it is difficult to make direct comparisons among neurotrophic factors, immunosuppressants, free-radical scavengers and anti-apoptotic agents as efficacious drugs for neural transplantation. Some views, however, can be expressed about two of these drugs. Although GDNF has been demonstrated to be ineffective in one PD patient [44], the efficacy of combined neural transplant and GDNF remains to be determined. Also, although Freed and colleagues [3] claim that immunosuppression might not be crucial for neural transplantation therapy, most of the transplanted PD patients enrolled in other centers that exhibited clinical improvement have received at least short-term immunosuppression. There is a potential for adjunctive use of these graft survival-enhancing drugs in neural transplantation therapy (Fig. 1). Future studies should characterize the optimal dose that results in enhanced graft survival accompanied with improved functional outcome. Single or combined drug regimens can be initiated before transplantation by pretreating the donor cells. Finding ways to permeate the BBB, thereby allowing the entry of compounds into the brain at a later stage post-transplantation will further advance the utility of these adjunctive pharmacological agents for neural transplantation therapy.

Summary

Alternative graft sources and novel drugs have been tested in the laboratory setting with successful results, and a large

number of therapeutic approaches have been explored for their clinical application. These strategies either protect against or rescue from neuronal cell death. Both protective and reparative therapies could be combined to enhance treatment for PD. Transplantation therapy holds considerable promise as a therapeutic regimen for PD, but several technical issues need to be optimized. The advent of an alternative graft source might circumvent the logistical and ethical issues surrounding the use of fetal cells for neural transplantation therapy. The use of novel drugs, alone or in combination with neural transplantation, might also promote enhanced therapeutic efficacy against neurodegeneration. Technical issues on long-term gene expression, regulation and dose control are equally important and need to be addressed. These issues, however, should be resolved using the 'post-lesion therapy' paradigm. Such an approach, which closely resembles the clinical progression of the disease, is the most logical way in validating the efficacy of such treatment intervention. All the approaches listed here will remain unproven delivery systems unless they are validated in a clinically relevant fashion.

In summary, accumulating evidence suggests the potential of a variety of therapeutic approaches to treat basal ganglia disorders, specifically PD. Indeed, several of these strategies have progressed to clinical trials. However, these approaches remain experimental therapies that would require rigorous research efforts and limited clinical trials to validate their potential for treatment of PD.

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