The challenges of administering cell-based therapies to patients with Parkinson’s disease
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Although the concept of cell-based therapy for Parkinson’s disease has been around for more than three decades with proof-of-concept studies in man having been achieved, it still remains a controversial experimental therapy. In this review, we discuss the reasons for this and the challenges that this approach generates in the treatment of Parkinson's disease in terms of adopting better strategies by which to develop this whole therapeutic area, an approach that is becoming more necessary as the era of stem cell therapies start to become a clinical reality. NeuroReport 00:000–000 © 2013 Wolters Kluwer Health | Lippincott Williams & Wilkins.
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Introduction
Although Parkinson’s disease (PD) is a complex and heterogeneous disorder, two important pathological hallmarks can be found in all cases: the aggregation of the protein α-synuclein into Lewy bodies and the degeneration of the nigrostriatal dopaminergic system. Although this can result from a single gene defect [1], for the majority of cases the cause is unknown. Clinically, the disease manifests itself through motor features (bradykinesia, rigidity, rest tremor and a gait disorder) [2] and a range of non-motor problems such as dysautonomia, depression and sleep disturbances [3], as well as mild cognitive impairments that can lead to dementia in a significant number of cases [4]. Current pharmacological therapies essentially strive to replace the lost dopaminergic stimulation to the striatum and work very well in the short term, but in the long term cause side effects such as dyskinesia and motor fluctuations [5]. The fact that motor symptoms and disease duration correlate with the loss of dopaminergic neurons within the substantia nigra [6,7], coupled with the efficacy of dopaminergic drugs in treating PD symptomatically, supports the hypothesis that replacing this degenerated system with new dopaminergic neurons would provide long-lasting motor benefits, whilst not curing it. Although proof-of-concept studies for this experimental therapy have been conducted, many challenges need to be overcome to allow any cell-based therapy to be taken to the clinic as a routine therapy in PD. These challenges will be discussed throughout this focused review.

The biological concepts behind cell replacement therapy for Parkinson's disease and the challenges they present
In the adult brain, it is estimated that 75% of all dopaminergic neurons reside in the ventral midbrain (VM) [8,9]. During embryogenesis, VM dopaminergic neurons arise from the floor plate region of the mesencephalon [10] and give birth to three different dopaminergic neuronal subtypes: A8, A9 and A10. It is the A9 subtype that constitutes the substantia nigra pars compacta, which projects to the caudate/putamen and which is preferentially targeted in early PD [11,12]. The molecular mechanism underlying the functional diversity of these neuronal subtypes is not well understood, nor their selective vulnerability to disease processes such as PD.

The nigrostriatal dopaminergic neurons projecting to the striatum form synapses with the medium spiny output neurons in a highly arborized fashion [13]. These nigral dopaminergic neurons have a pacemaker firing pattern that obviously determines how they release dopamine at the target site – a characteristic that cannot be replicated by the current oral and enteral dopaminergic replacement therapies and which may underlie some of the long-term complications of these therapies. One way of better trying to recapitulate this physiological release of dopamine is to use nigral dopaminergic cells grafted into the striatum.

Currently, a clinical diagnosis of PD can only be made at relatively late stages of dopaminergic terminal denervation in the putamen, because it is only at this stage that the system can no longer compensate and motor features emerge. However, within a few years (typically 4–5) of diagnosis, the loss of putaminal dopamine is complete [14] and as this evolves there is evidence of significant local oxidative stress and inflammation. This is important because for any cell therapy to be successful in PD, grafted neurons placed into the striatum need to survive this environment and then integrate well into the circuitry, a process that may take years. Linked to this is the age of the recipient of the graft as there may be
a reduction in the trophic support capacity of the central nervous system with aging [15,16], which may help explain why younger patients in receipt of human foetal ventral mesencephalon (hfVM) grafts have done better than older recipients.

In this respect, hfVM transplants have now been shown to give motor improvements in PD patients for more than 10 years, accompanied with restoration of normal dopamine tone in the grafted striatum [17]. However, whether such favourable results will be seen with transplants using less well-differentiated dopaminergic neuroblasts from stem cell sources remain to be seen.

This is especially pertinent, given that of late it has been shown that pathological forms of α-synuclein – Lewy bodies and Lewy neurites – can be found in grafts of hfVM in patients [18–20]. This suggests that grafted cells in the PD striatum can be affected in a similar manner to the host nigral dopaminergic neurons, possibly through a process that involves the prion-like spread of α-synuclein from the host into the graft. This has now been explored experimentally where it was demonstrated that the transfer of α-synuclein from the host to the graft can occur [21]. What drives this process is unknown but it does suggest that any cell transplant placed into the striatum will achieve some degree of α-synuclein pathology over time [18]. It is therefore essential that this process is better understood as it will impact ultimately on this whole field of therapeutics. However, this having been said, the long-term survival and functional benefits of hfVM grafts have been shown in some patients.

The cells being considered for grafting in Parkinson’s disease and the challenges with using them

Foetal ventral midbrain tissue

The ideal cell for replacement therapy in PD is the human A9 nigral neuron, which is the dopaminergic subtype that is believed to provide maximal innervation and motor benefits [22] – and the obvious source of such cells would be hfVM. The rationale behind the use of this tissue is that it contains postmitotic cells, which are already committed to the dopaminergic phenotypic fate and with this establish functional synapses in the striatal transplant site. Although this tissue has been used with some success in the clinic ([17] and see above), not all patients have exhibited a positive outcome and, in some cases, side effects have developed including graft-induced dyskinesias (GIDs) [23–26]. Although the underlying mechanism of these dyskinesias is not well understood, it is believed that they are, at least in part, the result of the inclusion of significant numbers of serotoninergic neurons in relation to low numbers of relevant dopaminergic neurons [27]. This is supported by the beneficial effects of buspirone, a 5-HT1A agonist, on these GIDs [17,28]. In addition to these side effects there are, and have always been, problems with the usage of hfVM tissue relating to the ethical issues of its harvest from termination of pregnancies and the practical problems that large numbers of foetuses are required to generate sufficient dopaminergic neurons for a single PD patient. As such, the search has always been on for a more practical, and ideally more ethically neutral, source of cells for grafting.

Embryonic stem cells

Embryonic stem (ES) cells are derived from the inner cell mass of the embryo and have the advantage of being highly proliferative and pluripotent, which means that they can differentiate into any somatic cell type of the human body. This provides a considerable advantage, given that there is no limitation in their availability as they can be expanded and maintained in culture for long periods of time. This cell type also potentially allows for the selection of a specific cell type, unlike the case of hfVM where over 99% of the tissue grafted is the non-nigral dopaminergic cells of the developing VM.

Transplantation of dopaminergic cells derived from ES cells in a rodent model of PD has shown that they can survive, differentiate and extend processes beyond the graft core and establish synaptic contacts with the host striatum [29,30] with functional, behavioural, benefits. However, the ability to achieve this has been limited by the low efficiency in generating dopaminergic neurons and the extent to which they are truly authentic dopaminergic A9 neurons with appropriate fibre outgrowth into the grafted striatum. Nonetheless, more recent protocols using extrinsic factors to programme the cells has shown promise in generating larger numbers of what look like more authentic nigral neurons without any evidence of tumour formation [31,32]. Although these results are encouraging, the use of ES cells is still limited by an inability to generate sufficient numbers of A9 nigral dopaminergic neurons with extensive fibre outgrowth, as well as the ethical issues linked to their derivation in the first place.

Patient-specific reprogrammed cells

Given the important limitations in using foetal VM and ES cells, the use of patient-specific-derived cells has been investigated as an alternate source that would circumvent some of these issues. Two different approaches are currently being studied: the reprogramming of patients’ somatic cells (e.g. skin fibroblasts) to a pluripotent stage and a further conversion of these induced pluripotent stem (iPS) cells into dopaminergic neurons, or the direct conversion of patient-specific somatic cells into dopaminergic neurons. Although both approaches do not have the same ethical issues as ES cells and foetal tissue [33] and avoid the risk of immune rejection, they still present problems for clinical translation. These include the risk that the cells so derived are not fully reprogrammed to the right fate and that they will develop the pathology of
the host from which they originate – namely α-synuclein pathology in the dopaminergic neurons so derived. In this respect, direct reprogramming of patient-specific somatic cells might be more likely to retain disease-specific vulnerability. This remains speculative, as more studies are needed to assess this potential concern. Nonetheless, grafted iPS cells have been shown to functionally integrate into the host brain in an animal model of PD and differentiate into dopaminergic neurons with behavioural recovery [34]. However, their capacity to do this as well (on a cell-to-cell basis) as hfVM tissue is lacking and there remain concerns of uncontrolled cellular proliferation and the development in some cases of teratoma-like structures [34,35].

Transplantation of directly differentiated dopaminergic neurons in rodent models of PD has also provided some evidence of motor improvements but these cells do not seem to integrate well into the host striatum [36], perhaps because of their more mature stage when grafted. Thus, although differentiated neurons are less likely to cause teratomas, development of protocols that would allow for better graft integration and improve levels of dopaminergic differentiation are required in order for these cells to be considered as true contenders for cell replacement therapy in PD.

Finally, other challenges that will also need to be overcome before any stem cells can be used in patients with PD relate to:

1. the important variability in differentiation efficiency across cell lines [37,38];
2. the possibility of DNA mutations during the reprogramming [39,40];
3. and the epigenetic profile in the case of iPS cells, which is maintained throughout the transdifferentiation protocol and which could make the cell differentiate back to its somatic original state [38, 41–43].

**What has been shown to date with cell-based therapies for Parkinson's disease?**

The use of cell-based treatments for PD has been in the clinical arena for nearly 30 years starting with adrenal medullary transplants in 1982. However, although many different types of cells have been used for grafting in PD, the ones that have proven most successful are allografts of hfVM. Using this approach in the late 1980s/1990s, a number of open-label studies were undertaken, which showed that hfVM transplants:

1. derived from aborted foetuses aged 6–8 weeks old survived long term in the PD brain [44,45];
2. made and received connections from the host brain and restored circuitry and activation of motor cortical areas [46,47];
3. had long-term clinical benefits to patients [17].

As a result of these encouraging results, NIH funded two double-blind placebo-controlled trials [23,25], which in contrast showed:

1. that the transplants were not efficacious at least in terms of successfully achieving the primary endpoints designated in the trials;
2. that significant numbers of patients developed side effects in the form of GIDs, which were so severe in some cases that additional deep brain stimulation surgery (DBS) was required to correct it.

As a result of this, it was felt that further trials with hfVM tissue should be suspended until such time as the trial results and GIDs could be better explained. A position that has been reinforced in the opinion of some commentators by the discovery of Lewy body-like pathology in transplants – an observation which suggests that they will ultimately succumb to the disease process (see above).

Over the ensuing years, a great deal of discussion and work has gone into better understanding this disparity between the open-label and double-blind placebo-controlled trials [48]. As a result, a new trial has been planned funded in the EU (TRANSEURO) that seeks to reinvestigate this approach but which importantly is different to what has gone before in so much that:

1. it will graft younger patients early in the disease course;
2. it will use tissue that has been prepared to optimize the dopaminergic to serotoninergic ratio and by so doing minimize the risk of GIDs;
3. it will ensure that the tissue is grafted across the whole posterior putamen to avoid dopaminergic hot spots which have also been linked to GIDs [49];
4. a 3-year endpoint and 1 year of active immuno-suppression.

By doing so, it is predicted that more consistent robust results will be seen in the absence of significant GIDs. However, even if successful, this trial will not herald an era when hfVM grafts will be a mainline treatment because of the reasons stated above. Nevertheless if successful, this trial will open the way for the next generation of cell-based therapies, which almost certainly will take their origin from stem cell sources.

**What are the challenges in trials of cell-based therapies for Parkinson's disease?**

The desire to take a cell-based therapy to the clinic for PD has much to commend, given the success to date with hfVM transplants in some patients and the fact that PD has a well-defined dopaminergic pathology that responds to dopamine replacement therapy. However, there are...
a number of challenges that present themselves with the translation of such therapies to the clinic, independent of any issues to do with the quality of the cell being grafted (see above). This includes:

(1) the type of cell and the evidence that it forms authentic nigral dopaminergic neurons in sufficient numbers;
(2) the ethical implications inherent in using cells of human embryonic and foetal origin [33];
(3) the risks of using pluripotent or multipotent stem cells sources in terms of cell proliferation and mass effects in the central nervous system secondary to this postgrafting;
(4) the optimal time and type of patient for the therapy, especially as those patients most responsive to dopaminergic cell-based therapies are the very same individuals who do well with the more conventional dopaminergic drug therapies and DBS. Indeed the ideal patient may be a young patient within the first few years of diagnosis but this creates a dilemma as to whether an experimental therapy should be used in patients with most to lose and in whom benefits may take years to become significant;
(5) the number of patients and the primary endpoint of any trial especially given that the cells can take years to mature and integrate and can only be done in small numbers of patients initially because of the invasive nature of the procedure of engraftment;
(6) the design of the trial as to whether it should be open-label, double-blind placebo-controlled or trialled head to head with other therapies such as DBS [50]. Each has their own merit and obviously in a first into man trial, a small open-label study is all that can be done, but at what point does one feel the therapy is optimal and should be trialled in a more rigorous way with the risk of failure for technical, rather than therapeutic, reasons;
(7) the funding of any such trial, as the involvement of commercial enterprises will ultimately be needed if this is to be a mainline treatment. However, once involved, the design of the trial and the speed with which it is moved to bigger trials may be influenced as much by financial pressures as scientific and clinical questions.

Thus, the translation of any novel cell therapy for PD is not straightforward and requires the involvement of scientists, clinicians, statisticians, ethicists and patient advocates. Indeed this whole approach has to be seen as an interactive and iterative one, as the move from the laboratory to the clinic requires a degree of calculated guesswork (e.g. number of cells to be grafted). As such, there is a high probability that the first patients in receipt of a new generation of stem cell-based grafts will have been given suboptimal therapies, especially as safety will be a major concern and thus underdosing is a likely issue. Nevertheless, the ability to reason and design trials on the basis of the available data and educated guesswork is needed and what has to be avoided is the adoption of entrenched positions ahead of the data.

Conclusion
Although cell-based therapies for PD have not yet been optimized, important insights have been learned from preclinical studies as well as the trials that have already been undertaken. Because we know that this therapy can in theory provide substantial benefits for PD patients, at least in some cases, the overall challenge is to find a way to take advantage of the information we already have and use it to inform us as to what we need to do next. This is essential as we try to develop better therapies and build a new, improved trial approach around stem cell-derived dopaminergic neuronal-based treatments.

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Conflicts of interest
There are no conflicts of interest.

References


