Future Therapies – How Close is Tomorrow?

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Abstract

Gene therapy has the potential to treat the symptoms of Parkinson's disease (PD) or even modify disease progression, while cell-based therapeutic approaches in PD involve cell replacement through transplantation or by recruitment of endogenous adult stem cells. Gene therapy studies demonstrate approximately 30 % improvement in motor function and potentially a low incidence of dyskinesia, but the risk of operation-induced adverse effects is unknown. Currently, there is a lack of placebo-controlled and long-term data to clarify the effectiveness of these strategies, and a move away from adeno-associated viral vectors to lentiviral vectors could bring more positive results in future. Cell-based therapy data provide 'proof-of-principle' in PD, but ethical and scientific considerations restrict the use of standard intrastriatal cell replacement strategies that use primary cells or tissue. Stem cells and induced pluripotent stem cells are promising cell sources for autologous cell replacement, and integration of transplants into the substantia nigra may be important for long-term survival, stability and functional outcome. In summary, we are a long way from achieving PD-modifying gene therapy, but symptomatic gene therapy may be a realistic option in the near future (Phase II trials are ongoing). Cell-based therapies have already been proved-in-principle, but many unanswered questions exist.

For several decades, levodopa-based treatments have been the mainstay of Parkinson's disease (PD) therapy. However, current treatments do not prevent disease progression and there is no cure. In an attempt to find disease-modifying treatments, research into biotechnological treatment approaches such as gene and (stem) cell-based therapy, is gaining momentum in the field of PD. Gene therapy has the potential to restore dopamine synthesis capacity in the striatum or modulate basal ganglia circuitries. In addition to these symptomatic therapies, gene therapy has potential for disease modification, with the delivery of neurotrophic factors. In addition to gene therapy approaches, neurotrophic factors could potentially be delivered by intracerebral (putaminal) infusion. Major stem cell-based therapeutic approaches in PD are cell replacement of diseased dopaminergic neurons through transplantation (restorative approach) or by recruitment of endogenous adult stem cells (regenerative approach).[1]

Gene Therapy

To date, only one virus (recombinant adeno-associated virus serotype 2 [AAV2]) has been approved by the US Food and Drug Administration (FDA) for use in the treatment of brain diseases. Although the virus can be safely employed in applications involving the human brain, one methodological disadvantage is that only one gene can be introduced per vector (two or more vectors are required to treat the patient with more than one gene). Furthermore, surgical infusion using a stereotactic procedure that is similar to deep brain stimulation (DBS) is required for viral vector delivery into the brain.[2] Ongoing developments to address the aforementioned technological issue include novel AAV (serotype 5) vectors that are able to incorporate larger genes, and lentiviral vectors that have a higher capacity for more than one gene. Lentiviral vectors have undergone safety testing in non-human primates with positive results,[3] but clinical data on their efficacy and safety in PD or other brain diseases are lacking.

Clinical data now exist for all three gene therapy strategies listed earlier (restoration of dopamine synthesis, modulation of basal ganglia circuitries and disease modification).

Restoring Dopamine Synthesis

Three different strategies have been proposed for restoration of dopamine synthesis capacity (see *Figure 1*): dopamine replacement

Figure 1: Strategies for the Restoration of Dopamine Synthesis Capacity[4]

Dopamine replacement

Continuous levodopa delivery

Pro-drug approach for dopamine production

- Three or novel not approved vectors needed
- Ectopic dopamine production possible (dyskinesia? neurodegeneration?)
- No clinical data yet

- Two or novel not approved vectors needed
- Continuous levodopa levels in putamen (no dyskinesia?)
- Encouraging preclinical data
- No clinical data yet

- See text for details

5HT = 5-hydroxytryptamine; AADC = aromatic L-amino acid decarboxylase; BH$_4$ = tetrahydrobiopterin ; DA = dopamine; DOPA = dihydroxyphenylalanine; GCH1 = GTP cyclohydrolase I; L-DOPA = levodopa; TH = tyrosine hydrolase; Tyr = tyrosine. Copyright (2009) with permission from Elsevier.

(relies on the introduction of three genes that will result in the production of dopamine); continuous levodopa delivery (intrastriatal gene transfer of the dopamine-synthetic enzyme tyrosine hydrolase [TH] combined with the exogenous administration of the cofactor for TH, tetrahydrobiopterin, or with co-expression of its rate-limiting synthetic enzyme, GTP cyclohydrolase 1); and a pro-drug approach for dopamine production (introduce one gene encoding the enzyme aromatic L-amino acid decarboxylase [AADC] to metabolise exogenous levodopa to dopamine).[4] Across all strategies, the viral vectors are implanted or injected into the striatum to induce the production of proteins solely in the striatal neurons (not the dopaminergic neurons). The dopamine replacement strategy may confer a risk of dyskinesia or enhanced neurodegeneration due to excess dopamine, whereas in the continuous levodopa delivery approach, levodopa is metabolised to dopamine, and therefore, there is less likelihood of dyskinesia.

To date, clinical data exist only for one of the three possible dopamine replacement strategies; that is, the pro-drug approach. A recently published open-label, non-placebo-controlled clinical trial evaluated the safety and tolerability of AADC expression (introduced via AAV2) in 10 patients with PD (five received a high dose and five received a low dose).[5] The results revealed an approximately 30 % improvement in motor score (total Unified Parkinson's Disease Rating Scale [UPDRS] and UPDRS III) both off and on medication and no relevant induction in dyskinesia.[5] In a subgroup of patients (n=5), positron emission tomography imaging confirmed expression of the AADC enzyme six months post-introduction in the high- and low-dose patient cohorts.[6] Although the gene therapy was generally well tolerated, a safety concern was the high frequency of operation-induced adverse events, primarily three reports of intracranial haemorrhage (30 % of patients) and self-limited headache.[5] It remains unclear whether the risk of intracranial bleeding is higher with gene therapy than with other neurosurgical procedures such as DBS.

Clearly, the efficacy and safety of the three strategies for dopamine replacement via gene therapy require further clinical studies.

Modulating Basal Ganglia Circuitries

The gene therapy strategy of modulating basal ganglia circuitries involves the delivery of the glutamic acid decarboxylase (GAD) gene into the subthalamic nucleus via the AAV2 vector. The resulting increase in the GAD enzyme results in increased levels of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) within the subthalamic nucleus. GABA inhibits activity in this region, thereby reproducing the effects of STN-DBS (and restoring normal physiological function to the basal ganglia circuitry). Results were favourable in a Phase I safety trial using this gene therapy in 12 patients with PD.[7] Significant improvements in UPDRS scores were observed at three months and these persisted until 12 months after surgery. There was a substantial reduction in thalamic metabolism that was restricted to the treated hemisphere, and there was a correlation between motor scores and brain metabolism.[7] No gene therapy-related adverse events were observed. Results from an ongoing Phase II trial are expected in 2011.

Disease Modification with Gene Therapy

Two trials, to date, have evaluated the gene therapy approach of disease modification by neurotrophic factor delivery (AAV2-associated introduction of neurturin [CERE-120]) into the putamen. This approach aims to promote sprouting from the remaining dopaminergic neurons and to slow disease progression by reducing cell death. A Phase I safety trial involved 12 patients with PD, and results showed no clinically significant adverse events, and a 36 % improvement in UPDRS III score and increased 'on' time without troublesome dyskinesia.[8] However, this study was followed by a placebo-controlled, double-blind Phase II trial of 58 patients with PD, which failed to reach the pre-defined clinical endpoint of symptomatic relief after 12 months' follow-up (published data are awaited). In light of the observed preliminary findings from these disease modification studies, points for consideration are:

- Are the results from pre-clinical studies translatable to humans? Are the right animal models being used?

- Was the wrong endpoint evaluated in the Phase II trial (the factors introduced were associated with disease modification rather than symptomatic relief)?
- Was the neurotrophic factor applied too late in the course of the disease?
- Are longer term data needed?

However, these data are similar to that observed with glial-derived neurotrophic factor delivery studies using continuous intraputaminal minipump infusion.[9–12]

Summary on Gene Therapy

In gene therapy strategies, safe viral vectors are available from which stable and functional protein expression is feasible. Across strategies, studies demonstrate approximately 30 % improvement in motor function and potentially a low incidence of dyskinesia, but the risk of operation-induced adverse effects is unknown. Currently, there is a lack of placebo-controlled and long-term data to clarify the effectiveness of these strategies. However, as recently cited, ''…the number of promising gene therapy studies in progress [for PD] is encouraging''.[2]

Cell-based Therapy

Restorative Stem-cell Approach

Two double-blind, placebo-controlled trials serve as the clinical basis for the application of the restorative approach in PD.[13,14] The strategy used in these studies was the heterotropic transplantation method, which involved the introduction of the dopaminergic cells into the striatum (target area) as opposed to the place of origin. 'Proof-of-principle' for this approach was demonstrated by observations revealing integration and functioning of the transplanted cells as dopaminergic neurons and consequent partial clinical improvement in a subpopulation of patients. In contrast to the beneficial effects, it is apparent that there are serious problems associated with the cell transplantation approach including severe motor side effects (dyskinesia), rapid expansion of the PD pathology into the transplant including the observation that α-synuclein-positive Lewy bodies propagate from host to graft cells (raising the issue that PD has a prion-like component),[15,16] high tissue consumption with the procedure and ethical concerns regarding the use of embryonic tissue. Thus, there is a need for better cell sources and a more effective approach for introducing the cells into the target region so as to enable them to integrate into the circuitries of the basal ganglia. Developments that may increase the potential of restorative stem cell transplantation are better cell sources and an approach that ensures integration of the transplant into the circuitries of the basal ganglia.

There are many sources available from which to produce stem cells and subsequently the dopaminergic neurons that are required for cell replacement strategies in PD. Research groups have produced functional neurons from adult bone marrow, but failed to produce dopaminergic neurons[17] (interestingly, bone marrow stromal cells have been used to deliver genes encoding neurotrophic factors to the brain in rodent models[18]). Similarly, neural stem cells have been produced from epileptic surgery tissue, but as yet efforts to differentiate these into dopaminergic neurons have failed.[19,20] However, the use of adult adrenal gland tissue to produce functional dopaminergic neurons has met with significant success,[21] and efforts are underway to transplant these cells into PD animal models.

The other potential approach to the generation of stem cells that can be used to produce dopaminergic neurons, is through the development of induced pluripotent stem cells (iPSCs), which resemble embryonic stem cells. A variety of transcription factors are used for the generation of iPSCs, including oncogenes,[22] which results in a high risk of tumour formation. This risk is circumvented by restricting the factors to a single transcription factor, octamer-binding transcription factor 4 (Oct 4), using neural stem cells as the source (as they express all the other factors that are required for successful reprogramming) and using recombinant protein factors (to avoid genetic manipulation of the cells). This protocol was employed in our research group to induce reprogramming of neural stem cells and produce functional dopaminergic neurons, which when transplanted into severe combined immune deficiency (SCID) mice did not induce any tumour formation. It is evident that generation of functional dopaminergic neurons from human Oct-induced iPSCs is possible and can be achieved without viral vectors. Therefore, iPSCs are a promising cell source for autologous transplantation approaches in PD, but as yet there are no clinical data or pre-clinical data in primates.

A further potential source of cells is foetal brain tissue, but only the cells derived from mid-brain tissue are able to differentiate into functional dopaminergic neurons (the most complicated cell type to be produced from stem cells). By adapting the protocol to reduce the atmospheric oxygen tension *in vitro* to 3 % (to replicate physiological levels in the foetal mid-brain), it has been possible to show differentiation of functional dopaminergic neurons that partially reconstitute the dopaminergic pathway.[23–29] However, this strategy fails to induce mature dopaminergic neurons after transplantation in the striatum.[24] Thus, low phenotypic stability of the transplanted cells over the long-term poses a major challenge to the development of stem cell transplantation strategies in PD.

These limitations could potentially be overcome by using an orthotropic transplantation technique, where the cells are transplanted into the basal ganglia circuitry in order to provide these neurons with the stimulatory factors that they need to maintain their dopaminergic capacity. A new approach is to transplant neurons derived from iPSCs into the substantia nigra and incorporate optimised innovative biomaterial (derived from polyethylene glycol) between this area of the brain and the striatum with the aim of stimulating the projection of the transplanted neuronal axons from the substantia nigra to the striatum. Preliminary data indicate the ability of biomaterials to guide human stem cell axonal outgrowth.[30,31] These findings lend support to a tissue-engineering approach involving a combination of innovative biomaterials and autologous stem-cell-based methods to reconstruct the dopaminergic nigrostriatal pathway in PD.

Regenerative Approach

Endogenous regeneration is an interesting concept, but the use of this strategy is hindered by the fact that adult neurogenesis is restricted to two brain regions – the hippocampus and subventricular zone of the lateral ventricles – both of which are located at a distance from the mid-brain. These observations give rise to questions about whether adult neurogenesis exists in the mid-brain and whether there are neural stem or precursor cells with dopaminergic potential within the adult mid-brain. Neurogenesis was not identified in the mid-brain in investigations in healthy animals and PD animal models.[32] However, there is evidence to suggest the existence of quiescent stem cells in the

mid-brain region, which have been shown to differentiate into functional dopaminergic neurons in culture.[33] Quantitative cell culture showed that 0.3 % of cells in the peri-ventricular region of the lateral ventricle and 0.1 % of cells in the mid-brain region (in the fourth ventricle) were neural stem cells.[32]

Summary on Cell-based Therapy

Controlled clinical data exist to provide 'proof-of-principle' for cell replacement methods in PD, but ethical and scientific limitations restrict the use of standard intrastriatal cell replacement strategies using primary cells or tissue. Stem cells and iPSCs, in particular, are promising cell sources for autologous cell replacement, but the risk of tumour formation (and other safety issues) and the phenotype stability of transplants require elucidation. There is evidence to suggest that integration of transplants into the substantia nigra may be important for long-term survival, stability and functional outcome.

Summary and Conclusions

Referring back to the title of this article, tomorrow is some distance away for achieving disease-modifying gene therapy given the problems in translating data from animal models to the clinical situation, the lack of positive clinical data and the methodological limitations associated with designing suitable studies to accurately measure disease-modification. However, tomorrow is closer for symptomatic gene therapy because safe and suitable viral vectors are available, there are preliminary clinical data supporting 'proof-of-principle' and safety, and controlled Phase II trials are ongoing. Although such data exist for cell-based therapies, there are significant problems associated with heterotopic transplantation of primary dopaminergic cells or tissue. However, this area of research is not devoid of hope as stem cell-based autologous neural tissue engineering will eventually help to reinforce cell-based therapies, potentially by reconstructing the dopaminergic nigrostriatal system in PD. ■

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