

Models of repair mechanisms for future treatment modalities of Parkinson's disease

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ABSTRACT: Parkinson's disease is one of the most likely neurological disorders to be fully treatable by drugs and new therapeutic modalities. The age-dependent and multifactorial nature of its pathogenesis allows for many strategies of intervention and repair. Most data indicate that the selectively vulnerable dopaminergic neurons in the substantia nigra of patients that have developed Parkinson's disease can be modified by protective and reparative therapies. First, the oxidative stress, protein abnormalities, and cellular inclusions typically seen could be dealt with by anti-oxidants, trophic factors, and proteolytic enhancements. Secondly, if the delay of degeneration is not sufficient, then immature dopamine neurons can be placed in the parkinsonian brain by transplantation. Such neurons can be derived from stem cell sources or even stimulated to repair from endogenous stem cells. Novel molecular and cellular treatments provide new tools to prevent and alleviate Parkinson's disease. © 2002 Elsevier Science Inc.

KEY WORDS: Oxidative stress, Protein damage, Cellular damage, Age, Trophic factors, Prevention, Stem cells, Neurological diseases.

INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disease characterized clinically by bradykinesia, rigidity, and tremor at rest. The majority of motor abnormalities are associated with a specific loss of dopamine (DA) neurons in the substantia nigra pars compacta (SNc) and the secondary depletion of striatal DA levels [1]. While the loss of striatal DA correlates with the severity of clinical disability, clinical manifestations of PD are not apparent until 80–85% of SNc neurons have degenerated and striatal DA levels are depleted by 60–80% [98]. Administration of L-DOPA, the precursor of DA, initially relieves parkinsonian motor signs, but its long-term use is associated with severe fluctuations in drug response. Because pathologic changes precede the manifestation of clinical symptoms, it is reasonable to develop strategies to protect remaining DA neurons during the subclinical stage.

Clinical abnormalities and post-mortem findings in PD patients have led to various explanations for the observed DA cell loss [112,113]. Several lines of evidence suggest excitotoxicity coupled with a decline in mitochondrial energy metabolism as the cause of SNc DA degeneration [9,12,21,33,59]. Exposure to the mitochondrial toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or rotenone [12] results in death of DA neurons in the SN and depletion of DA and its metabolites in the neostriatum [21,55,87]. The neurotoxicity of MPTP is mediated by its active metabolite

1-methyl-4-phenylpyridinium (MPP⁺), which inhibits mitochondrial oxidative phosphorylation [109] at complex-1 of the electron transport chain [110]. MPP⁺ toxicity can be reduced by inhibition of glutamatergic input by glutamate antagonists or decortication, indicating that excitotoxicity is necessary, if not sufficient, to kill DA neurons in this paradigm [9,134,141]. The finding that the neurochemical, anatomical, and behavioral abnormalities of MPTP-induced and rotenone-induced parkinsonism closely resemble idiopathic PD suggests there may be a common final pathway of neuronal degeneration [18–20,46,62,81,131].

Post-mortem analyses indicate increased lipid peroxidation in the SNc of PD brains, implying either an excess production of neurotoxic free radicals or a failure of the normal protective mechanisms to clear these radicals [31,32]. Several detoxifying systems may be deficient in the PD brain, including decreased catalase and peroxidase activity [6] as well as reduced glutathione free radical scavenging [76]. Additionally, post-mortem and *in vivo* studies have demonstrated mitochondrial changes in PD patients. Similar to the inhibition of mitochondrial function produced by MPTP, defects have been found in complex I in SNc [126], platelet [116], and muscle [130] mitochondria preparations from PD subjects further supporting a relationship between oxidative stress and neuronal degeneration. Moreover, recent findings have documented the genetic and pathological involvement of α -synuclein in the PD neuronal pathology [18,46,62,81,131]. Several other protein abnormalities may contribute to the typical morphological changes seen in PD brains [18,46,62,81,131].

While there are multiple causes of neurodegenerative diseases including environmental, genetic, and age-associated factors, the treatments may be directed at similar underlying mechanisms via neuroprotective or reparative interventions. In a theoretical framework, one working model of neuronal damage and the prevention of cell death is the concept of "neuronal resilience" [70]. Depending on the status of the cell with respect to pre-traumatic events and gene-expression relevant to neuronal preservation, the neuron will exist far from or close to the threshold for irreversible neuronal damage. The neuron can thus be thought of as oscillating between protected and vulnerable conditions. This model of neuronal homeostasis suggests that a number of separate therapeutic measures, including delivery of neurotrophic factors (NTFs), may reduce the overall probability of degeneration in those neuronal populations that approach their specific threshold for degeneration [70].

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POSITRON EMISSION TOMOGRAPHY AND MAGNETIC RESONANCE SPECTROSCOPY MODELING

We conducted positron emission tomography imaging studies of modulation of dopamine transporter function and magnetic resonance spectroscopy studies of neurochemicals in an idiopathic primate PD model induced by long-term, low-dose administration of MPTP [19,20]. Striated MR spectra of control primate and human showed striking similarities and the same applies for MPTP-treated primate (6 months after cessation of MPTP) and PD patient striatum (68-year-old male; Hoehn-Yahr scale II; 510 mg/d L-DOPA). The choline/creatine ratio was similar in the MPTP model and human parkinsonism, suggesting a possible glial abnormality [19,20]. The progressive degeneration of dopamine reuptake sites observed in our PD model can be expressed by a time dependent exponential equation: $N(t) = N_0 \exp(-0.072 \pm 0.016t)$, where N_0 represents intact entities (dopamine re-uptake sites before MPTP) and 0.072 per month is the rate of degeneration. When the signs of PD appear, $N(t)$ is about 0.3–0.4 times N_0 . Interestingly, this biological degenerative phenomenon has similar progression to that observed in cell survival theory [19,20]. According to this theory and calculated degeneration rate, predictive models can be produced for regeneration and protective treatments.

NEUROPROTECTION BY NEUROTROPHIC FACTOR INTERVENTIONS IN PD RESEARCH

A range of NTFs has been shown to protect neurons against a spectrum of cellular insults and may thus have potential value in the treatment of neurodegenerative disorders [101,137]. Among the mechanisms by which NTFs protect are the maintenance of calcium homeostasis and the increase of antioxidant enzyme activities [25,102]. By decreasing cellular oxidative stress, or by interfering in the cascade to cell death, NTFs can reduce neuronal vulnerability and protect against ensuing neurodegenerative processes. Classically, NTFs are secreted during specific developmental stages by target tissues in limited quantity [8] and are important determinants of neuronal development and organization, affecting innervation of target tissue as well as survival of neurons [122]. Previously, it was thought that different neuronal populations were each responsive to only a single NTF. However, evidence indicates that there is overlap and redundancy, whereby a single NTF may affect more than one cell type, and a specific cell type may respond to several NTFs [84]. Moreover, actions of NTFs are associated not only with retrograde transport from the target tissue but also autocrine and paracrine mechanisms [82,105]. The site-specific NTF expression in the adult brain suggests various mechanisms of action in relation to the observed selective neuronal trophism. NTFs are important for neuronal maintenance in the adult brain, and insufficiency of such trophic support due to decreased NTF supply or impaired target cell response may account for some of the cell death in neurodegenerative diseases [7,54].

Initial findings that FK506 has trophic capacity *in vitro* and *in vivo* [29,47,93,128] sparked an interest in development of immunophilin ligands that possess neurotrophic activities, yet are not immunosuppressive. Based upon the FKBP/FK506 complex structure, several novel small-molecule immunophilin ligands have been designed which bind the immunophilin FKBP12, yet do not interact with calcineurin, and are thus devoid of immunosuppressive activity. These non-immunosuppressive immunophilin ligands demonstrate neurotrophic activity analogous to that obtained with FK506; they potentiate the effects of NGF on PC12 cells and sensory neurons in culture by promoting neurite extension [135].

We and others have now evaluated these compounds in models relevant to PD (see below).

The effects of such non-immunosuppressive immunophilin ligands may be mediated by their binding with FKBP12 and subsequent effects on Ca^{2+} homeostasis, consistent with the understanding that an optimal Ca^{2+} concentration is involved in neurotrophic effects [72]. FKBP12 complexes with several Ca^{2+} channels ryanodine receptor [71], inositol 1,4,5-trisphosphate receptor [24], and transient changes in concentration of neuronal intracellular Ca^{2+} can trigger various processes including structural modifications, neurotransmitter release, modulation of synaptic transmission, excitotoxic cell death, and gene expression [100]. Intracellular Ca^{2+} concentrations can be altered by flux from internal stores, and distinct Ca^{2+} channels in subcellular regions of the neuron generate highly compartmentalized Ca^{2+} signaling [136], possibly contributing to the observed trophic effects.

EFFECTS OF PEPTIDERGIC NEUROTROPHIC FACTORS

The supplementation or replacement of a DA NTF may protect or slow the neuronal degeneration of PD. Several NTFs have shown trophic activity in the DA system, including brain-derived neurotrophic factor (BDNF), neurotrophin (NT)-3, NT-4/5, basic fibroblast growth factor (bFGF), transforming growth factor- β (TGF- β), and glial cell line-derived neurotrophic factor (GDNF) [63–65,79,91,103,121]. The effects of the neurotrophins BDNF [63,64,80], NT-3 [64], and NT-4/5 [64,65] as well as GDNF [91] on fetal DA neurons were first demonstrated *in vitro*. Platelet-derived growth factor (PDGF) [111], TGF- β [121], and bFGF also increase DA cell survival *in vitro*, yet the effect of bFGF is thought to be mediated by glia [37,79,103]. In addition to promoting survival of DA neurons in culture, the administration of these factors to the intact adult rat brain is associated with significant behavioral and neurochemical alterations. Supranigral delivery of BDNF enhances striatal DA turnover and decreases nigral DA turnover, as well as causes contralateral rotations and locomotor activity in amphetamine treated rats [4,99]. The chronic administration of BDNF above the SN enhances the firing rate and number of electrically active DA neurons [129]. The localization of mRNA for BDNF and its receptor, TrkB, to the SN in adult brain suggests that BDNF may maintain SNc neuronal function in the intact brain, perhaps in an autocrine or paracrine manner [5,43,64,104,127]. Also, exogenous BDNF delivered to the striatum can act on SNc DA neurons via receptor mediated retrograde transport [108].

Similar to the *in vivo* effects of BDNF, supranigral infusion of NT-4/5 results in increased striatal DA turnover and release as well as contralateral rotation following the administration of amphetamine [2], and NT-3 increases amphetamine-induced contralateral turning and decreases SN DA turnover [99]. Likewise, intranigral GDNF administration in intact adult rats increases spontaneous and amphetamine-induced locomotor behavior. These behavioral changes are associated with increased DA levels and turnover in the SN and increased DA turnover and decreased DA levels in the striatum [61]. In addition, sprouting of tyrosine hydroxylase (TH)-positive fibers near the injection site and increased striatal TH fiber staining were noted [61]. By injecting GDNF into the striatum, this factor is retrogradely transported to the SN DA neurons, suggesting that GDNF may act as a target-derived NTF [140]. These actions suggest that NTFs may be able to augment DA neuronal function in the adult brain. Of relevance to the neurodegenerative processes of PD, pretreatment of DA neurons with BDNF protects against the neurotoxic effects of MPP+ and 6-hydroxydopamine (6-OHDA) *in vitro*, perhaps by increasing levels of the antioxidant enzyme glutathione reductase [10,63,133]. NT-4/5 [65], bFGF

[115], GDNF [60], and TGF- β [85] also protect against the toxic effects of MPP+ *in vitro*.

Several DA NTFs can prevent cell death in models of neurodegeneration *in vivo*. BDNF has shown variable efficacy in protection *in vivo* lesion paradigms. In rats with partial lesions of the nigrostriatal pathway induced by striatal infusion of 6-OHDA, concomitant supranigral administration of BDNF enhances striatal DA metabolism and reverses lesion induced rotational asymmetry [3]. Yet, BDNF or NT-3 did not alter SN DA levels nor protect against the loss of striatal DA nerve terminals in this partial lesion paradigm. Initial studies using BDNF failed to show protection of DA neurons in the SNc following axotomy of the medial forebrain bundle in rat [78,88]. However, another study suggests that BDNF is neuroprotective in the axotomy paradigm [50]. BDNF-secreting fibroblasts implanted in the mesencephalon of adult rats attenuate the SNc DA cell loss caused by subsequent administration of the mitochondrial complex I inhibitor MPP+ [11], and increase DA levels in the SNc [44]. Similarly, intrastriatal grafts of BDNF-secreting fibroblasts prevent DA neuronal degeneration associated with intrastriatal administration of 6-OHDA [90]. GDNF is able to protect against SN neuronal degeneration in MPTP-treated mice [48] as well as in rats following axotomy of the medial forebrain bundle [11] or striatal 6-OHDA administration [75,124]. Interestingly, GDNF was shown not only to be neuroprotective, but also regenerative because administration following MPTP administration resulted in regeneration of TH fibers [83,139]. In addition, GDNF administration to the SN 4 weeks after partial 6-OHDA lesioning of the MFB decreased apomorphine-induced rotational asymmetry, increased SN DA and DOPAC content, and spared 10% of the SN DA neurons [16,58]. Additional *in vivo* studies have demonstrated that NT-4/5 prevents 6-OHDA denervation-induced changes in striatal neurotransmitter gene expression [125]. Continuous infusion of ciliary neurotrophic factor (CNTF) near the SN prevents DA neuronal degeneration in the SNc following transection of the nigrostriatal pathway in adult rat [51]. However, in contrast to overall cellular protection caused by GDNF and BDNF, TH expression was only slightly preserved.

CELL TRANSPLANTATION THERAPY

Cell replacement therapy seeks to replace the loss in synaptic signaling caused by the neuronal degeneration. It has been shown that fetal ventral mesencephalic neurons transplanted to the caudate/putamen of PD patients can significantly reduce the need of L-DOPA treatment and improve symptoms. However, the fetal cell grafting procedure is limited to a few centers worldwide and will not become a standard treatment until some major issues are solved. The current major problem is the use of fetal tissue that raises ethical concerns and is impracticable because tissue from several fetuses is needed due to low neuronal survival after grafting. It is also of importance to achieve increased axonal outgrowth and synaptic reinnervation from the grafted cells. Recent findings in stem cell research have indicated that stem cells might be a very potential cell donor source for cell replacement therapy. In addition, new insights into axon guidance mechanisms will provide tools for stimulating outgrowth and achieving appropriate target innervation from the grafted cells. Although it is clear that the procedure can work, the technical aspects of cell implantation and selection of appropriate patients are obstacles to a reliable clinical procedure as has been shown in recent clinical trials [42,68].

HOW CAN STEM CELL BIOLOGY RESEARCH HELP PARKINSON PATIENTS?

Most living systems undergo continuous growth [14]. We see maintained growth in adult human bodies; for example, in the bone

marrow that recruits stem cells capable of dividing into most of the cells necessary for blood and immune systems throughout life. In a more limited sense, we also see part of entire organs being regenerated, such as the liver and similarly specialized systems. Cells in the lining of the gut are shed on a daily basis with replacements growing in from cell layers below. In the skin, the basal cell layers of the dermis provide a plentiful source of growth that covers our bodies; that also signifies a continuous growth process. Even though recent cloning experiments have illustrated that each adult cell nucleus containing DNA has the material for establishing all cells of a whole organism, even a mammal; we are more used to the specialized cells mentioned above that divide to maintain or increase growth of organ systems in the body [138]. In asexual cell division and multiplicative division of micro-organisms, such as bacteria, the genetic material is identically reproduced and expressed in specifications determined by a genetic plan. At the beginning of building a multicellular body, the sexual association between the egg and sperm generates a fertilized cell that is capable of cell divisions that grow logarithmically. After the first few rounds of cell divisions, this cluster of cells (in the range of 250 cells) representing the previously fertilized egg-cell is capable of imbedding itself in the wall of the uterus in mammals [15,30]. At this stage, each of the cells in the cluster is usually capable of forming any part, or the whole of the entire body plan [56]. This type of cell is therefore denoted stem cell, or in this case, embryonic stem cells. From this initial group of stem cells, all other cells that form the living body are generated. The developmental sequential orchestration of the growth of the body into its specialized parts and unique form and function follows a strict pattern in the embryo and neonate [49,52]. Nevertheless, as previously mentioned, many cells with the potential of division and growth into specialized cell systems also remain in the adult organism. Recently, such divisible (yet non-malignant or carcinogenic) cells have gained increased attention. The idea that such multipotent cells present in the blood stream, or even in the brain, are still capable of multiple cellular fates has intrigued biologists and the public. In particular in the brain, in addition to the well-known fact that olfactory epithelium and a few other brain regions (including the dentate gyrus of the hippocampus) have dividing cells there may also be dividing cells capable of other types of growth or repair [14]. This has generated much speculation. On the other hand, more established fields of stem cell biology, such as bone marrow based analysis of the formation of blood cells has already acquired a sophisticated appreciation of such continuous cell division as a necessary condition for maintenance and adaptive function of many cellular systems.

In any event, the understanding of the control of specific cell fates as the brain develops, or as other specialized organ systems mature, is of fundamental importance in biology [52,86,120]. A new therapeutic methodology is being developed, involving fetal cell implantation to the living brain in order to replace intrinsic systems that do not spontaneously regenerate, such as the dopaminergic system [70]. This new treatment discipline has recently merged with the more recent work for obtaining potential stem cell-derived dopaminergic cells for transplantation to PD. This is of practical and therapeutic interest because the optimal cells for replacement, such as highly specialized fetal dopaminergic cells capable of repairing an entire degenerated nigro-striatal system, are hard to obtain and almost impossible to generate in sufficient numbers for a medically effective treatment or with a reliability necessary for the treatment of a large number of patients [30,70,119]. In a more limited scientific context, the concept and methodology of stem cell-derived dopamine cells intrigues both neurobiologists and clinically oriented scientists, insofar as it could both explain the biology of the developing dopamine cells and

what controls its fate, as well as generating a procedure for obtaining such cells in abundance for clinical applications.

Thus, to study the creation of dopamine neurons from embryonic stem cells to their highly specialized form in the adult brain provides a window to neurobiologists for fully understanding the health and maintenance of such cells. It follows that the new biological phenomenon illuminated by embryonic stem cell work for generation and control of genes and transcription factors regulating dopamine cells function has potential impact on many new treatment avenues for PD and related disorders. First, the PD treatment approaches potentially available through current methodologies can be viewed at several intervention levels; at which current techniques can attack the disease process, compensate or reverse its effects. Starting at the genetic level, a number of genes related to the development or control of dopaminergic identity and specialization (e.g., *Nurr-1* and sonic hedgehog protein) act in concert with transcription factors and downstream genetic activation of specific transmitter enzymes (for instance, TH and dopa-decarboxylase) in dopaminergic neurons [14,30,66]. These genes and effects are under feedback and dynamic control. Some of these proteins expressed by these genes are characteristic or even unique to the dopaminergic neuron affected in PD [14,66]. Therefore, at the molecular level, therapeutic intervention involving gene therapy and intra-cellular signaling molecules can potentially be investigated in the embryonic stem cell experimental system. The identification and understanding of the events that control the activation of a dopaminergic cell could potentially allow scientists and clinicians to “reverse engineer” the disease process, given that a deteriorating DA cell most likely will go through stages of dysfunction over months or even years in which various systems are switched off or put into a dormant or vegetative state. This could involve reversing the course of degeneration from a dysfunctional cell into a fully active neuron in a PD patient, once pathological processes have been reduced and cleared [69]. The understanding of the specific and dynamic cell biology of dopaminergic neurons would allow a more sequential strategy for treating and restoring function in PD patients. The current cell therapy tools available to pre-clinical and clinical scientists in attacking the problem of parkinsonism, and providing ways of intervening to benefit the patient, are in many ways still exploratory or in a discovery phase [69].

Therapies ranging from the traditional chemical substance-based pills (oral drugs) that substitute for lost DA by providing the (a) precursor, levodopa, or DA (b) agonists in various assortments or activations or blockade of enzymes (c) involved in the biosynthesis of DA; are currently complemented by tools involving (d) neurosurgical methods alleviating circuitry disrupted or dysfunctional by the primary loss of dopaminergic function. On the horizon are potential (e) NTs, or other substances or agents that would provide a slowing or reversal of the degenerative process itself. (f) Gene therapeutic agents could also be inserted in a molecular biological sense; to directly affect genes that are dysfunctional in a debilitated neuron (see previous discussion on transcription factors in the embryonic stem cell).

Of these methodologies, the second revolution in the understanding of treatment options for PD (the first being the discovery of L-DOPA) is neural cell transplantation, which has opened up a completely new avenue for brain repair. Instead of chemically controlling a degenerated system, this method allows reconstruction and renovation of the DA system by a new set of cells performing the normal function of those that had previously perished.

In summary, stem cell biology is valuable in a discovery process of several new or potential treatments for PD. For example, research on embryonic stem cells may demonstrate the genetic

transcription factors that control the specific genes participating in the orchestrated function and cell development. Genetic programs have dynamic components; for example, concerted actions of growth factors or NTs that act as molecular switches required for initiating and maintaining the function of a DA neuron. The knowledge of how DA neurons can be formed would allow a reasonable process to be established for industrially producing a large number of such cells. These cells could be used for effective cell transplantation or cell therapy in which needed cells could be implanted under local anesthesia to brain regions that have lost more than 60–80% of the normal human set (500,000–1,000,000 DA cells in the human brain, substantia nigra region) [69]. Finally, in trying to understand the remarkable complexity of growth of brains and bodies, the knowledge surrounding the stem cell provides a rich source for study. The stem cell biology related to brain development and repair can be approached by methods using either adult or embryonic stem cells potentially capable of generating new neurons, after selective expansion in cell culture systems or in the living brain. The investigation of embryonic stem cells also provides a system in which transcription factors responsible for directing the typical dopaminergic cell fate in the nigro-striatal systems can be determined. This allows a sophisticated understanding of the factors controlling the specialization and health of such cells. All of these investigations help clarify pathological stress, toxic events, or genetically induced cell dysfunction.

In conclusion, the rapidly developing understanding of pathological mechanisms in PD is enhanced by in-depth knowledge of the DA neuron from stem cells, via progenitor cells, to adult DA neurons. This provides an understanding of the vulnerable cell responsible for PD and how to effectively intervene to reverse this disease.

GENE THERAPY?

Recent advances in aging PD models using lentiviral transfer of GDNF [83] has created hope and interest in gene delivery (therapy) as a future treatment modality for PD. Many advancements in viral-based vectors for gene delivery to cells of the central nervous system have been achieved [17,28,41,89,96,106,114,123,142,143]. However, improvements still must be made with respect to safety and efficiency of gene transfer to neurons. What are the desired features of viral vectors in gene delivery to the brain? First, a large transgene capacity is often needed within a vector to include a gene(s) of interest and its appropriate regulators. Second, high transduction efficiency is needed to transfer a gene of interest to a population of neural cells, given the limitations in the maximal volumes that can be delivered. Third, stability of transgene expression is required in many applications, and is affected by how the transgene is maintained within the host-cell nucleus (free, episomal, or integrated), long-term regulation of promoters, and immune responses to antigenic proteins encoded in the virus or transgenes [22,23,34,35,117,145]. Fourth, the appropriate dose of transgene product can be critical [17], and inclusion of sequences within the vector that can regulate the transcription of the transgene is often required for this control [114,39,53,5792,97]. Fifth, the cell specificity of gene transfer within the nervous system (to neurons versus glia, or to more specific phenotypes of each) will depend on cell-specific promoters [73,77,118,132], expression of viral vector-specific receptors [107], or route of axonal transport of the vector in the brain [13,27,94]. Finally, for clinical application of viral vector-mediated gene transfer, lack of both toxicity and inflammatory immune response is essential [36,38,41,74,95,144].

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