

Neuroprotective Therapy in Parkinson's Disease: Current Status and New Directions from Experimental and Genetic Clues

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Despite successful treatment of Parkinson's disease (PD) with a wide variety of symptomatic therapy, the disease continues to progress and drug-resistance symptoms become the predominant factors producing the disability of PD patients. Neuroprotective therapies have been tested, but clinically effective drugs have not been found yet. New insights gained from studies of genetic forms of PD point to the common pathogenic mechanisms that have been suspected in sporadic forms of the disease and may provide new approaches for the future neuroprotective therapies.

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INTRODUCTION

Parkinson's disease (PD) is a progressive motor disorder caused by the neurodegeneration involving many brainstem nuclei with preponderance of dopaminergic neuronal loss in the *substantia nigra pars compacta* (SNpc). PD affects every 1:1,000 to 1:10,000 individuals, with an average age of onset of 55 and markedly increased prevalence and incidence with advancing age¹ making it the second most common neurodegenerative disease after Alzheimer's disease. Pathological hallmarks of a PD brain are the degeneration of dopaminergic neurons in the midbrain and the presence of cytoplasmic inclusions called Lewy bodies (LB). Although post-mortem studies reveal the loss of SNc neurons, patients diagnosed with PD do not show symptoms until the threshold of SNc neuronal loss

is reached, about 50-70%.² The clinical symptoms dramatically impair the patients' quality of life, including motor symptoms such as bradykinesia, resting tremor, rigidity and gait abnormalities, and non-motor symptoms such as dementia and depression. Symptomatic treatment of PD by dopaminergic medications is one of the most successfully therapies for neurodegenerative disorders. However, development of the motor response complications and medication-resistant symptoms ultimately limits the symptomatic therapy. Therefore, recent intense interests in neuroprotective therapy hope to stop the progression of the disease process. So far the neuroprotective therapies have been elusive, in part complicated by overshadowing symptomatic effect that obscures discernable neuroprotective effect.

The purpose of this article is to review the current status and understand the rationale and potential future

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directions of neuroprotective therapy in PD. We will discuss the insights that we have gained from studying the pathogenesis of PD, ranging from potential environmental factors to genetic studies. This is followed by a discourse of how the potential pathogenic players are linked together by their cell biological function and interaction with intrinsic factors inherent in dopaminergic neurons. We will focus our consideration of clinical therapeutic agents in this review to those that are designed to interfere with pathogenesis of PD, in line with our discussions on the etiology and pathogenesis of PD.

PATHOGENESIS OF PD

Age is the biggest risk factor for PD, and yet, how senescence contributes to the pathogenesis remains enigmatic. The sporadic form accounts for most cases and the genetic component has been only recently appreciated.³ Although less than 5-10% of PD cases are familial, the convergence of implicated mechanisms from genetic mutations and environmental factors has vastly facilitated the studies on PD pathogenesis. The etiology of PD in most cases probably cannot be explained by a single cause, but by a combination of genetic susceptibility and environmental insult. A popular theory implicates to the role of oxidative stress and mitochondrial dysfunction. This is supported by the discovery of toxins that damage dopaminergic neurons selectively and display affinity to disrupt mitochondrial functions and generate reactive oxygen species (ROS). Another theory suggests the role of protein misfolding and aggregation, lending support from the formation of Lewy bodies and the abnormal accumulation of α synuclein. Lastly, the selective loss of dopaminergic neurons poses an interesting hypothesis that dopamine itself can contribute to toxicity in PD. After all, normal metabolism of dopamine increases level of intracellular ROS. The overall effect of these varying pathways is to increase vulnerability of dopaminergic neurons in SNpc.

1. Environmental factors

Epidemiological studies indicate both factors that increase and those that decrease the risk of developing PD. Exposures to pesticide, rural living, farming, and drinking well water augment the risk of developing PD.⁴ In cellular and animal models, these chemicals and related compounds produce experimental PD and recapitulate the selective vulnerability of nigrostriatal dopaminergic neuron. The main effects of various farming chemicals underscore a pathogenic theme: inhibition of mitochondrial electron transport chain and increased levels of ROS.

MPTP is a highly lipophilic synthetic neurotoxin that gets oxidized by monoamine oxidase B (MAO-B) into MPP⁺ in the brain. MPP⁺ enters dopaminergic neurons via high affinity binding to the dopamine transporter (DAT), as well as norepinephrine and serotonin transporters.^{5,6} Once inside the neuron, MPP⁺ can translocate into vesicles by vesicular monoamine transporter (VMAT),⁷ concentrate within the mitochondria,⁸ or remain in the cytosol.⁹ The ratio of DAT to VMAT expression level is thought to determine the selectivity of MPP⁺ in damaging dopaminergic neurons, where VMAT acts to sequester MPP⁺ from harmful interaction within the cells. Thus, a cell type with higher DAT to VMAT ratio is more vulnerable to MPP⁺ toxicity than a cell type with a lower DAT to VMAT ratio.¹⁰ MPP⁺ binds to and inhibits mitochondrial complex I.¹¹ The inhibition of complex I activities leads to enhanced ROS generation, significant ATP depletion, and apoptosis.¹² MPTP sounds like a magic bullet for PD research but two major caveats exist in MPTP models. First, MPTP toxicity is primarily based on its selective uptake into DA neurons. The intracellular processes may not share common pathways with PD. Second, most animal models of MPTP are acute and do not contain classic Lewy Bodies, possibly due to rapid cell death before aggregate formation.¹³ Regardless, MPTP models have been the corner stone of PD research^{14,15} and suggest that the intrinsic properties of a dopaminergic neuron play a role in its demise.

Other similar toxins have been utilized as PD models. Paraquat is an herbicide that generates superoxide radicals. It is structurally similar to MPP⁺ with one extra N-methyl-pyridinium group. Systemic injection of

paraquat in mice causes mild SNc degeneration and inclusion bodies.^{16,17} Paraquat crosses the blood brain barrier via amino acid transporter.¹⁸ Rotenone is a highly toxic and common insecticide that binds and inhibits mitochondrial complex I at the same site as MPP+.¹⁹ Unlike paraquat and MPTP, rotenone's lipophilic property allows easy crossing of the blood brain barrier and into the cells to exert its neurotoxic effects. Therefore, its selective toxicity against dopaminergic neurons of SN suggests enhanced vulnerability of these neurons to mitochondrial complex I inhibition. There are, however, conflicting reports regarding rotenone's effect, with one group demonstrating selective dopaminergic degeneration and inclusion bodies by chronic low-dose injection in rodents,²⁰ and others observing a more wide spread pathology.^{21,22}

Epidemiological studies have also provided important insights into possible neuroprotective mechanisms in PD. Caffeine consumption²³ and cigarette smoking²⁴ have been inversely correlated with the risk of developing PD. The use of nonsteroidal anti-inflammatory agents,²⁵ Vitamin E intake²⁶ and vigorous exercise²⁷ have been associated with decreased risk of PD. These provide rationale for neuroprotective therapies.

2. Genetic factors

One could argue that familial and sporadic forms of PD may have entirely different etiologies, but evidence points to potential shared pathways in the degenerative process. Therefore, understanding the genetic forms may provide new insights into the mechanism of neurodegeneration in PD and potential therapeutic approaches. A similar theme to other neurodegenerative disorders such as Alzheimer's disease has emerged: genetic abnormalities either in aberrant protein aggregation or in the proteins that process these aggregated protein may lead to the disease. A summary of all genes that have been discovered so far is provided in the Table 1 and more relevant genetic forms are discussed below.

1) α synuclein

In 1997, the discovery of a missense mutation with an alanine to threonine substitution at position 53 (A53T) of α synuclein opened the field of PD genetics.²⁸ Two other mutations including alanine to proline substitution at position 30 (A30P) in a German family²⁹ and E46K in a Spanish family³⁰ were identified. Besides genetic mutations, triplication of α synuclein gene was also found

Table 1. Summary of established genes linked to familial PD

Locus	Gene	Inheritance	Phenotype	Age of onset	pathology
PARK1	α -synuclein	AD	Parkinsonism, dementia, hallucination	40	LB - also cortical
PARK2	Parkin	AR	EOPD: slow progression, early onset dystonia, sensitivity to L-DOPA >50% of EOPD;	20-40	No LB (some with syn + inclusion, tau)
PARK3	2p13	AD	Typical PD Low penetrance, haplotype affects onset age	60	LB, plaques, tangles
PARK4	α -synuclein	AD	Tremor, dementia susceptibility gene	30	LB, vacuoles
PARK5	UCH-L1	AD	Typical PD susceptibility gene	50	?
PARK6	PINK1	AR	EOPD, dystonia uncommon	30-40	PET abnormality
PARK7	DJ-1	AR	EOPD, anxiety	30-40	PET abnormality
PARK8	LRRK2 (dardarin)	AD	Typical PD 1-2% of sporadic form	60	Variable LB, tau

AD: autosomal dominant, AR: autosomal recessive, LB: Lewy body, DLB: diffuse LB, EOPD: early onset PD, No pathology is known for PARK5-7.

to associate with Parkinson's disease.³¹ These PD families demonstrate that excessive amount of wild-type α -synuclein leads to abnormal accumulation, and mutations may lower the threshold for the pathological process.

The exact mechanism of α -synuclein-dependent pathogenesis is still unclear, but it appears that the mutation results in a gain-of-function and α -synuclein aggregation leads to dopaminergic neuronal death. α -Synuclein is a major fibrillar component of LB in both familial and sporadic PD. Overexpression of α -synuclein A53T inhibits proteasomal activity, and α -synuclein mutants increase the sensitivity of cells to proteasomal inhibition.³²⁻³⁴ Transgenic mouse models of α -synuclein show that overexpression of wild-type human α -synuclein leads to cytoplasmic inclusions in the SN and the loss of dopaminergic terminals in the basal ganglia without the loss of dopaminergic cells.³⁵ Mice expressing human A53T α -synuclein developed progressive motoric dysfunction and adult-onset neurodegeneration only in dorsal midbrain, deep cerebellar nuclei, brainstem, and spinal cord-regions associated with aberrant α -synuclein aggregation.³⁶ Although mouse models of synucleinopathy have not shown particular selectivity to dopamine neurons, *in vitro* data suggest specific interaction of dopamine and α -synuclein pathology. Study with α -synuclein aggregation *in vitro* demonstrates that synuclein mutations favor the formation of potentially toxic protofibrils.³⁷ Expression of mutant α -synuclein can cause dopamine-dependent toxicity at a lower concentration compared to wild-type α -synuclein.³⁸ Dopamine inhibits fibrillization and stabilizes α -synuclein protofibril intermediates, leading to an accumulation of the toxic protofibrils *in vitro*.³⁹

Implication of the role of α -synuclein in PD goes beyond the few families with mutations or triplication, but extends into a large group of neurodegenerative disorders, now referred to as synucleinopathies including diffuse Lewy body disease.

2) Parkin

The loss-of-function mutations in parkin associate with early-onset parkinsonism without Lewy body formation.^{40,41} Parkin gene encodes an E3 ubiquitin ligase

protein that plays an important role in the ubiquitin-proteasome system, and the mutations in parkin abolish its ligase activity.^{42,43} Parkin conjugates polyubiquitin chain in a substrate-specific manner and targets the substrate for proteasome degradation. A lack of E3 ligase activity will cause aberrant protein accumulation and can lead to cell death. The physiological role of parkin implies that proteasome pathway can contribute to the pathogenesis of PD. However, how substrates of parkin promote selective dopaminergic cell death is unclear, especially since the expression of the substrates is not strictly limited to dopamine neurons in the SNc. Mice deficient in parkin exhibit mitochondrial dysfunction, increased protein and lipid peroxidation, as well as nigrostriatal functional abnormalities, but without significant loss of dopaminergic neurons.^{44,45}

3) UCH-L1 (Ubiquitin C-terminal Hydrolase-1)

Another protein of the ubiquitin pathway is implicated in PD-UCH-L1. The I93M dominant mutation in UCH-L1 was found in only one family with inherited PD,⁴⁶ thus representing a rare contributor to PD and possibly a polymorphism.⁴⁷ However, UCH-L1 polymorphism (S18Y) shows statistically significant inverse association with PD.⁴⁸ UCH-L1 hydrolyzes ubiquitylated peptides to generate free ubiquitin monomers that are recycled when proteasome-targeted peptides are degraded.^{49,50} Interestingly, both I93M and S18Y decrease the UCH-L1 ligase activity and lead to an impairment of the ubiquitin-proteasome system. *UCH-L1* knockout mice show ubiquitinated deposits and axonal degeneration, but no dopaminergic neurodegeneration.⁵¹ While ubiquitin-proteasome system remains critical in understanding PD pathogenesis, as is demonstrated with α -synuclein and parkin, strengthening the pathogenic role of mutant UCH-L1 requires further confirmation in other families.

3) DJ-1

The link between DJ-1 and PD was initially found in two consanguineous families, where *DJ-1* gene was truncated in one family and the other family harbored a potential loss-of-function mutation, L166P.⁵² Crystal structure analysis of DJ-1 finds that the proline substi-

tution at position 166 destabilizes the dimer interface important for DJ-1 activity.⁵³ Currently, the exact function of DJ-1 is unknown but a role in oxidative stress has been suggested. DJ-1 expression protects cells from oxidative stress, accompanied by modification into a more acidic form.^{53,54} DJ-1 is presumed to have a redox-dependent activity, and overexpression of wild-type DJ-1 in neuroblastoma cells significantly decreased visible α synuclein aggregates compared to L166P mutant.⁵⁵ *DJ-1* knockout mice do not show dopaminergic neurodegeneration but exhibit age-dependent and task-dependent motoric behavioral deficits that are detectable by 5 months of age,⁵⁶ as well as detectable changes in striatal dopaminergic function consisting of increased dopamine reuptake rates and elevated tissue dopamine content.^{56,57} The connection between dopamine neuron losses in the absence of DJ-1 needs further study; however, the roles of DJ-1 enhance our understanding that oxidative stress and protein chaperone activity are critical aspects of PD pathogenesis.

4) PINK1 (PTEN-induced putative kinase 1)

Recently, missense (G309D) and nonsense (W437X) mutations in PINK1 were identified in families with early-onset PD.⁵⁸ Data from exogenous PINK1 overexpression suggest that PINK1 localizes to the mitochondria.^{58,59} PINK1 contains motifs that indicate kinase activity. Because the kinase domain spans a large portion of PINK1, both G309D and W437X mutants are thought to generate a kinase-dead protein. *In vitro* kinase activity experiment demonstrates G309D mutant has less activity than wild-type.⁵⁹ This lack of kinase activity seems to enhance some forms of mitochondrial dysfunction when the cells are stressed, as measured by changes in mitochondrial membrane potential.⁵⁸ Determining the function of PINK1 is currently under hot pursuit, with its mitochondrial localization hinting at the common pathogenesis as the sporadic form.

5) LRRK2 (leucine-rich repeat kinase-2; dardarin)

LRRK2 is the latest gene that is cloned with mutations involved in late-onset parkinsonism in an autosomal dominant pattern.⁶⁰ *LRRK2* encodes a large protein with multiple functional domains, including a MAPKKK

kinase domain; yet the true function eludes researchers. Interestingly, neuropathology of the affected patients showed selective neuronal loss and gliosis in SN. However, the pathological features vary from those without Lewy Bodies to tauopathy and synucleinopathy. LRRK2 may participate in the phosphorylation of proteins implicated in PD such as α synuclein and tau.⁶⁰

Other genes have been identified to influence the age-of-onset or susceptibility to develop PD include sepiapterin reductase (PARK3),⁶¹ tau,⁶² and PARK10.⁶³

3. The Common Thread

As discussed in previous sections, disruptions in cellular homeostasis, whether caused by environmental insults or genetic mutations, can lead to PD. Environmental factors suggest the participation of oxidative stress and mitochondrial dysfunction in aberrant accumulation of proteins such as α synuclein and tau. The presumed functions of the genes whose mutations produce PD underline the potential role of mitochondria (PINK1), oxidative stress (DJ-1), and protein degradation pathway (parkin, UCH-L1, DJ-1) in PD pathogenesis, and emphasize α synuclein as one of the key molecules that are abnormally processed in the pathogenesis of PD. At first glance, they appear to be distinct and unrelated, though this is not the case. They are in fact intimately connected and any one or combinations of these factors may trigger the degenerative process in PD.

For example, the main intracellular source of ROS for most cells comes from the mitochondrial electron transport chain (ETC). ROS is generated under normal conditions, but it is accelerated upon mitochondrial dysfunction. It can change the mitochondrial membrane potential and release cytochrome c into the cytosol. ROS can cause protein damage and lead to protein misfolding and degradation. Mitochondria also regulate apoptosis, as many apoptosis-associated proteins reside in the mitochondria, such as caspases and cytochrome c. It is found that during apoptosis, caspases cleave subunits of the proteasome that would impair the proteasome activity and augment accumulation of proteasome substrates.⁶⁴ Lastly, perturbing mitochondrial ETC affects ATP

generation that will negatively influence a wide variety of cellular functions including proteasomal activity and cell viability.

The protein quality control machinery in the cells assures that nascent polypeptides are properly folded into the correct conformation.⁶⁵ The misfolded proteins or unfolded proteins are retained in the endoplasmic reticulum (ER) and are either refolded by chaperone proteins or degraded through ER-associated degradation via the proteasome. Another integral aspect of the protein quality control is the ubiquitin-proteasome pathway (UPS). Inhibiting the proteasomes will increase protein accumulation and can lead to ER stress or trigger apoptosis. Recently, cell culture study demonstrated proteasome inhibition impairs mitochondrial electron transport chain and increases mitochondrial ROS.⁶⁶

Currently, there exist therapeutic agents targeted at each pathway and are part of ongoing clinical trials. However, most of the past and current neuroprotective trials were based on data from experimental models that focused on dopaminergic cell loss in acute toxic models in rodents and nonhuman primates. New models that are based on genetic mutations or other novel mechanisms such protein processing abnormalities may provide better indicators of a successful clinical translation. In addition, developing a successful neuroprotective agent may require the elucidation of how each cellular pathway interacts with one another in the overall pathogenesis.

4. Dopamine's contribution to vulnerability

Even though degeneration in PD occurs predominantly in the dopamine neurons, environmental factors may affect neurons globally and familial genes are ubiquitously expressed throughout the nervous system. Thus, how these factors contribute to selective dopamine vulnerability remains an enigma. As aforementioned, differential protein expression profile within the nigrostriatal dopaminergic neuronal population might explain selective vulnerability. The role of dopamine metabolism can be another explanation. A circulating hypothesis names dopamine cytotoxicity as a culprit in dopaminergic death in PD, citing dopamine is a key contributor to the selective vulnerability. Tyrosine is converted into

L-DOPA by tyrosine hydroxylase (TH), which is in turn decarboxylated by aromatic L-amino acid decarboxylase (AADC) to dopamine. Free dopamine can either be sequestered into vesicles by VMAT2 or oxidized into dopamine quinone (DAQ). Dopamine oxidation by monoamine oxidase (MAO) produces hydrogen peroxide.⁶⁷ Among those dopamine released into the synaptic cleft, some bind to postsynaptic receptors, some are degraded by catechol-O-methyl-transferase (COMT) in the cleft, and the rest are recycled by DAT. Dopamine uptake increases the probability of generating DAQ and oxidative stress. Therefore, one can think of dopamine, in addition to being a signal molecule in motor activity, reward system or emotions, as a pro-apoptotic neurotransmitter. The oxidation of dopamine can activate JNK and the release cytochrome c from the mitochondria, and ultimately result in the activation of caspase-9 and -3.^{68,69} Though, one should bear in mind that there is no evidence that dopamine is toxic *in vivo*, especially at physiological concentrations in normal animals without lesions. Some investigators have noted intrinsic defense mechanism against oxidative stress in dopaminergic neurons to counteract their increased exposure to oxidants.^{70,71} In addition, dopaminergic neurons are not the only neuronal population affected⁷² and the contribution of non-dopaminergic deficits in early stages and to overall disabilities of advanced stages of PD is appreciated increasingly. Furthermore, not all dopaminergic neurons are affected equally in PD.³

In a recent study comparing the gene expression profiles between rat SN and ventral tegmental area (VTA) dopamine neurons by genechip microarray, the dopamine neurons in SN express more mRNAs related to energy metabolism than VTA neurons.⁷³ The expressions of genes involved in protein catabolism, apoptosis, and oxidative stress are not significantly different between SN and VTA. Another study found that mitochondrial complex I inhibition may lead to energy stress in dopaminergic neurons whereas the same insult results in oxidative stress in nondopaminergic neurons.⁷⁴ The ATP levels were higher in dopaminergic neurons than nondopaminergic neurons. These studies indicate that SN neurons could potentially be operating at higher metabolic activity and thus are less capable of compen-

sating slight perturbations in energy homeostasis. Simultaneously, a higher metabolic activity translates into more oxidative stress for the neurons. Thus, linking the intrinsic properties of dopaminergic neurons to genetic and environmental risk factors could enhance our understanding of PD pathogenesis. Such efforts are underway using either normal or PD brains.^{75,76}

NEUROPROTECTIVE THERAPY

In continuation with our discussions on the etiology and pathogenesis of PD, we choose to focus on the clinical therapeutic agents that are intended to be neuroprotective and address only the trials that attempt to intervene with the pathogenic mechanisms specific to PD. For example, cell transplantation is a very promising neurorestorative therapy, but does not specifically interfere with the pathogenic process and will not be discussed here. The therapeutic agents are grouped by the biological pathways that they are presumed to act upon (Fig. 1). The mechanisms of these agents may involve actions on multiple points in the presumed pathogenic pathway of PD. Therefore the classification of these agents used below is based on its primary putative mechanism and somewhat arbitrary.

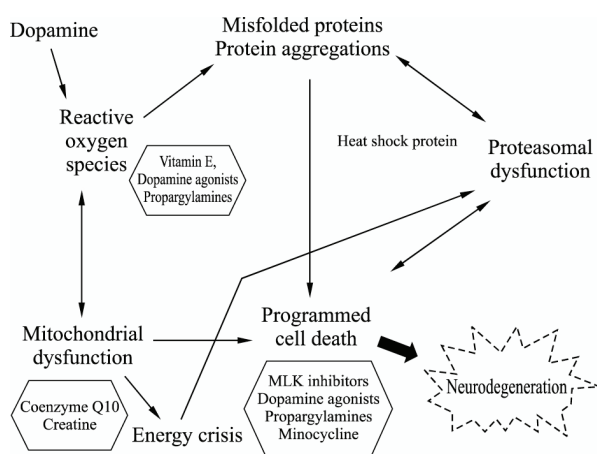


Figure 1. Potential mechanisms of neurodegeneration and neuroprotective therapies. This schematic outlines the proposed pathogenesis of PD and the sites of action for the neuroprotective drugs. MLK: mixed lineage kinase.

1. Antioxidants

The prominent role of oxidative stress in PD, as patients show decrease in glutathione and evidence of oxidative damage, provides the rationale for testing antioxidants as potential neuroprotective agents. One small uncontrolled study attempted intravenous administration of reduced glutathione,⁷⁷ but an established therapy to elevate the intracellular glutathione levels in neurons is not available. Vitamin E (α -tocopherol), a biologically active free radical scavenger, has been tested in DATATOP (Deprenyl and Tocopherol Antioxidant Therapy of PD) study.⁷⁸⁻⁸⁰ *De novo* PD patients were randomized to treatment with 10 mg selegiline, a MAO inhibitor, 2000 IU tocopherol, both agents, or placebo. Tocopherol provided no significant benefit over placebo. Although oxidative stress is implicated in many disorders, ROS are necessary for normal cellular signaling pathways and only specific cellular compartments may be affected by the damaging effects of oxidative stress. Current antioxidants are indiscriminate in its site of action and may not provide normalization of the reactive oxidants when and where they are needed.

2. Mitochondrial enhancers

Evidence supporting the association between mitochondrial dysfunction and PD derives from mitochondrial function measurements in PD brains and platelets^{81,82} and from toxin models that we discussed above. Two bioenergetic agents, coenzyme Q10 and creatine, have shown to protect dopaminergic neurons in MPTP mouse models.^{83,84} Coenzyme Q10 is naturally produced electron acceptor for mitochondrial complexes I and II. The level of coenzyme Q10 is decreased in serum and platelet mitochondria of PD patients.⁸⁵ Trial involving coenzyme Q10 at dosages of 300 mg/d, 600 mg/d, or 1200 mg/d were administered to patients for 16 months or until levodopa treatment is needed.⁸⁶ Coenzyme Q10 slowed the progression of functional deterioration, only at the highest dose used, 1200 mg/d, and the drug was safe and well-tolerated. A larger trial with coenzyme Q10 is planned to confirm this preliminary finding of a modest benefit in a small study and test the efficacy of a higher

dose. Creatine is a nutritional supplement that serves as an energy reservoir and acts as an antioxidant. Based on the positive results from the study of creatine in MPTP-treated rat and safety data from trials in Huntington's disease,⁸⁷ NIH/NINDS is conducting a study on the effect of creatine in PD.⁸⁸

3. Anti-apoptotic agents

One direct intervention to neuronal loss in PD is to prevent apoptosis. While there are many apoptotic pathways, the mixed lineage kinase (MLK) - c-jun N-terminal kinase (JNK) signaling cascade has attracted attention in dopaminergic neuronal death.⁸⁹ MLK acts upstream in the activation of c-jun and a MLK inhibitor, CEP1347, was tested for its effect in preventing dopamine neuron cell death. In animal models of parkinsonism, such as MPTP model, CEP1347 reduced the loss of SN dopaminergic neurons.⁹⁰ In acute MPTP model, CEP11004, an analogue of CEP1347, also attenuated the increase of cyclooxygenase-2 and the loss of TH-positive neurons.⁹¹ CEP1347 demonstrates a neuroprotective effect; however, it is known that acute MPTP treatments do not induce apoptosis and MPTP-treated animals only exhibit degeneration in the nigrostriatal pathway. Therefore, the promise of MLK inhibitors in PD patients could be limited as is shown in recent trials with CEP1347. In two small phase II studies of safety and tolerability, CEP1347 was well-tolerated and safe. When given to PD patients, CEP1347 did not have detectable effect on PD symptoms or L-dopa pharmacokinetics.⁹² A recent phase II/III trial involving 800 patients was, however, stopped because of the lack of any beneficial effect, although there were no safety concerns.⁹³

Other agents such as adenosine antagonists, nicotine, minocycline (inhibits microglial inflammation and anti-apoptotic), and neuroimmunophilin (GPI-1485) are considered or being tested for neuroprotective trials in PD,⁹⁴ partly based on the rationale derived from epidemiological evidence for protective effects of caffeine, cigarette smoking, and anti-inflammatory drug use.

4. Dopaminergic agents

MAO-B inhibitors were initially considered because of their ability to prevent the oxidation of MPTP and dopamine into their metabolites. Selegiline significantly delayed the need for levodopa treatment in DATATOP study mentioned above. Unfortunately, the protective effects of selegiline was not sustained as patients treated with selegiline reached disability endpoint faster than control patients.⁹⁵ It is unclear if selegiline protected against degenerating neurons or exerted a symptomatic effect.⁹⁶ To avoid the confounding effects of prolonged symptomatic benefit despite washout period, a delayed start design was used with rasagiline, another MAO inhibitor with a higher potency and modified structure that does not metabolize into amphetamine derivatives.⁹⁷ Those who started treatment of rasagiline for 12 months showed less functional decline than those whose treatment were delayed for 6 months. Although the randomized delayed-start analysis suggests potential disease-modifying activity of the drug, this study was relatively short in duration and its design has its own caveats. Further studies are necessary to address its neuroprotective effect. The presumed mechanisms of propargylamines such as selegiline and rasagiline include antioxidant and antiapoptotic effects in addition to MAO inhibition.⁹⁸

Dopamine agonists were used to treat PD as symptomatic agents because of its longer half-life than that of L-DOPA. Nonetheless, experiments showed protection of dopaminergic neurons and neuroprotective effects.⁹⁹ The site of neuroprotection is unclear, but dopamine agonists are proposed to affect levodopa turn-over rate through the activation of presynaptic autoreceptors, to scavenge free radicals as antioxidants, and to increase cell survival by an antiapoptotic mechanism.¹⁰⁰ Two trials suggested the neuroprotective potential of dopamine agonists in patients with early PD. One study, CALM- PD (Comparison of the Agonist Pramipexole versus Levodopa on Motor Complications in PD), investigated the nigrostriatal function and presynaptic density of dopamine transporters between pramipexole and levodopa.¹⁰¹ This was accomplished by using single photon emission CT (SPECT) to assess the striatal

uptake of β CIT. Patients were given either 300 mg/d levodopa or 1.5 mg/d pramipexole but no placebo controls in the trial. In another agonist study, REAL (Ropinirole as an Adjunct to Levodopa), positron emission tomography (PET) was used to study striatal uptake of fluorodopa.¹⁰² In both studies, fewer motor complications were observed with dopamine agonists than L-DOPA whereas patients taking levodopa had better motor scores improvement. In addition, patients taking dopamine agonists had a slower rate of deterioration than those on levodopa on two different types of imaging parameters in two different studies. Although these results imply neuroprotective effect in addition to pharmacodynamic effect on motor complications, the lack of untreated controls raises a possibility that L-DOPA may have accelerated the deterioration of imaging parameters.¹⁰³ In addition, the effect of the dopaminergic agents on the imaging parameters themselves and the large variability of the imaging data make the interpretation of these biomarker data rather challenging.¹⁰⁴

5. NMDA antagonist

Another cause of PD that is not discussed here is excitotoxicity associated with glutamate receptor and calcium influx. No controlled clinical trials with a presumably weak noncompetitive NMDA antagonist, amantadine have addressed its neuroprotective effect in PD patients. Remacemide¹⁰⁵ and riluzole¹⁰⁶ trials were discontinued due to the lack of efficacy. The current lack of efficacious and specific agents limits the exploration of the protective effects of NMDA receptor antagonists. Deep brain stimulation of subthalamic nucleus was put forth as a neuroprotective therapy in addition to having its well-established symptomatic effect because of its ability to limit the excitatory input from the subthalamic nucleus to the dopaminergic neurons. However, there is no clinical data demonstrating such effect and recent imaging show continuing decline of fluorodopa uptake on PET in patients with deep brain stimulator.¹⁰⁷

6. Protein Chaperones

As we discussed above in the setting of several genetic forms of PD, abnormal protein processing and aggregation of pathogenic proteins such as α synuclein play an important role in PD. When molecular chaperone, heat shock protein 70 (Hsp70) is overexpressed in drosophila PD model, Hsp70 prevented dopaminergic neuronal loss associated with α -synuclein. The interference with endogenous chaperone activity accelerated alpha-synuclein toxicity in drosophila.¹⁰⁸ Similar results were obtained in MPTP mouse model overexpressing Hsp70 by adenovirus, which support the idea that increasing chaperone activity may potentially be beneficial in treating PD.¹⁰⁹ Several experimental compounds such as geldanamycin alter the expression of chaperones and may prevent aggregation of abnormal proteins.¹¹⁰ Protein chaperones represent hopeful therapeutic agents for future PD treatment.

CONCLUSION

Intricate interplays among the implicated cellular pathways may underlie the apparent setbacks of neuroprotective therapy. Future therapies have to explore these interactions in a more comprehensive manner. In addition, intrinsic factors of the susceptible neuronal population have not been well elucidated. The importance of these factors is evident from genetic mouse models that have failed to reproduce selective dopaminergic neuronal degeneration. Understanding these intrinsic factors will have to consider the effect of dopamine, aging, and human versus rodent differences, to name a few. New models of PD should incorporate the interaction of various pathogenic processes and intrinsic factors that make dopaminergic neurons particularly protective and vulnerable. Modeling the chronic nature of the disease will be critical since chronic degeneration involves different molecular pathways than acute degeneration. Furthermore, therapeutic intervention at a sufficient level and at specific anatomical and intracellular locus may require novel methods of delivery such as gene therapy. The future has plenty of challenges, yet it has never looked more optimistic for PD patients.

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