Parkinson's disease: A review

» Abstract

Parkinson's disease is a common movement disorder seen in neurological practice, but the diagnosis and management is challenging. The diagnosis is clinical and sometimes difficult, considering a large number of motor and non-motor symptoms in PD patients. The medical management of PD patients is difficult, as choices of drugs are limited and levodopa is the mainstay of treatment. However, levodopa-induced dyskinesia (LID) is commonly seen in Parkinson's disease patients treated with levodopa. This side effect is usually encountered after a long duration of treatment, but occasionally, this may be seen even after a few days or months of treatment. Different types of surgical approaches, including unilateral pallidotomy and deep brain stimulation, have given very good results in PD patients, who cannot be managed by medications alone.

Keywords: Deep brain stimulation, dopaminergic drugs, Parkinson's disease

Key Message: This review addresses the epidemiological features, pathophysiology, genetics, pathology, and the clinicoradiological presentations in patients suffering from Parkinson's disease and also studies the therapeutic options available in treating this progressive disease.

Parkinson's disease (PD) is a chronic progressive neurodegenerative disorder characterized by early prominent death of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and wide spread presence of alpha synuclein (aSyn), an intracellular protein. Dopamine deficiency in the basal ganglia leads to classical Parkinsonian motor symptoms viz, bradykinesia, tremor, rigidity and later postural instability. PD is also associated with non-motor symptoms, which may precede motor symptoms by more than a decade. These non-motor symptoms become troublesome symptoms in the later stages of PD. Currently, the mainstay of PD management is pharmacological therapy; however, these symptomatic therapies have major limitations in advanced disease. Many disabling features develop later in the course of the disease including non-motor symptoms, dopamine resistant motor symptoms and motor complications of long-term dopamine therapy. Although there have been remarkable advances in the medical and surgical treatment for PD, definitive disease modifying therapy is lacking. However, researchers are hopeful that they will be able to identify the potential targets for disease modification. In this review, we will be discussing the epidemiology, clinical features, pathophysiology, diagnosis and management (medical and surgical) of PD. Experimental therapies have so far yielded only limited test results and will not be discussed here.

Epidemiology

The incidence and prevalence of PD increases with advancing age, being present in 1% of people over the age of 65 years.[1] Early-onset Parkinson's disease (EOPD) is defined as the onset of parkinsonian features before the age of 40 years. It accounts for 3-5% of all PD cases. It is classified into the 'juvenile' (occurring before the age of 21 years) and 'young-onset' PD (YOPD, occurring in the age range of 21- 40 years).[2] PD is twice as common in men than in women in most populations.[3],[4] A protective effect of female sex hormones is observed. The presence of gender - associated genetic mechanisms or/and gender -specific differences in exposure to environmental risk factors might explain this male preponderance.[3],[4]

There is no homogenous and large epidemiological data on PD from India. Razdan *et al*., reported a crude prevalence rate of 14.1 per 100,000 amongst a population of 63,645 from rural Kashmir in the northern part of India. The prevalence rate over the age of 60 years was 247/100,000.[5] A low prevalence rate of 27/100,000 was reported from Bangalore, in the southern part of India, and 16.1/100,000 from rural Bengal, in the eastern part of India.[6],[7] Bharucha *et al*., reported a high crude prevalence rate of 328.3/100,000 among a population of 14,010 Parsis living in colonies in Mumbai, Western India.[8]

» Pathophysiology

Genetics of PD

Genetic forms of PD represent only 5–10% of all cases.[9],[10] Presence of family history, early onset of disease and specific clinical features (e.g., dystonia as a presenting symptom) arouse suspicion of the presence of the genetic form of the disease in a patient. A genetic basis can be seen in >10% of YOPD individuals and the proportion of genetically defined cases rises to >40% if the onset of disease is before 30 years of age.[11],[12] The major genes identified and proved to be causal in PD include Parkin (PARK2), Leucine rich repeat kinase2 (LRRK2/PARK8), Alpha synuclein (SNCA-PARK1/PARK4), PTEN induced putative kinase 1 (PINK1/PARK6), DJ1 (PARK 7), ubiquitin C-terminal hydrolase like 1 (UCH-L1), and ATPase type 13A2 (ATP13A2).[9],[10],[13]

» Genetics of Pd in India

Mutations reported in the Parkin gene are the highest and vary from 1.96% to 39.1% among Indian case series.[14] Mutations are absent in SNCA, and less frequent in DJ1, PINK1 and LRRK2.[14] Mutations in the Parkin gene have been implicated to cause the autosomal recessive (AR) early onset PD and vary

considerably between subjects from different geographical locations in India.[15],[16],[17],[18],[19]

Chaudhary *et al*., in 2006 observed that Parkin mutations accounted for 14.3% cases of familial PD, 6.9% cases of young onset (age of onset ≤40 years), and 5.9% cases of late onset (age of onset ≥41 years) sporadic PD.[16] Padmaja MV *et al*., in 2012 reported Parkin mutations in 68% of the early onset PD cases in a study from the southern part of India.[19] Differentiating Parkin positive young onset PD patients from Parkin negative patients on clinical features alone is not possible.[9],[10]

DJ-1 mutations (seen in AR Parkinsonism) are responsible for early onset of PD symptoms with a benign course. These mutations are characterized by a good response to levodopa and are usually associated with the presence of dystonia. The prevalence of DJ1 mutations (AR) in patients with PD is modest (~5%) in the Indian population.[20] Two other Indian studies looked at the prevalence of DJ1 mutations in PD patients.[21],[22] One study reported a prevalence of 3.9% of the DJ1 variants,[21] while another study failed to identify any pathogenic mutations.[22]

The LRRK 2 (autosomal dominant [AD] Parkinsonism) is the most common cause of 'familial' as well as 'sporadic' PD globally, with the frequency of mutations being 5-7% in patients with a family history of PD.[23] LRRK2 mutations, however, have been less frequently seen in India.[24],[25],[26],[27],[28] The most frequent and best-studied LRRK2 mutation is the substitution of glycine by serine at 2019 position (c.6055G>A).[29] The study by Vijayan B *et al*., could not find any contribution by G2019S mutation in the pathogenesis of PD.[25] Punia *et al*., saw similar results in a previous study from the northern part of India (composed of a heterogeneous population), which reported LRRK2 mutations in <0.1% of PD cases.[27]

A point mutation of the SNCA gene causes early onset of PD (AD), and its over-expression causes the development of PD symptoms at a later age in the fourth or fifth decades in the affected members.[9],[10] SNCA mutations, however, rarely contribute to PD in India.[24],[30] A limited study with 100 PD cases from north Karnataka, India, suggested that SNCA mutations might be population specific and, therefore, might not be playing a causal role in all the populations studied.[31] The PINK1 gene, coding for a mitochondrial complex, has been implicated in the causation of an AR form of Parkinsonism.[32] The contribution of PINK1 variants in the causation of PD is limited in India.[33],[34] Tamali Halder *et al*., observed that 1.8% (2/106) patients suffering from PD in northern India harbor the PINK1 variants.[34]

In 2016, Sudhaman *et al*., discovered a novel frame shift mutation in the podocalyxin- like (PODXL) gene as a likely cause of early onset Parkinsonism (AR) in one Indian family, in whom the test for mutations in the Parkin, PINK1 and DJ1 genes was negative.[35] New mutations are being identified on a daily basis and have added to the spectrum of causation of genetic PD. However, the contribution of genetic testing in the management of PD is limited and there is no influence of positive genetic testing on the decision-making regarding treatment.

» Neuropathology

The pathophysiology of PD involves loss or degeneration of the dopaminergic neurons in substantia nigra pars compacta (SNpc) and the accumulation Lewy bodies, which are abnormal intracellular aggregates containing proteins, like alpha-synuclein (aSyn) and ubiquitin.[36],[37] About 60-70% of neurons in SNpc are lost before symptoms occur.[38] Research has revealed that the pathogenic process in PD involves regions of the peripheral and central nervous system in addition to the dopaminergic neurons of the SNpc. Lewy body pathology starts in cholinergic and monoaminergic brainstem neurons and in the neurons of the olfactory system, but involves limbic and neocortical brain regions with disease progression.[39],[40] Loss of dopaminergic neurons that was initially restricted to SNpc becomes more widespread by the time end-stage disease has been established.[41],[42]

Motor circuit changes in PD

Selective loss of dopaminergic neurons in the striatum causes impairment of motor control in persons with PD. The motor circuit of PD consists of corticostriatal projections from the primary motor cortex, supplementary motor area, cingulate motor cortex and premotor cortex, terminating on the dendrites of the striatal medium spiny neurons.[43],[44] The direct pathway is a monosynaptic connection between the medium spiny neurons that express dopamine D1 receptors and GABAergic (gamma amino butyric acid-ergic) neurons in the globus pallidus internus (Gpi) and the substantia nigra pars reticulata (SNpr). The 'indirect' pathway originates from medium spiny neurons that express D2 receptors, which project to the globus pallidus externus (Gpe), and reaches the Gpi via the subthalamic nucleus (STN) as a glutamatergic relay. Through these two pathways, the striatal dopaminergic tone regulates the GABAergic output activity of the basal ganglia. There is reduction in the D1 mediated direct pathway activity and an increase in the D2 mediated indirect pathway activity, resulting in a net increase in the firing rate of basal ganglia output neurons (GABA), which over-inhibit downstream thalamocortical and brainstem areas.[43],[44]

Changes in cerebellar activity and in the interaction between the basal ganglia and cerebellum contribute to the pathophysiology of tremor in PD.[45] Abnormalities of balance and gait are due to dysfunction of the basal ganglia output via projections into the midbrain locomotor region (pedunculo-pontine and cuneiform nuclei).[46]

Gut and PD

Parasympathetic nerves and enteric nervous systems are among the structures earliest affected by the aSyn pathology. Dysfunction of the brain-gut-microbiota axis in PD may be associated with non-motor symptoms that are evident before the classical motor symptoms, supporting the hypothesis that the pathological process spreads from the gut to the brain.[47] Gut microbiomes play an important role in regulating movement disorders, and alterations in the microbiota might be a risk factor for PD. Sampson *et al*., using mice that overexpress aSyn, reported that alterations in the gut microbiota were required for motor deficits, microglial activation, and aSyn pathology to develop.[48] Antibiotic treatment ameliorated, while microbial re-colonization promoted the pathophysiology in adult animals, suggesting that postnatal signaling between the gut and the brain modulates the onset and course of the disease.[48] Oral administration of specific microbial metabolites [e.g., short chain fatty acids (SCFA)] to germ-free mice promoted the development of neuroinflammation and motor symptoms. Investigations have shown that alterations in the gut microbiome is related to several clinical features. A recent Finnish study has shown that alterations in the microbiota composition, in particular, the abundance of Enterobacteriaceae is positively associated with the severity of postural instability and gait difficulty in PD patients.[49] Keshavarzian *et al*., and Unger *et al*., observed that faeces derived from patients with PD contained less short chain fatty acids (SCFA) including butyrate, that produce bacteria that could exert anti-inflammatory properties.[50],[51] An increase in the intestinal permeability and the dysfunction in the intestinal symbiosis have also been proposed as the mechanisms responsible for the development and progression of PD.[52] Recently, Hill-Burns *et al*., observed significantly altered abundance of several taxa in 197 patients with PD. They demonstrated the independent effects of PD medications on these microbiomes, thereby providing further leads in the pathophysiology and treatment of PD.[53]

» Clinical Diagnosis and Natural History

Parkinson's disease is defined clinically by the presence of bradykinesia in combination with at least one more manifestation: muscular rigidity, rest tremor or postural instability (the latter being a feature of the more advanced form of the disease).[54] Motor symptoms starts unilaterally, and asymmetry persists throughout the course of the disease. Non-motor symptoms are seen in a large proportion of patients. Some of these non-motor symtpoms can antedate the onset of cardinal motor symptoms by years.[55] These non-motor symptoms include sleep disorders [for example, frequent waking, rapid eye movement sleep behavior disorder (RBD), and day time somnolence], hyposmia, disturbance in autonomic function [orthostatic hypotension, urogenital dysfunction, and constipation], cognitive impairment, mood disorders and pain.[55] The Sydney Multicentre Study of Parkinson's disease reported dementia (83%), hallucinosis (74%), symptomatic hypotension (48%), constipation (40%) and urinary incontinence (20%) in 71% of patients with PD who had survived for >20 years after the onset of the disease.[56] Freezing of gait, postural instability and falling and choking were reported in 81%, 87% and 48% of the patients, respectively.[56]

Alhough there is no consensus regarding the classification of PD subtypes, clinical observations suggest the existence of two major subtypes: tremor-dominant PD (with a relative absence of other motor symptoms), and non-tremor-dominant PD (which includes phenotypes described as the akinetic-rigid syndrome and the postural instability gait disorder, PIGD). Tremor-dominant PD is often associated with a slower rate of progression and less functional disability than the non-tremor-dominant Parkinson's disease.[57]

Almost 90% patients with PD experience non-motor symptoms during the course of their illness that usually do not respond well to dopamine therapy.[58] Mood disorders and constipation almost double an individual's risk for developing Parkinson's disease in the later years.[59] Idiopathic RBD carries a high risk for the development of PD and other α-synucleinopathies.[60] The average latency between the onset of RBD and the occurrence of Parkinsonian motor symptoms is 12–14 years.[61]

Autonomic symptoms (mentioned above) increase with a higher age, with disease severity, and with higher doses of dopaminergic medications. Urinary symptoms include urgency, frequency, nocturia, and urge incontinence, with urinary storage problems being commoner than voiding difficulties. Urinary symptoms are more frequent and occur earlier in multisystem atrophy (MSA) when compared to PD.[62] Painful sensory symptoms are seen in two-third of PD patients and are thought to be due to abnormal nociceptive processing.[62]

There is a six-fold increased risk for dementia (subcortical type) in patients with PD and this occurs later in the course of the disease course.[63] Upto 60% of patients with PD develop dementia within 12 years of its diagnosis.[63] Hyposmia occurs in approximately 90% of patients with an early-stage PD and antedates the typical motor symptoms by several years.[64] Occurrence of hyposmia may predict a higher risk of developing PD, and olfactory testing might help in differentiating PD from other parkinsonian syndromes.

» Imaging

Early PD is a diagnostic challenge with the existence of wide differential diagnoses that consists of disease that are not associated with nigral degeneration or striatal dopamine deficiency. The commonly used UK Parkinson's Disease Society Brain Bank (UKPDSBB) Clinical Criteria has a diagnostic accuracy of only about 80% at the first visit following the development of early PD in a patient.[65] Thus, functional imaging is necessary to confirm the clinical diagnosis and to

understand the underlying pathophysiology.

123I-ioflupane single-photon emission computed tomography [SPECT] (also known as DaTscan) is useful to assess the density of the presynaptic dopaminergic terminals within the striatum as it helps to differentiate PD from disorders that exist without the presence of presynaptic dopaminergic terminal deficiency.[66],[67]

^{18} F-DOPAL-6-fluoro-3, 4-dihydroxyphenylalnine (^{18} F-DOPA) positron emission tomography (PET) scan assesses the presynaptic dopaminergic integrity and accurately reflects the monoaminergic disturbances in PD. A retrospective analysis of 27 patients who underwent ^{18} F-DOPA PET scan for motor symptoms suspicious of PD showed its sensitivity as being 95.4% (95% confidence intervals [CI], 100%-75.3%), specificity 100% (95% CI: 100%-59.0%), positive predictive value (100% (95% CI, 100%-80.7%), and negative predictive value 87.5% (95% CI, 99.5%-50.5%).[68]

[^{123} I] N-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl) nortropane (FP-CIT) is a selective and potent dopamine transporter imaging [DAT] agent. Correlation between the values obtained on FP-CIT single photon emission computed tomography (SPECT) and F-DOPA PET for striatal uptake in patients with different stages of PD has been proven.[69],[70] Dopamine transporters get downregulated as an early response to reduction in the amount of endogenous dopamine concentration and this results in a decreased striatal binding of FP-CIT in the early phases of PD. FP-CIT might, therefore, be more sensitive than the F-DOPA scan for detecting early striatal dopaminergic deficits. A study compared the sensitivity and specificity of the contralateral striatal and putaminal uptake based on the findings of FP-CIT SPECT and F-DOPA PET in patients with Parkinson's disease and healthy controls and found it to be 100% in the early phase of the disease. When only caudate uptake was considered, the specificity remained 100% for FP-CIT but reduced to 90% for F-DOPA, while the sensitivity was 91% for both the scanning techniques.[71] However, techniques that rely solely on dopamine imaging are not sufficient to diagnose Parkinson's disease because they do not reliably distinguish PD from other parkinsonian syndromes associated with nigral degeneration, such as atypical parkinsonism.

The standard magnetic resonance imaging (MRI) has a marginal role in establishing the diagnosis of PD; however, the high and ultra-high-field (7 Tesla) MRI combined with advanced techniques, such as diffusion tensor imaging, are being explored for determining an early diagnosis of Parkinson's disease.[72] MRI helps to identify patients with symptomatic parkinsonism, and also helps to show specific changes in the basal ganglia and infra-tentorial structures in patients with atypical Parkinsonism.[73] Myocardial sympathetic denervation, assessed with PET or SPECT using noradrenergic tracers, is seen in PD, but not in patients with atypical parkinsonism or other PD mimics.[74]

» Cerebrospinal Fluid (CSF) and Blood Tests

Currently, there is no clinically useful CSF based test for the diagnosis of PD. There were several studies that assessed the levels of proteins in CSF (e.g., the levels of different α-synuclein species) but the sensitivities and specificities of these tests have been low.[75] Though, lower plasma level of apolipoprotein A1 often correlates with a greater severity of motor symptoms, its utility as a blood biomarker is not established till date.[76]

The major roadblock in PD research is absence of good biomarkers with a high sensitivity and specificity to diagnose the disease in the early or even the prodromal stage; and, no single measure currently fulfills all the necessary criteria for a biomarker in PD.[77] Disease modifying therapies would be most effective if patients are diagnosed and treated during this prodromal period. The possible clinical markers include RBD diagnosed by polysomnography, and olfactory dysfunction measured by standard methods, such as the University of Pennsylvania's smell identification test.[61]

Pharmacologic management

The major objective of PD research is to develop disease-modifying therapy that can slow or stop the neurodegenerative process. However, there is no existing definitive disease-modifying therapy to achieve this aim.

Dopaminergic therapy

The American Academy of Neurology (AAN) recommends initiating one of the following available drug therapies once the patients develop functional disability.[78] The medical therapies available for treatment of motor symptoms include levodopa/carbidopa, dopamine agonists (both ergot and non-ergot types), monoamine oxidase-B (MAO-B) inhibitors, injectable dopamine agonist (apomorphine), catechol-O-methyltransferase (COMT) inhibitors, N-methyl-D-aspartate (NMDA) receptor inhibitors, and anti-cholinergics. In the later stages of PD, drug delivery can be supplemented via alternative routes [79],[80],[81],[82] (e.g., intrajejunal infusions, subcutaneous injections or transdermal patches). Continued motor fluctuations and dyskinesias indicate the patient's candidacy for deep brain stimulation (DBS).

Dopaminergic therapy is highly effective in bradykinesia and rigidity but monoamine MAO B inhibitors are only moderately effective. Dopamine agonists and levodopa help to reduce disease progression and disability. Tremor responds to anticholinergic drugs like trihexyphenidyl but has a poor and inconsistent response to dopamine replacement therapy.[83],[84]

» Levodopa and Novel Levodopa Formulations

The mechanism for major motor symptoms in PD is the depletion of striatal dopamine due to loss of dopaminergic neuron in the SNpc. Administration of levodopa to substitute striatal dopamine was a major breakthrough in the treatment of PD, and since then, multiple additional targets for dopaminergic therapies have been identified. Levodopa is considered as gold standard therapy and almost all patient require this particular treatment during the course of their illness.[85]

Long-term use of levodopa is complicated by motor fluctuations and dyskinesias. Mechanisms underlying these motor complications are still unclear. One accepted hypothesis for this manifestation is the involvement of both presynaptic and postsynaptic mechanisms that eventually lead to non-physiological pulsatile striatal dopamine receptor stimulation, causing various maladaptive neuronal responses.[86],[87] Erratic drug delivery due to the short half-life of levodopa, as well as variability in its absorption and blood–brain barrier transportation also play an important role in the development of motor complications.[82]

Levodopa bioavailability can be improved either by developing more effective oral formulations (e.g., sustained release formulations) or by devising innovative routes of administration (e.g., intestinal infusion, transcutaneous administratin via mini pumps or by inhalation). RYTARY/IPX066 is a novel levodopa-carbidopa (LD/CD) oral formulation combining the immediate-release and extended-release LD/CD. This has been approved in the USA and the European Union. IPX066 is composed of LD/CD micro-beads designed to dissolve at various rates that allows for a quick absorption and sustained levodopa release over an extended period of time. Studies have shown that IPX066 administration improved the symptoms in patients with both early and advanced PD.[88],[89],[90],[91],[92],[93] Significant improvement in the unified Parkinson's disease rating scale (UPDRS) scores has been reported without the development of worsening troublesome dyskinesias, using this preparation when compared to other levodopa formulations.[88],[89],[90],[91],[92],[93]

Levodopa-carbidopa intestinal gel (LCIG) is an approved therapy for inpatients with advanced PD. LCIG is delivered continuously by a percutaneous endoscopic gastrojejunostomy tube (PEG-J), through a portable infusion pump. It reduces L-dopa-plasma level fluctuations and thereby decreases the motor complications.[80],[81],[94] Recently, researchers are evaluating the 'accordion pill' (AP09004), an extended release LD/CD formulation with gastroretentive properties.[95],[96],[97] Other levodopa formulations currently active in studies include ND-0612, ODM-101, CVT-301 and cyclops. ND-0612 is a proprietary liquid formulation of LD/CD that enables subcutaneous administration via a small patch-pump device; and, ODM-101is a new oral formulation of levodopa/carbidopa/entacapone that contains a higher amount of carbidopa (65 or 105 mg).[98],[99],[100] CVT-301 and cyclops are levodopa inhalation powders. As they possess a rapid onset of action, they are promising candidates for the treatment of PD.[101],[102]

Although, the highest levels of symptomatic relief is provided by levodopa, to delay the ensuing complications, MAO-B inhibitors/dopamine agonists can be considered as the initial therapy. A randomized trial of newly diagnosed PD patients failed to show the long-term benefit of levodopa sparing therapy.[103] This study, however, had limitatons characterized by a lack of generalizability, as patients <60 years of age, who were at a high risk of developing dyskinesias, were not well represented.[104]

Dopamine agonists

Dopamine receptors mainly target the D2 receptor family. The initial members of this family of drugs were ergoline derivatives. Ergoline drugs raised cardiac and pulmonary safety concerns and the currently used agents are all non-ergoline drugs, e.g., pramipexole, ropinirole, apomorphine, piribedil, rotigotine. Dopamine agonists induce less pulsatile striatal dopamine receptor stimulation than levodopa and can markedly reduced the risk of motor complications when they are being used as initial monotherapy.[84],[105],[106]

Apomorphine has both D1 and D2 receptor activity and a potency equal to that of levodopa.[66] Continuous subcutaneous apomorphine infusion reduces the motor response fluctuations and levodopa induced dyskinesias.[107] Another drug, rotigotine, is available as a transdermal patch formulation that permits a continuous drug delivery.[79]

Both levodopa and dopamine agonists are associated with nausea, daytime sleepiness and edema, but the adverse effects are more frequent with dopamine agonists. Dopamine agonists are known to cause impulse control disorders and drug induced hallucinations (especially in elderly people with cognitive impairment), and therefore, they are better avoided in the high-risk groups.

MAO B inhibitors

MAO B inhibition leads to an increase in the synaptic dopamine concentration and in symptomatic efficacy. Selegiline, a selective irreversible MAO B inhibitor, has proven its efficacy as an adjunct to levodopa since the 1970s.[108] Results from the MONOCOMB study showed that selegiline monotherapy in early-phase PD retarded the progression of the disease. In advanced PD, selegiline had levodopa-sparing qualities and was reasonably well tolerated on long-term usage.[109] In a recent trial conducted in Japanese patients with early PD, selegiline monotherapy significantly reduced UPDRS part I + II + III scores.[110]

Rasagiline, another irreversible MAO B inhibitor, is a well-known add-on therapy in patients with motor fluctuations.[105] A head-to-head 3-year retrospective case control study, analyzing the efficacy of MAO B inhibitors in PD, reported an equal efficacy in controlling motor symptoms in PD patients on optimized therapy.[111] MAO B inhibitor therapy was associated with a significant reduction in the levodopa requirements and a lower frequency of dyskinesias.[111]

Safinamide is a reversible MAOB inhibitor with antiglutaminergic properties. Safinamide gives enhanced control over motor symptoms in advanced PD and improves the quality of life.[112] In a recent randomized control trial, safinamide, when used as an adjunct to levodopa, improved the ON time without causing troublesome dyskinesias, and reduced the incidence of 'wearing off' phenomenon.[113]

Catecohol-O-methyl transferase (COMT) inhibitors

Current levodopa preparations contain carbidopa or benserazide to prevent peripheral metabolism of dopamine and, therefore, these drugs enhance the bioavailability of the former medication. This shifts the peripheral metabolism of levodopa to a secondary pathway that involves COMT. Inhibition of the COMT pathway will further increase the bioavailability and the half-life of levodopa, thus, helping patients with motor fluctuations.[114] Triple therapy with levodopa/carbidopa/COMT inhibitor increases the ON time, reduces the OFF time, and significantly improves the quality of life.[115] Use of tolcapone is restricted due to its side effects. Entocapone, a safer alternative, is currently available but is less efficacious. In phase II trials, nebicapone has been found to be more efficacious than entacapone and is safer than tolcapone.[116] Opicapone, in a once-a-day oral dose regimen, has also been proven to reduce the OFF time and to increase the ON time without troublesome dyskinesias, in patients suffering from advanced PD.[117]

» Non-Dopaminergic Pharmacological Targets

Symptoms of late stage PD (both motor and nonmotor) respond poorly to dopaminergic therapy. The reason may be abnormalities in other non-dopaminergic neurotransmitters like acetylcholine, glutamate, norepinephrine or serotonin.[118] Motor fluctuations, levodopa induced dyskinesias, freezing of gait, postural instability and falls, treatment-resistant tremor, swallowing and speech disturbances are among the symptoms that require treatment with non-dopaminergic agents.

Acetylcholine deficiency, due to degeneration of cholinergic neurons, leads to dementia, gait abnormalities and falls.[119] The trial of donepezil instituted for treatment of falls is related to the hypothesis of the existence of an abnormal cholinergic system in PD that is responsible for the frequent falls.[120] Rivastigmine, a cholinesterase inhibitor, is used for PD associated dementia.[121] The usefulness of rivastigmine in treating gait abnormalities and frequent falls is being evaluated.[122]

Depression in patients with PD responds to all types of antidepressant medications and there is limited evidence to recommend tricyclic antidepressants over selective serotonin reuptake inhibitors. The role of noradrenergic medications needs to be firmly established in clinical trials.[123] Psychotic symptoms in Parkinson's disease respond well to clozapine.[123],[124] Apart from quetiapine, all other atypical neuroleptics worsen Parkinsonism by blocking the striatal dopamine D2 receptors. A serotonergic effect of clozapine in treating psychosis is strongly supported by the recent positive results using the 5hydroxytryptamine 2A (HT2A) inverse agonist, pimavanserin.[125]

Amantidine is an N-methyl-D-aspartate (NMDA) receptor antagonist used for levodopa-induced dyskinesias.[83],[84],[105] Guidelines differ regarding the efficacy of amantadine in the management of levodopa-induced dyskinesias. Movement Disorders Society's evidence-based review reported that amantadine was 'efficacious' in the treatment of dyskinesias, whereas the American Association of Neurology (AAN) guidelines concluded that amantadine was 'possibly effective'[83],[126] A recent controlled trial investigated the efficacy and safety of 274 mg of ADS-5102 (amantadine) extended-release capsules (equivalent to 340-mg amantadine hydrochloride) for levodopa-induced dyskinesia. The study reported a significant decrease in levodopa-induced dyskinesia and an improvement in the OFF time.[127]

Patients with PD are troubled by autonomic dysfunction, particularly in the late stage. Pharmacological therapy directed towards the autonomic nervous system includes the use of mineralocorticoid, fludrocortisone, as well as the use of adrenergic agents (such as midodrine and etilefrine), the noradrenaline precursor (that is, droxidopa) to treat orthostatic hypotension; anti-muscarinics (such as oxybutynin, tolterodine or trospium chloride) to treat urinary urgency or incontinence; and, pro-kinetic drugs to improve constipation.[84],[123],[128]

Tyrosine kinase inhibitors for the treatment of PD

Recently, investigations have shown that the levels and activation of Abelson non-receptor tyrosine kinase (c-Abl) were upregulated in the brain tissue of patients with PD. Karuppagounder *et al*., evaluated the *in vivo* efficacy of a brain penetrant, c-Abl tyrosine kinase inhibitor, in the acute 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)-induced model of PD and found that nilotinib prevented dopamine neuronal loss and behavioral deficits following MPTP induced intoxication.[129] Nilotinib reduced c-Abl activation, levels of Parkin substrate and neuronal cell death. Imam *et al*., tested the

efficacy of INNO-406 (a second generation irreversible Abl kinase inhibitor) and found that INNO-406 was capable of preventing the progression of dopaminergic neuronal damage in a toxin-induced C57 mouse model of PD.[130] Researchers have demonstrated that c-Abl inhibitors (nilotinib, imatinib, and to a lesser extent, bafetinib) could prevent the loss of dopamine neurons, improve motor behavior, inhibit phosphorylation of Cdk5, regulate α-synuclein phosphorylation and reduce the levels of Parkin substrate.[131] Brain permeable c-Abl inhibitors can serve as potential therapeutic agents for the treatment of PD and other neurodegenerative disorders.

Surgical treatment

Deep brain stimulation (DBS) of either the subthalamic nucleus (STN) or globus pallidus interna (GPi) is a well-known treatment for patients with motor complications.[132],[133],[134] For treatment of tremors, thalamic DBS is a viable option. Surgical treatment is preferred when motor fluctuations and dyskinesias become disabling despite responsiveness of the motor symptoms to levodopa. The average time before DBS is performed is about 10–13 years after the diagnosis of Parkinson's disease has been established. Findings of the EARLYSTIM trial, a multicenter randomized control trial showed that DBS in the early course of disease (mean disease duration 7.5 years, with motor fluctuations for <3 years) could improve the patient's quality of life and several secondary outcome measures more than the best medical therapy.[135]

DBS is reversible and can be adjusted for disease progression. The presence of dementia, acute psychosis and major depression are the exclusion criteria for DBS.[136] Bilateral DBS of the STN improves the UPDRS II (activities of daily living) and UPDRS III (motor) scores, on an average, by 50–60% compared with the preoperative medical OFF state. The total daily dopaminergic drug dosage is reduced by about 60% following the institution of DBS, and dyskinesias decrease by 60–70%.[137],[138] Subthalamic nucleus (STN) DBS was associated with decreased requirement of levodopa doses.[139] The mortality of DBS is <0.5% and the important adverse events include intracranial bleeding or device-related complications (such as infections and lead misplacements, among others).[140]

Non-pharmacological therapies available for PD include exercise, education, support groups, speech therapy and nutrition. Evidence from literature recommends their usage earlyon in the course of the disease.

» Conclusion

Parkinson's disease is one of the most common neurodegenerative diseases affecting the aging population and is associated with an increased morbidity and mortality. Awareness of the disease manifestations, the treatments, and the progressive long-term course of the disease is necessary for the optimal management of the cases. Tremendous progress has been made in understanding the neuropathology of PD and its progression throughout the nervous system. However, none of these treatments is curative. PD remains a progressive disorder that eventually causes severe disability due to the increasing severity of treatment-resistant motor problems and non-motor symptoms. Modifying factors that lead to the disease progression and in further delaying its disability are the key unmet needs to be addressed by the current and future research efforts.

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Conflicts of interest

There are no conflicts of interest.

Introduction

Parkinson's disease (PD) involves the progressive loss of do- paminergic neurons in the substantia nigra, leading to motor symptoms including akinesia, resting tremor, rigidity and postural instability (Jankovic, 2008; Lees, Hardy, and Revesz,

2009). While the dopamine precursor L-DOPA is the most effective symptomatic treatment for PD, chronic L-DOPA treatment typically results in the development of drug- induced dyskinesias (Cenci, Ohlin, and Odin, 2011).

In order to avoid dyskinesias, early to mid-stage PD is of- ten treated with dopamine D2 and D3 receptor agonists (Stowe et al., 2008). Among these, ropinirole (Figure 1) is frequently used as a monotherapy or adjunctive treatment due to its low dyskinesia liability (Brooks et al., 1998; Rascol et al., 2000). However, the efficacy of D2/3 agonists often wanes in late-stage PD, requiring these drugs to be combined with other medications and administered more frequently in later-stage PD patients (Connolly and Lang, 2014).

While a number of symptomatic treatments for PD are available, there is limited evidence that any medications are disease-modifying (Valera and Masliah, 2016). The histo- pathological hallmark of PD is the presence of Lewy body pro- tein aggregates, which may proliferate in dopaminergic neurons when excessive oxidative stress leads to mitochon- drial and lysosomal dysfunction (Wirdefeldt et al., 2011). In support of this theory, retrospective analyses have sometimes found a negative correlation between dietary antioxidant vi- tamin intake and PD progression, although the effects are not consistent across all studies (Zhang et al., 2002; Knekt et al., 2010; Wirdefeldt et al.,

2011). Ongoing clinical trials are examining antioxidants in PD patients, and it was recently reported that administration of the antioxidant coenzyme Q10 was able to reduce disease progression over a

2 year period (Yoritaka et al., 2015).

As an improvement over available pharmacotherapies, we have recently developed a novel series of compounds de- signed to optimize symptomatic efficacy and provide disease-modifying properties (Johnson et al., 2012). One of the most promising of these 'multi-functional' compounds, D-512 (Figure 1A), is a high-affinity D2/3 agonist (Km < 3 nM) with motor-stimulating properties in rats that last three times as long as ropinirole when acutely administered i.p. on an equimolar basis (Santra et al., 2013). Additionally, D-512 Ropinirole

provides in vitro and in vivo neuroprotection against the dopa- minergic neurotoxins, ostensibly through reducing oxidative stress within dopaminergic neurons (Santra et al., 2013; Shah et al., 2014; Voshavar et al., 2015).

Even though acute administration of D-512 enhances spontaneous motor activity, the acute and chronic effects of this compound on the motor symptoms of PD have not been examined. Likewise, it is not clear if D-512 produces signifi- cant levels of drug-induced dyskinesia, a major concern con- sidering that, in general, the most effective PD medications have the greatest dyskinesia liability (Cenci et al., 2011; Connolly and Lang, 2014). In the present study, we used the rat 6-hydroxydopamine (6-OHDA) model of PD to examine the potential superiority of D-512 to ropinirole by directly comparing the profile of each agent for motor stimulation and reversal of PD symptoms.

Discussion

The novel multifunctional $D_{2/3}$ agonist, D-512, was compared with the clinically used $D_{2/3}$ agonist, ropinirole, for the treatment of PD symptoms in rats. Higher plasma levels and brain uptake were observed for D-512 over equimolar doses of ropinirole (Figure 1). Both compounds increased spontaneous movements by a similar magnitude immediately after injection, but the duration of motor activation was longer for D-512 (Figure 3). Only D-512 was able to significantly reverse symptoms of forelimb akinesia, a cardinal feature of PD

metabolized compounds lead to frequent 'wearing off' epi- sodes, creating such a significant clinical problem that many

patients use specialized medical devices offering continuous drug delivery (Nyholm et al., 2005). As shown in Figure 1, D-512 was present in the brain and blood plasma at more than twice the concentration of ropinirole 1 h after injection of equimolar amounts of each drug. Indeed, the brain concentration of D-512 was greater at 4 h post-injection than was observed for ropinirole at 1 h post-injection, suggesting that D-512 remains active in the CNS longer than ropinirole.

Spontaneous motor activation

Research in early-stage PD patients and in animal models of PD has shown that ropinirole produces an anti-Parkinsonian response of similar magnitude and duration to L-DOPA (Brooks et al., 1998; Pearce et al., 1998; Rascol et al., 2000; Ravenscroft et al., 2004). The present data suggest that the initial magnitude of the motor response is comparable be- tween D-512 and ropinirole. Both drugs increased discrete movements during the first hour after drug administration (2–3× above untreated Parkinsonian animals on days 3, 10 and 17; Figure 3B, D, F). There was some suggestion that

ropinirole (5.1 μmol·kg ^{1}) may have a more rapid onset since

it alone induced significant increases in distance travelled during the first hour of testing on day 17 (Figure 3E). How- ever, D-512 clearly exhibited longer-lasting locomotor activa- tion than ropinirole: D-512 enhanced both distance travelled and discrete movements for all 4 h on all test days, while ropinirole had no significant effect after 2 h on any test day (Figure 3).

Unilaterally Parkinsonian rats exhibit a mild tendency to preferentially turn ipsilateral to lesion, whereas in the same animals, anti-Parkinsonian compounds strongly and dose- dependently induce rotations contralateral to lesion (Ungerstedt, 1971). This has led to widespread use of contra- lateral rotations as a measure of anti-Parkinsonian efficacy (Lundblad et al., 2002; Smith et al., 2012; Breger, Dunnett, and Lane, 2013). Both ropinirole and D-512 caused some contralateral turning, but statistically significant increases relative to vehicle were only observed with D-512 (Figure 5).

Indeed, chronic D-512 at 9 μmol·kg ^{1} continued to signifi-

cantly induce rotations at the 240 min time point, when mea- surements ceased (Figure 5D, F, H). These effects are in

agreement with previous work showing that D-512 caused longer-lasting rotations than ropinirole when the drugs were administered acutely (Santra et al., 2013).

Even though it is common to measure contralateral rota- tions as a proxy for anti-Parkinsonian efficacy, the interpreta- tion of these behavioural effects is complicated by the fact that rotations appear to reflect a combination of anti- Parkinsonian efficacy and dyskinesia liability (Lane et al.,

2006). For example, rotations are dose-dependently increased by L-DOPA even when the dose is increased above the thera- peutic maximum (Smith et al., 2012; Breger et al., 2013). For this reason, we chose to also examine motor ability with the forepaw adjusting steps test, an assessment that more closely . (Figure 4). Rotational behaviour, a marker of anti-PD efficacy, was greater for D-512 than for ropinirole even though both drugs produced dyskinesia of similar severity (Figures 5 and 6).

Pharmacokinetic profile

The development of long-lasting anti-Parkinsonian agents is critical as drugs with short half-lives are less convenient for patients and may be more prone to elicit dyskinesia (Jankovic, 2005; Olanow, Obeso, and Stocchi, 2006). Rapidly

measures Parkinsonian symptoms (Olsson et al., 1995; Chang et al., 1999).

Reversal of forelimb akinesia

The forepaw adjusting steps test assesses the ability of a rat to rapidly initiate and terminate movement and may be the best indicator of anti-PD efficacy in a rat model of PD because performance is strongly diminished after dopaminergic lesion and restored by dopamine replacement therapy (Olsson et al., 1995). In the present investigation, untreated 6-OHDA-lesioned rats averaged 85% fewer steps with their lesioned forelimb than observed in sham-lesioned animals (Figure 4). Ropinirole did not significantly alter the number of steps relative to vehicle. This result is similar to a previous report showing that quinpirole, a close analogue of ropinirole, did not increase performance on the forepaw adjusting steps test (Olsson et al., 1995). Even though acute treatment with D-512 did not affect motor performance, chronic administration at 3 and 9 $\mu mol \cdot kg^{1}$ was able to increase stepping relative to vehicle on days 15 and 22 (Figure 4C, D).

Dyskinesia liability

Treatment of PD is complicated by the fact that there is a consistent positive correlation between the magnitude of anti-PD effects provided by a drug and the severity of dyskinesia it elicits (Stowe et al., 2008; Cenci et al., 2011). In the present study, dyskinesia was similar between ropinirole and D-512. Examining total dyskinesia on each test day, the low and high doses of ropinirole produced equivalent dyskinesia to the low and high doses of D-512 respectively (the sole exception being that the low dose of D-512 caused more dyskinesia than the low dose of ropinirole on day 15; Figure 6A, C, E, G). However, the time series for each test day shows that the high dose of ropinirole produced a magnitude of dyskinesia that was equal to or greater than that of D-512 in the period immediately after injection (Figure 6B, D, F, H). By contrast, the duration of dyskinesia caused by D-512 was greater than that of ropinirole on days 8, 15 and 22. Thus, it may be that ropinirole causes greater peak dyskinesia than D-512, but D-512 extends the time during which dyskinesia is present. In this sense, the dyskinesia data are consistent with other behavioural assays in suggesting that the anti-Parkinsonian effects of ropinirole extinguish within 2 h, while the anti-Parkinsonian effects of D-512 last for at least 4 h (Figures 3–5).

Although dopaminergic agonists that target $D_{2/3}$ receptors are considered to have low dyskinesia liability, our data are consistent with previous research in demonstrating that

the risk is present. Studies in Parkinsonian primates have shown that ropinirole causes dyskinesia, but the severity is re- duced relative to L-DOPA (Pearce et al., 1998; Maratos et al.,

2001). Similarly, early-stage PD patients who are otherwise drug-naïve can experience dyskinesia when given ropinirole as monotherapy, but the odds are much lower than if the pa- tient was started on L-DOPA monotherapy (Rascol et al.,

2001). Although we did not compare ropinirole or D-512 to L-DOPA in the present study, our laboratory has consistently reported that a therapeutic dose of L-DOPA in rats (6 mg·kg ^{1}) induces median peak dyskinesia scores of 5–6 (on the abnor- mal involuntary movements scale) that are stable for approx- imately 1–2 h (Bishop et al., 2012; Ostock et al., 2015; Lindenbach et al., 2016). Considering that, in the present

study, neither drug caused dyskinesia greater than a median score of 2.5, it seems reasonable to conclude that both ropinirole and D-512 are less dyskinesiogenic than L-DOPA.

It is unclear why $D_{2/3}$ agonists produce dyskinesia, but the reason may relate to the interactions between D_1 and D_3 re- ceptors. While D_1 receptor knockout is sufficient to almost completely abolish dyskinesia, D_3 receptor knockout reduces dyskinesia severity by less than half (Darmopil et al., 2009; Solis et al., 2015). D_1 and D_3 receptors form tetrameric protein complexes. Within these complexes, D_3 activation attenuates the effect of D_1 receptor agonists on canonical G-protein- dependent cAMP formation but potentiates the effect of D_1 agonists on G-protein-independent MAPK activity (Ferre et al., 2014; Guitart et al., 2014). This finding is relevant for dyskinesia since induction of MAPK activity appears to pro- mote the development and expression of dyskinesia (Pavon et al., 2006; Santini et al., 2007). In support of this notion, D_3 receptor knockout in vivo reduces L-DOPA-induced dyski- nesia, at the same time reducing the ability of L-DOPA to stimulate the MAPK pathway (Solis et al., 2015). Thus, mono- therapy with a $D_{2/3}$ receptor agonist may elicit some degree of dyskinesia, albeit less than L-DOPA, by enhancing constitu- tive activity of canonically D_1 receptor-mediated signalling pathways.

Clinical implications

While $D_{2/3}$ agonists are often used to treat motor symptoms in early-stage PD, accumulating evidence suggests that these compounds may also benefit non-motor symptoms. PD pa- tients often have clinically significant anxiety, depression and/or apathy, with prevalence estimates for at least one symptom ranging from 20 to 60% (Gallagher and Schrag,

2012). $D_{2/3}$ agonists such as ropinirole or pramipexole re- duce non-motor PD symptoms, even though traditional anti- depressants that target the 5-HT or noradrenaline transporter are often ineffective (Pahwa et al., 2007; Chaudhuri and Schapira, 2009). These clinical findings are bolstered by animal work showing that dopamine depletion with 6-OHDA causes increased phenotypic expression of anx- iety, depression and apathy, which can be reversed by treat- ment with dopaminergic agonists, especially those targeting the D_3 receptor (Bonito-Oliva, Masini, and Fisone, 2014; Carnicella et al., 2014; Favier et al., 2014). Further studies are needed to assess if D-512 is more effective than ropinirole or pramipexole, in terms of relieving non-motor PD symptoms.

D-512 in vitro, but not ropinirole, attenuated dopaminer- gic cell loss after 6-OHDA if the two drugs were administered

concomitantly (Santra et al., 2013; Shah et al., 2014). Similar neuroprotection was found in vivo when D-512 was co-administered with the monoamine toxin MPTP (Shah et al.,

2014). These effects might be due to antioxidant properties and other neuroprotective properties of the indole and aminothiazole moieties in the D-512 molecule (Johnson et al., 2012; Santra et al., 2013; Shah et al., 2014; Voshavar et al., 2015).

In the present study, the most obvious benefit of D-512 over ropinirole is an enhancement of peak-dose efficacy and an extension of the duration of action. Stimulation of spontaneous movement by ropinirole was complete after

2 h, while the effects of ropinirole on motor performance and rotations did not reach statistical significance. By con- trast, D-512 significantly enhanced spontaneous locomo- tion, motor performance and rotations throughout the

4 h of testing. Dyskinesia was observed with both drugs. However, D-512 showed a consistently greater ratio of drug-stimulated rotations relative to dyskinesia, suggesting that the efficacy to side-effect ratio of D-512 was greater than that of ropinirole (Figure 7). This is consistent with the theory that increasing the half-life of dopaminergic ag- onists makes them less prone to cause dyskinesia due to more consistent stimulation of dopamine receptors across time (Olanow et al., 2006).

There is growing evidence that D-512 has a better profile to other dopamine receptor agonists in terms of pharmacoki- netics (Figure 1), symptomatic efficacy (Figures 3–7) and neu- roprotection (Santra et al., 2013; Shah et al., 2014; Voshavar et al., 2015). Considering that $D_{2/3}$ receptor agonists remain front-line treatments for early-stage PD, our results suggest that further investigation of D-512 could prove useful in im- proving the treatment of PD.

Introduction

Parkinson's disease (PD) is the 2nd most common progressive neurodegenerative disorder after Alzheimer's disease, mostly affecting the elderly population. The primary clinical hallmark of the disease is severe motor dysfunction. PD is clinically characterized by

tremors, bradykinesia, rigidity and postural instability, all representative features of motor dysfunction characteristic of the disorder (Carlsson 1959, Parkinson 2002). Cardinal motor symptoms associated with the disease are due to the selective loss of dopaminergic neurons within the substantia nigra pars compacta (SNpc) (Barbeau 1962, Bertler & Rosengren

1959, Hornykiewicz & Kish 1987). Loss of these cells is associated with significant changes in oxidative stress markers (Hornykiewicz & Kish 1987, Sofic *et al.* 1992), increased iron accumulation (Jellinger *et al.* 1992, Jellinger *et al.* 1990), and accumulation of intracytoplasmic protein-rich inclusions called "Lewy bodies" (Braak *et al.* 2003, Forno

1996).

The etiology of PD has not been fully elucidated. Aging (Hornykiewicz 1989), environmental toxins (Langston *et al.* 1983, Tanner *et al.* 2011), mitochondrial dysfunction (Schapira 1993), and genetic mutations (Chartier-Harlin *et al.* 2004, Polymeropoulos *et al.*

1997, Singleton *et al.* 2003) have all been shown to increase the risk for PD. Genetic mutations, however, only account for 5–10% of PD cases (Lesage & Brice 2009). Excessive formation and/or lack of detoxification of destructive oxygen radicals and hydrogen

peroxide (collectively referred as reactive oxygen species, "ROS") in critical areas of the brain are associated with neuropathology in the more common sporadic form of the disorder, likely occurring as a consequence of aging and/or environmental exposures over a lifespan (Hornykiewicz & Kish 1987). Amongst the various organelles and enzymes that can

generate ROS within the cell, mitochondria are responsible for more than 90% of ROS generation. Various environmental toxins associated with PD including rotenone, MPTP, and paraquat, all result in inhibition of mitochondrial complex I, leading to formation of

defects in the electron transport system. Mitochondrial dysfunction caused by environmental toxins and/or aging itself may result in leakage of electrons and cellular energy deficiency. Leaked electrons contribute to the generation of ROS. Energy deficiency and ROS together likely contribute to PD cell death (Chinta & Andersen 2008, Jenner 2003). The selective vulnerability of dopaminergic neurons in PD implicates dopamine (DA) itself as another major contributing factor in disease initiation and progression. DA auto-oxidation as well as its metabolism by monoamine oxidase B (MAO-B) can yield 6-hydroxydopamine (6- OHDA) and dopamine quinones which can increase ROS generation (Linert & Jameson

2000). The iron content in the SNpc of PD patients has also been shown to be elevated (Jellinger et al. 1992, Jellinger et al. 1990). Iron can act to generate highly reactive hydroxyl radical via the Fenton reaction. ROS generated by these various factors are highly unstable and can instantaneously oxidize biomolecules in their vicinity. Post-mortem analyses of the SNpc from PD patients versus controls indicate significant elevations in lipid peroxides, DNA oxidation, and protein carbonyls, indirect markers of oxidative burden (Zecca *et al.*

2004). Loss of antioxidant capacity within the PD SNpc may also contribute to increased ROS and subsequent damage; for example, levels of total as well as reduced glutathione (a thiol tripeptide) have been shown to be significantly depleted in the SNpc of brains of PD patients (Sofic et al. 1992).

Currently available clinical therapy for PD targets restoration of DA levels within the nigrostriatal tract, preventing symptomatic effects associated with the disorder without addressing the underlying neuropathology. L-DOPA, the first FDA-approved drug treatment

for PD which is still widely-utilized in patients with the disorder, is a precursor of DA that is converted in the brain by the enzyme dopa-decarboxylase (Cotzias *et al.* 1967). L-DOPA usage is unfortunately associated with side-effects including dyskinesia and its long-term

use can produce sudden "on-off" effects (Marsden & Parkes 1976). L-DOPA has also been reported to increase levels of oxidative stress and to enhance disease progression (Basma *et al.* 1995, Fahn 1996). DA agonists including pramipexole and ropinirole are also widely

used for treatment of the disease. They too provide only symptomatic relief and may only be helpful during the early phases of PD. The development of clinically viable drugs that act as disease-modifying agents rather than providing only symptomatic relief is therefore crucial for the treatment of this devastating disorder. PD is a complex disease with multiple pathogenic factors and thus it would be of great value to develop novel therapeutics that can act on various mechanisms associated with the overall disease process (Van der Schyf *et al.*

2007, Youdim 2010, Youdim 2013). In our continued efforts to discover multi-pronged therapeutics targeting multiple complex factors involved in PD neuropathology, we have developed a series of dopamine D2/D3 agonist compounds that possess potential antioxidant, iron-chelator, and neuroprotective properties (Li *et al.* 2010, Gogoi *et al.* 2011,

Johnson *et al.* 2012). Here, we describe the evaluation of one of our lead compounds, D-512 (Figure 1), a novel highly potent D2/D3 receptor agonist, as a novel symptomatic and

neuroprotective treatment agent for PD (Johnson et al. 2012). Recently, we have shown that D-512 significantly attenuates 6-OHDA- and MPP^+-induced neurotoxicity in dopaminergic

MN9D cells in a dose-dependent manner. Inhibition of caspase 3/7 activity and reductions in lipid peroxidation along with restoration of tyrosine hydroxylase levels in 6-OHDA-treated cells may partially explain D-512's mechanism of action (Santra *et al.* 2013). In this current study, we further explore the neuroprotective effect of D-512 in an alternative cellular

model, rat adrenal phaeochromocytoma PC12 cells (a rat pheochromocytoma line), against

6-OHDA-induced cytotoxicity, as well as possible mechanisms involved. 6-OHDA is a widely used toxin that mimics the generation of oxidative stress observed in PD. 6-OHDA induces neurotoxicity via its auto-oxidation and subsequent hydrogen peroxide generation (Blum *et al.* 2000, Soto-Otero *et al.* 2000). We have additionally carried out studies assessing the effects of pre-treatment with D-512 in an *in vivo* MPTP model of PD in terms of its ability to abrogate reductions in striatal DA levels, loss of DAergic SNpc neurons, and motor dysfunction associated with this model (Langston & Ballard 1983, Przedborski *et al.*

2000, Jackson-Lewis & Przedborski 2007).

Discussion

In our recent publications, we have shown that newly developed D2/D3 agonists may act as both symptomatic and neuroprotective agents for the treatment of PD (Biswas *et al.* 2008, Li et al. 2010, Gogoi et al. 2011). In this regard, we have recently characterized the neuroprotective effects of two lead compounds, D-512 and D-440, via assessment of their

effects on neurotoxicity associated with 6-OHDA and MPP^+ administration in DAergic

MN9D cells (Santra et al. 2013). We have now begun to further explore the possible mechanisms underlying D-512's neuroprotective effects in several additional studies. We have reported that D-512 inhibits the activity of apoptotic enzymes caspase 3/7 and restores the loss of tyrosine hydroxylase levels induced by the treatment with 6-OHDA *in vitro*. In order to better understand the scope of neuroprotection induced by D-512, we have carried out additional *in vitro* and *in vivo* experiments.

Oxidative stress has been strongly implicated in neurodegeneration associated with PD. 6-OHDA is a neurotoxin that produces oxidative stress in both *in vitro* and *in vivo* experimental PD models (Soto-Otero et al. 2000). 6-OHDA has been demonstrated to induce apoptosis in PC12 cells via release of cytochrome c and activation of caspase-3 (Ochu *et al.* 1998). In order to further validate our results on the neuroprotective effects of D-512 in MN9D cells, we carried out additional experiments using PC12 cells. 6-OHDA produces dose-dependent toxicity in PC12 cells, with 75 μM producing ~50% cell loss (Figure 2a). D-512 alone was found to have no effect on cell viability (Figure 2b). Interestingly, this lack of effect of different doses of D-512 on cell proliferation is different

from the drugs effect in MN9D cells where a significant cell proliferation occurred at higher concentrations of the drug (Santra et al. 2013). Compounds with trophic factor properties have

been shown to induce cell proliferation in MN9D cells (Signore *et al.* 2006). The fact that we do not observe any D-512-induced cell proliferation in PC12 cells underscores an important difference between the two cell types. D512 was found to be neuroprotective against 6-OHDA toxicity following both pre-treatment and co-treatment or pre-treatment alone (Figures 2c, 2d) in PC12 cells. Thus, our current data establish the neuroprotective property of D-512 beyond a single specific DAergic cell line.

In our earlier study, we demonstrated that D-512 has potent *in vitro* antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Johnson et al. 2012). In our current study, the capacity for D-512 to reduce intracellular ROS levels was assessed via the DCF- DA assay. As described in Figure 3b, as assessed by DCFH fluorescence, dose-dependent pretreatment with D-512 reduced the production of ROS generated by 6-OHDA. Treatment with drug alone however did not alter the levels of ROS compared to untreated controls (Figure 3a). These results suggest that D-512 is able to inhibit ROS produced by 6-OHDA. This may be due to its ability to either scavenge free radical species, or by enhancing the endogenous cellular antioxidant defense system, or a combination of both processes. In our efforts to further ascertain the anti-oxidant activity of D-512, a cell-based lipid peroxidation assay was carried out using sodium nitroprusside (SNP), a strong oxidizing agent. As shown in Figure 4, D-512 reduced lipid peroxidation induced by 200 μM SNP in a dose-dependent manner. These results further strengthened the suggestion that D-512 is capable of

neutralizing cellular ROS, thereby decreasing oxidative stress.

Oxidative stress can lead to DNA damage including fragmentation. Treatment with 6- OHDA has been previously shown to cause nuclear chromatin condensation and DNA fragmentation (Kim *et al.* 2011). In our hands, treatment of PC12 cells with 6-OHDA for 24 hours produced significant nuclear condensation compared to controls as assessed by Hoechst 33342 staining (Figure 5a). Pretreatment with D-512 reduced this significantly. Treatment with drug alone produced a similar profile as control, indicating no effects on DNA. Further DNA laddering experiments validated these findings. As shown in Figure 5b,

6-OHDA caused a significant fragmentation of DNA, while untreated control cells displayed no DNA fragmentation (Lane 2 and 3). Pretreatment with D-512 produced a dose-dependent reduction of DNA fragmentation with the highest dose (10 μM) producing the greatest effect (Lane 5, 6 and 7). Pretreatment with D-512 alone did not produce any DNA fragmentation (Lane 4).

To further validate the neuroprotective effects of D-512 noted in our *in vitro* studies, we carried out additional *in vivo* neuroprotection studies using the well-characterized MPTP mouse model. Systemic administration of MPTP results in selective destruction of DAergic SNpc neurons in both primates and rodents resulting in an acute Parkinsonism phenotype (Lee *et al.* 2009, Przedborski *et al.* 2004). MPTP has been demonstrated to exert its neurotoxic effect via selective inhibition of mitochondrial complex I activity resulting in both a reduction

in ATP synthesis and accumulation of reactive oxygen species. MPTP reproduces many hallmark symptoms of the disease, including inhibition of mitochondrial complex I activity, decreased GSH and increased oxidative stress levels in the SN, preferential neurodegeneration of the DAergic nigrostriatal system, striatal DA depletion, and motor control deficits. The MPTP mouse model is currently widely used to study the disease (Kaur et al. 2003).

As shown in Figures 6, pretreatment with D-512 improved MPTP-mediated effects on locomotor behavior as assessed by the pole test. Results from the pole test indicate that pretreatment with D-512 improves motor coordination as measured by a greater than 50% decrease in latency time compared to MPTP-only treated controls. This result clearly demonstrates that D-512 provides potent protection of motor function against the neurotoxic effects of MPTP. As shown in Figure 7A, neurochemical analysis of the striatum revealed significant protection (>24%) against MPTP-mediated losses in striatal DA content in the group pretreated with D-512 followed by MPTP compared to the MPTP-alone treated group. Protection against striatal DAergic loss correlated well with immunohistochemical analysis

of numbers of DAergic SN cells in the D-512 pre-treatment group compared to the MPTP- only group (Figure 8a–b). In order to assess whether D-512 had any effect on metabolism of MPTP to its neurotoxic metabolite MPP^+, we evaluated the level of MPP^+ in both drug treated/MPTP and control MPTP groups. The results indicate that D-512 did not influence the metabolic conversion of MPTP to MPP+ demonstrating that neuroprotection conferred by D-512 is not due to alterations in production of MPP^+. It is important to note that the

anti-parkinsonian effect of D-512 was exhibited at a dosage comparable to that used in the current MPTP study (Santra et al. 2013). This is highly relevant in the context of our

multifunctional drug development approach to address both symptomatic (relieving motor

dysfunction) and development of disease-modifying neuroprotective effects to slow or stop the progression of the disease.

Conclusion

We have shown that D-512 is neuroprotective in an *in vitro* model of DAergic neurons (PC12 cells) treated with the neurotoxin 6-OHDA. In our effort to understand the mechanisms underlying this protection, we carried out further *in vitro* studies with the drug. In both DCF-DA and lipid peroxidation assays, D-512 was found to be an inhibitor of ROS production, confirming its potent antioxidant activity. This may be mediated by the ability of the compound to scavenge ROS or by enhancing endogenous cellular antioxidant defense systems. Pretreatment with D-512 was also found to prevent nuclear condensation and DNA fragmentation, consistent with its ability to inhibit apoptotic mechanisms. Further *in vivo* studies using the mouse MPTP administration model demonstrated that D-512 conferred significant protection in terms of both loss of DAergic SNpc neurons and locomotor

behavior associated with MPTP. These results validate the multifunctional symptomatic and potential disease-modifying properties of D-512.

D-512: A Potential Dopamine Agonist for Parkinson's

An Investigational Drug That Battles Parkinson's Disease From the Beginning

Medications called dopamine agonists like Requip (ropinirole) and Mirapex (pramipexole) are commonly used to treat the symptoms of Parkinson's disease, especially in the early stages.

Dopamine agonists are usually prescribed by neurologists as either a means of lengthening the time it takes before a person has to start (or increase) their dose of levodopa. Levodopa is the most effective medication for Parkinson's, but its effectiveness decreases the longer someone is on the medication.

In addition to being inferior to levodopa in treating motor symptoms, dopamine agonists do nothing to slow down the disease.

These downsides have sparked researchers to develop a new dopamine agonist, called D-512, which not only appears superior to other dopamine agonists in terms of managing motor symptoms but may protect existing nerve cells, potentially putting the brakes on a person's disease (a remarkable feat).

It's important to understand that D-512 is in the very early phases of research. In fact, it's only been studied in animals. Still, it's a good first step towards finding a medication that battles Parkinson's disease from the beginning.

Overview of D-512

Parkinson's disease involves the loss of dopamine-producing nerve cells in a region of the brain called the substantia nigra. Since dopamine is a brain chemical (called a neurotransmitter) that is needed for the body to move, motor (movement-related) symptoms arise from this loss.

While there are a number of motor symptoms associated with Parkinson's disease, four cardinal ones are:

- Resting tremor
- Bradykinesia (a decreased ability to move)
- Rigidity
- Postural instability

As a dopamine agonist, D-512 binds to dopamine receptors, or docking sites, in the brain. By directly stimulating these receptors, D-512 mimics the brain chemical dopamine (so the brain thinks it has dopamine when it really doesn't).

D-512 is different from other dopamine agonists, though, because it has a higher affinity for dopamine receptors. This means it can bind more easily and more tightly, which makes it last longer.

In addition to having a higher affinity for dopamine receptors, D-512 is believed to protect the dopamine-producing nerve cells that are still living, presumably by reducing oxidative stress (a key feature to the "why" behind Parkinson's disease). By reducing oxidative stress, D-512 would be considered to have antioxidant properties.

In other words, researchers believe D-512 could be a disease-modifying treatment for Parkinson's disease because it may slow down its progression.

The Science Behind D-512: An Animal Study

In one study in the *British Journal of Pharmacology*, the brains of rats were infused with 6-hydroxydopamine (a dopamine neurotoxin to mimic the disease of Parkinson's in humans). Then, the rats were given either D-512 or Requip (ropinirole), and the effects were compared.

Results

Study results revealed a higher brain uptake and blood levels of D-512 than ropinirole.

Moreover, while both D-512 and ropinirole increased spontaneous movements (in the rats) to a similar degree right after injection, the duration of motor activation was longer for D-512 than ropinirole.

More specifically, the anti-Parkinsonian effects of ropinirole lasted only about two hours whereas the anti-Parkinsonian effect of D-512 lasted for at least four hours.

Side Effect: Dyskinesia

D-512 was observed to cause dyskinesia, but to the same severity as Requip (ropinirole), in the rats. Dyskinesia refers to abnormal movements like writhing or twitching that are out of a person's control.

It's important to note that while dyskinesias are a common side effect of levodopa, occurring in around 50 percent of people with Parkinson's disease at five years, they are much less common in people taking dopamine agonists.

In fact, research reveals that dyskinesias, when a person is taking dopamine agonists alone, occur in about 5 to 7 percent of people with Parkinson's—and if dyskinesias do occur, they are generally milder in severity and occur later.

Bottom Line

All in all, dyskinesias are not a huge problem in people taking dopamine agonists alone (without levodopa), so there is still likely an improved benefit-side effect ratio of taking D-512, as compared to other dopamine agonists like Requip (ropinirole).

Remember, this is an animal study, so it's too early to make any conclusions yet. The bottom line here is that the effects of D-512 need to be translated into human use.

Dopamine Agonists and Their Role in Non-Motor Symptoms

In addition to treating motor symptoms in early-stage Parkinson's disease, scientific evidence suggests that dopamine agonists benefit non-motor symptoms, especially mood problems like anxiety, depression, and/or apathy.

Dopamine agonists may also improve certain autonomic problems like sexual function or sweating, as well as specific sleep problems in Parkinson's disease like restless leg syndrome or sleep fragmentation.

This is promising, as experts are focusing more and more on non-motor symptoms, as they often start earlier than motor symptoms and can be debilitating.

That said, it's unclear whether D-512 would be superior to traditional dopamine agonists like Requip (ropinirole) or Mirapex (pramipexole) in easing these non-motor symptoms.

A Word From Verywell

In the animal study mentioned, the biggest benefit of D-512 over Requip (ropinirole) is that it lasts longer and is better at its peak effect.

Still, more studies are needed to better understand if a compound like D-512 is truly better than current dopamine agonists for treating people with Parkinson's.

Besides motor symptoms and side effects, other factors need to be considered like a person's quality of life, the time postponed to starting levodopa, and whether D-512 is truly disease-modifying (can it protect the dopamine-producing nerve cells that are still living).