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An Attempt to Unearth the Chemical and Molecular Pathogenetic Mechanisms behind Causation of Parkinson's Disease After Two Centuries Since its First Description

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Abstract

Parkinson's disease (PD) is an age-related neurodegenerative disorder that affects approximately 1 million persons in the United States. It is characterized by resting tremor, rigidity, bradykinesia, gait disturbance and postural instability. Its pathological features include degeneration of dopaminergic neurons in the substantia nigra pars compacta coupled with intra-cytoplasmic inclusions known as Lewy bodies. Neuro-degeneration and Lewy bodies can also be found in locus ceruleus, nucleus basalis, hypothalamus, cerebral cortex, cranial nerve motor nuclei, and central and peripheral components of autonomic nervous system. The appearance of Lewy-body-like inclusions in nigro-striatal terminals might be followed by retrograde degeneration, further accumulation of aggregated proteins in nigral cell bodies and, finally, reactive gliosis and cell death. In familial forms of Parkinson's disease, linked to mutations in α-synuclein, it is proposed that a loss of normal function of this protein, as well as a toxic effect of altered forms of the mutant protein, promote the accumulation of dopamine in cytoplasm. This would result in oxidative stress, leading to the onset of neurodegenerative changes mentioned above. Finally, we review evidence for a role of α-synuclein in synaptic vesicle recycling and suggest that impaired function of this protein might lead to accumulation of dopamine in cytoplasm. This could be the final deleterious event that triggers the death of nigral dopaminergic neurons in PD.

Keywords: Parkinson's disease; Chemical; Molecular; Mechanisms; Alpha synuclein; MPTP; Genetics; Dopamine; Oxidative stress; Mitochondrial dysfunction; Excitotoxicity; Inflammation; Apoptosis; Pathogenetic

Introduction

Parkinson's disease (PD) initially described by Mr James Parkinson in his book "An Essay on the Shaking Palsy" in 1817 is one of the first two most common neuro-degenerative disorders, affecting a particular set of elderly people. There, he elaborates on the clinical features of six different individuals and described it as a disease entity. In a small monograph, Mr Parkinson amazingly described their presentation and it's progressively worsening nature, calling it "the shaking palsy"; also called "paralysis agitans". It was because of the tireless efforts of the French neurologist Mr Charcot that the disease was named as "Parkinson's disease" and the cardinal features of this disease entity include flexed posture, tremor at rest and shuffling gait. PD is most common in older adults, with its peak around sixth to seventh decade. According to Elbaz et al., the probability of developing PD progressively increases with age, with around two percent lifetime risk. A family with an affected individual is known to double the risk to its members. Twin studies by Tanner et al., debate that onset of PD before an individual crosses his fifth decade is more likely to establish a genetic relationship, and that the incidence and prevalence rates are higher in males [1]. History and neurological examination forms the main basis of diagnosing a case of PD and currently none of the laboratory tests can definitely establish the diagnosis of PD. Progression of PD can be suspected when one notes the development of motor fluctuations or dyskinesia in a PD patient, independently or together with a requirement for larger and /or more frequent drugs or combinations of drugs. There are certain motor symptoms of PD, which may not respond to medicine adjustments, such as: postural instability and falls, freezing of gait, fatigue, dysarthria, dysphagia and at times, tremors [2].

Levodopa was introduced much later, as a reliever of the symptoms of PD, since it took nearly 14 decades for the cross-verifying reports to conclude, with various drug formulations and combinations, before levodopa was accepted as a therapeutic agent [3,4] . Cotzias et al. in 1967 postulated that, very high doses of D, L-Dopa [5] were tried initially, before the use of L-Dopa (since D,L-Dopa turned out to be myelotoxic), and finally, addition of carbidopa, a decarboxylase inhibitor let us to use much lower dose of L-Dopa with consequent minimization of peripheral side-effects [6]. The epoch of L-Dopa and other dopamine agonists, as an initial therapy for treating motor symptoms continues till date [7]. Mr Benabid AL's revolutionary work lead to the introduction of deep brain stimulation (DBS) for the surgical treatment of PD's motor symptoms, in which high frequency thalamic stimulation was demonstrated to suppress tremors [8]. It was later proposed that the dominant pathologic network activities and the chemical effectors, of both neuronal and

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non-neuronal components had significant roles to play. Following identification of sub-thalamic nucleus as the treatment target, DBS has been widely used as a safe, non-pharmacological approach for the treatment of PD and it is now considered to treat many other diseases too. Further betterment in either medical or surgical management of patients with PD depends largely on our understanding of the chemical and molecular pathogenetic mechanisms underlying the disease. Hence the present review was undertaken to address this critical issue and also with an intention to open more avenues for the possibility of development of biomarkers, aiding in its early diagnosis and better management.

Literature Review

Biochemical Pathology of PD

In nearly two centuries since its first description, we have gained quite a lot of knowledge about this crippling disease, including the location of primary lesion, its principal deficit and its varied clinical manifestations. However, it is only in the last few years or so that insights have begun to emerge regarding its causative factors and possible treatment options. PD is known to affect many parts of the nervous system. Although Mr. Parkinson, during his initial description, focused completely on its motor symptoms, off late, we have realized that the disorder is not so simple. PD's main pathological hallmark is a pronounced loss of dopamine producing neurons in SNc (substantia nigra pars compacta) and VTA (ventral tegmental area), resulting in a drastic depletion of dopamine in corpus striatum. In addition, there is a progressive loss of other types of mono-aminergic neurotransmitters also: like norepinephrine in locus coeruleus' pigmented neurons and serotonin in raphe nucleus of pons and medulla. Mono-aminergic chemical loss explains the early motor symptoms of bradykinesia, rigidity and tremor; but the later motor symptoms of loss of postural reflexes, flexed posture and freezing episodes exhibit poor correlation with dopaminergeic (DA) deficit. Further, reduced thalamic cholinesterase activity indicates reduced cholinergic output of pedunculo-pontine nucleus (PPN) and this explains the characteristic parkinsonian gait. Cortical loss of ACh probably contributes to dementia [9].

There exist multiple circuits within the basal ganglia, probably affected, resulting in patients presenting with a wide array of dysfunctions in addition to its classic motor symptoms. These include a number of psychiatric and cognitive domain dysfunctions, like dementia, hallucinations, depression, irritability, apathy, anxiety and delusions. Dementia is off late recognized as one of the main non-motor presentations of PD. It remains a major cause of disability and there exists no effective symptomatic treatment as of now, unlike that of motor symptoms. Demented PD patients exhibit a worsening clinical course, and are more likely to be hospitalized sooner or later. Before death, even mild PD patients exhibit a loss of nearly 60% of their dopaminergic neurons, and it's this, together with probable dysfunction of residual neurons, which accounts for ~80% DA loss in corpus striatum. Once released out of synapse, a fraction of DA diffuses into the extracellular space, where it action time is extended as a result of comparative deficiency of sites with high-affinity dopamine uptake. When dopamine levels become too low to be rapidly compensated or when some physiologic, pharmacologic or environmental challenges are encountered, neurologic deficits being to appear. These deficits can subside only if additional compensations occur at a more rapid rate than the underlying neuro-degeneration, such as recruitment of additional dopamine receptors, induction of the synthesis of tyrosine hydroxylase, its sprouting or itsregeneration [9]. Clinico-pathologically, Parkinson's is defined by Lewy bodies (LBs), intra-cytoplasmic inclusions found in and around substantia nigra (SN), dorsal vagal nucleus and nucleus basalis of Meynert [10,11]. Lewy neuritis, is also seen in corpus striatum, autonomic ganglia, peripheral nervous system and other regions of CNS, including cerebral cortex though not frequently [1]. An important element of these lewy bodies is α–Synuclein [3].

Etiopathogenesis of PD

Abnormal aggregation of α–Synuclein [12] is suspected as a key event in DA neuronal cell death in PD. Additional pathologies contributing to the development of PD includes extensive axonal pathology and abnormal aggregation of α-synuclein [13] Till date, the reason for selective susceptibility of mesencephalic dopaminergic neurons for the development of PD remains unanswered. Few researchers have described the inhomogeneity of dopaminergic neurons as a possible explanation [14]. Such diversityis not limited just to its functions and afferent and efferent connections, but also to diverse gene expressions with a dynamic molecular variability. Based on these, experts have reconsidered the current understanding on calcium homeostasis, voltage gated L-type calcium channels and its bidirectional functions, ATP sensitive metabolically gated potassium channels, their activity patterns and its associated feed-back/feed-forward nigral signaling mechanisms and their probable contribution to neurodegeneration [15].

Role of α-Synuclein in causation of PD

α-Synuclein is a tiny protein, made up of 140 amino-acids, normally occurring in an unfolded state. But at high concentrations, it can form oligomers called Protofibrils, that seed in a nucleation-dependent manner. Immuno-cytochemistry has shown that α-synuclein is a major component of Lewy bodies both in patients with sporadic and familial PD, even without corresponding gene mutation. This points out to the fact that α-synuclein accumulation may be central to development and even progression of PD. It is therefore proposed that various etiological triggers lead to a cascade of events that involves misfolding or loss of normal function of α-synuclein and in dopaminergic neurons, promotes an elevation of cytosolic dopamine [2].

α-Synuclein can even associate with synaptic vesicle membranes forming Protofibrils. These protofibrils permeabilize the synaptic vesicles that store dopamine by forming pore-like assemblies on their surface. This allows leakage of vesicular DA into cytosol, thus enriching cytosolic dopamine to enhance oxidative stress. A vicious cycle is at play because cytosolic dopamine forms adducts with α-synuclein that slows the re-conversion of protofibrils to fibrils. These oligomers then lead to more permeabilization of synaptic vesicles and more cytosolic DA. These changes in turn, could lead to formation of ROS (reactive oxygen species) and its resultant harmful effects, protofibrillar α-synuclein accumulation or saturation of the pathway of UPP. Alterations in the structure or function of α-synuclein and changes in DA homeostasis can aggravate each other and set off a feed forward vicious cycle. Mutations or polymorphisms in other proteins involved in vesicle biogenesis or recycling too might promote this cycle. Alternatively, effects of normal or accelerated aging on nigral neurons might lower the function of UPP or lead to neuronal atrophy and cytoplasmic crowding of wild-type synuclein, promoting its aggregation. Using microtubular transport, they lucratively produce juxta-nuclear inclusion bodies known as "aggresomes", thought to be a way by which the cell guards itself from toxic effects. Through lipid rafts, it may even interact with synaptic membrane, involved in signal transduction, membrane trafficking and cytoskeletal organization and thus play an important role in vesicle

sorting and regulation of catecholamine metabolism. α-Synuclein can induce stress even in endoplasmic reticulum, eventually leading to cell death. It can even participate in protein degradation pathways such as the ubiquitin proteasomal pathway (UPP) and chaperone mediated autophagy (CMA). In this direction, it is proposed that primary proteasomal defect or even oxidant damage to proteins sufficient enough to prevent their clearance by normal proteasomal mechanisms may account for accumulation of non-mutated α-synuclein in sporadic PD patients. Further, it has been noted that nigral neuronal apoptosis is associated with enhanced expression of α-synuclein [13].

Plasma membrane bound phospholipase D2 (PL-D2) can release phosphatidic acid by hydrolyzing phosphatidyl choline, in response to an external stimulus. By modulating PL-D2 activity, synaptic vesicle formation might be regulated by α-synuclein at the pre-synaptic terminal. The released phosphatidic acid in turn, recruits adaptor molecules and triggers vesicular budding. The ability to recycle synaptic vesicles is one of the main features of CNS synapses, which, because of their size, must reuse a small pool of synaptic vesicles when neuronal activity is ongoing. In DA neurons, a drastic reduction in the vesicle number resulting from α-synuclein mutations could lead to building up of cytosomal dopamine - especially so if the feedback inhibition of tyrosine hydroxylase was aberrant. This could lead to acceleration of dopamine-induced oxidative stress. In addition to deficits in vesicle-recycling machinery resulting from altered regulation of PL-D2, mutant α-synuclein could also lead to cytosolic accumulation of neurotransmitter by permeabilizing dopaminergic vesicles. While the first mechanism is thought to result from the failure of normal function of α-synuclein, vesicle permeabilization might represent a secondary gain-of-the-function effect of protofibrillar α-synuclein. These two pathways are not mutually exclusive, given that elevations in cytosolic dopamine resulting from altered vesicular storage function would further potentiate vesicle permeabilizing effect of α-synuclein (protofibrillar).13A fragment of α-synuclein protein, known as NAC (non-beta amyloid component) has been successfully isolated from senile plaques found in brains of Alzheimer's disease patients and this NAC is known to emerge from the non-amyloid component precursor protein (NACP), homologous to α-synuclein [16].

In PD, Mr. Heiko Braak did a great work of describing the expansion of synuclein pathology in six distinctive stages along anatomical connections [17]. Brain structures first affected include: DMN (dorsal motor nucleus) of vagus, brainstem's intermediate reticular zone and anterior olfactory nucleus. It is only in the later stage that synuclein aggregates form in substantia nigra and this leads to the theory that these originate in nose or gut and then propagate to caudal brainstem and/or temporal lobe [18]. Recently, a group of researchers discovered that α-synuclein assembles not only with various structural characteristics, but that its conformation and even seeding propensity could lead to discrete histopathological and behavioural phenotypes [19,20]. This even explains the different clinico-pathological phenotypes of synucleiopathies in PD, MSA (multiple system atrophy) and dementia with Lewy-bodies. Since the dissemination of virus-mediated α-synuclein expression in vagus, follows a diffuse pattern both into and throughout the brain [21], the theory of prion-like mechanism has been debated, because it requires templating of α-synuclein, which is endogenously expressed. Braak's stages of synucleiopathy, clearly identifies PD as a multi-system disorder. PD can no more be considered as an exclusive movement disorder since the preceding symptoms of constipation, depression, orthostatic hypotension, olfactory impairment and REM-sleep behavior disorder are noticed well in advance [22]. Previously, many of these symptoms were considered as risk factors to the development of Alzheimer disease. However, now they are considered as prodromal symptoms of Parkinson's, for which α-synuclein may essentially serve as a potential biomarker. Correlating α-synuclein concentration in CSF with disease phenotype assumes importance [23]. Although aggregated α-synuclein and its spatio-temporal dissemination are the hallmarks of Parkinson's, exact mechanism by which it leads to progressive neuronal dysfunction followed by death remain unclear. Numerous factors are described to take part in the causation of PD, including a host of endogenous factors environmental factors and genetic factors.

Environmental factors contributing to Etiology of PD

The very thought of the role of environmental factors in the causation of Parkinson's disease gained momentum when it was discovered in 1983 that MPTP exposure by itself is enough to induce Parkinsonism. MPTP is highly lypophilic, can cross BBB and can form toxic MPP+ within non-dopaminergic cells, glial cells and serotonergic neurons. Upon its uptake, it causes a defect in mitochondrial complex-I, resulting in inability to transport electrons. 2Subsequently, over few years, amitochondrial complex I defect was exposed in substantia nigra (SN) of PD patients [24]. Concurrently, nigral iron toxicity [25] and oxidative stress [26] were described as main contributors to the selective dysfunction and death of DA neurons. Environmental factors more frequently associated with PD include: rural residence, well-water drinking, herbicide/pesticide exposure, industrial chemicals, farming, wood pulp mills etc. Certain infectious agents (H5N1 avian influenza and few other viruses)upon entry into CNS cause activation of microglia and finally α-synuclein aggregation. Rotenone is an effective and high-affinity complex I inhibitor. Paraquat leads to oxidative and nitrative stress. Paraquat's chemical structure is similar to that of MPP+. Exposure to a deadly duo of paraquat and maneb (agricultural pesticide), has been shown to exacerbate degeneration of dopaminergic neurons. In such a context, long term exposure to combination of various toxins would lead to higher risk of developing Parkinson's than any single toxin. Ziram, a pesticide, acts by inhibiting UPP and β-Hexa-Chloro-Cyclo-Hexane is also known to cause PD [9].

Genetics as an Etiologic factor of PD

It took nearly 180 years after Mr James Parkinson first described the disabling disease before mutation of any gene associated with PD was identified [27]. Twin studies have reported that it's not just the environmental factors inhibiting mitochondrial complex I, but also the genetic makeup of an individual, that contribute to anyone's risk of developing PD [28]. As of now, nearly 5-10% of Parkinson's patients have familial etiology [2] Genetic factors play an important role in its pathogenesis, especially of young-onset PD3 and they cause a more violent form of PD. Genes associated with PD are known to play vital roles in many cellular functions including mitochondrial function, ubiquitin-proteasomal pathway (UPP), autophagy (lysosomal pathway) and membrane trafficking. All forms of PD might involve dopamine induced oxidative stress and a disruption of α-synuclein folding or processing. Over the last decade, more than sixteen loci and eleven causative genes are identified. Main mutations reported with familial type of PD are those with: *PARK1* (α-Synuclein), *PARK2* (Parkin), *DJ-1, PINK13, LRRK23, PARK5, CYP2D6, MAO-A* and *B* [9].

PARK1 or *SNCA* gene was one of the first genes to be identified and described. It encodes α-synuclein, a component of LBs (Lewy bodies). In addition to that, various other proteins, like synphilin, ubiquitin, proteasome subunits, heat shock proteins and neurofilaments, are found in LBs of PD. Further, Ca^{2+}/calmodulin–dependent PK II and CDK-5,

have both been immune-localized to LBs. An extra antigenic element of LBs is expression of ubiquitin and proteasome, which represents the cell's effort to degrade the aggregate of abnormal protein [13] *PARK2* or Parkin mutations account for ~50% familial young-onset PD (followed by mutations in DJ-1 and PINK1). The clinical presentation of such PDs are a little different from those of common PD. Pathologically, SNc and locus ceruleus exhibit selective degeneration and gliosis. More than70 mutations involving point mutations, exon rearrangements and numerous deletions are identified. Homozygous parkin mutations cause Juvenile Parkin-related PD, while heterozygotic mutation causes late onset PD [9].

Many monogenic patterns of PD, few by means of dominant inheritance (VPS35 and LRRK2 mutations), and few others as recessive forms (Parkin, DJ-1, PINK1, PLA2GB, ATP13A2 and FBXO7 mutations) have been elaborated [29]. Among almost-all monogenic forms, LRRK2 mutations are very much prevalent and such Mendelian forms throw light on pathophysiology of hereditary as well as idiopathic PD [3]. LRRK2 and its link with α-synuclein focus on protein synthesis and vesicular trafficking as two major leading molecular mechanisms [30]. Till date, the exact cause of Parkinson's remains unexplained in many patients and is proposed to be a result of the combination of environmental, lifestyle and multiple genetic factors [29]. Epigenetic mechanism, particularly DNA methylation is shown to enhance individual susceptibility for developing idiopathic PD [31]. Many PD gene products are involved in regulation of lysosomal autophagy system [32]. Mutations occurring in PINK1, Parkin and Fbxo7 might affect mitophagy [33,34] and those in α-synuclein, Vps35 andLRRK2 have implications for autophagy. Carriers of gaucher's disease (due to mutations in β-gluco cerebrosidase gene GBA1) also have an increased risk of developing PD, and its symptoms strongly resemble those of idiopathic PD. Decreased activity of β-glucocerebrosidase leads to accumulation of glucosyl-ceramide and so lysosomal dysfunction. Activity patterns and intracellular Ca^{++} homeostasis of dopaminergic neurons of SN, as shown in numerous PD models are not just crucial for dopamine release, but even alter their mitochondrial and lysosomal activity, vulnerability to degeneration and levels of metabolic stress [3], rendering mitochondrial iron homeostasis [3], quality control andPINK1, the mitochondrial kinase, important factors in PD [34]. Human mesencephalic cells over-expressing mutant α-synuclein i.e., Ala53Thr seem to contain higher levels of extravesicular, cytoplasmic dopamine and are completely resistant to amphetamine-induced cell death. Resistance to the neuro-degeneration that was induced by numerous other toxins have also been noted in Huntington's disease's trans-genic mouse model, wherein human huntingtin gene's exon 1 with its expanded trinucleotide repeat is expressed [2]. There are certain PD families with AR (autosomal recessive) inheritance or even few monogenic forms. The p.A53T, p.A30P, G51D, E46K and G50D mutations of α-synuclein and α-synuclein gene duplication and triplication are known to lead to autosomal dominant form of inherited PD [29].

The relationship of PD syndrome with both α-synuclein mutations and MPTP exposure suggests that either genetic or environmental factor can cause PD. However, it's less likely that in majority of PD cases, it will be a single causative factor. This very concept has eventually given rise to "double hit hypothesis". It posits that Parkinson's may result due to an interplay between various genetic mutations and/or combination of an environmental toxin and a mutant gene. Although it is less likely that a mutation in α-synuclein can account for numerous cases of Parkinson's disease, this very discovery may permit development of transgenic animals and thus provide ample opportunities to understand the process of neuronal cell death in a better way [2].

Endogenous factors contributing to Etiology of PD

PD usually occurs in later half of middle age, with a marked increase in the prevalence in elderly and a loss of DA of striatum and SN with age. The timing and pattern of such losses differ between aging and PD. The loss of nigral neurons with aging is linear and predominantly in SNc's dorsal tier, while in PD it's exponential and mainly in ventro-lateral tier. In addition, Substantia Nigra in PD patients contains abundant reactive microglia, which is much less frequent in age-matched brains of controls, indicating a very active destruction. Thus, increased age cannot directly be a risk factor for Parkinson's and its precise role in pathogenesis of PD is yet to be elucidated.16Gene mutations described above are not restricted to the cells that succumb in PD. Therefore, there must be some other factors that make these cells, more vulnerable. There are some unique features of dopamine neurons, and it is plausible that one or more of these may contribute to selective susceptibility of these cells. Three of them assume importance: the presence of dopamine, the interaction between dopamine and α-synuclein and autonomous firing of SNc dopaminergic neurons following influx of calcium ions to trigger the action potential.

Dopamine induces oxidative stress (OS)

Dopamine can be auto-oxidized to oxyradicals (semiquinones and quinones) and dopamine can be enzymatically oxidized by MAO-A to form the intermediate dihydroxy-phenyl-acetaldehyde (DOPAL) and final metabolite, homovanillic acid (HVA). DOPAL has been found to enhance aggregation of α-synuclein. The cell's defense is to condense the oxidized products and turn them into insoluble Neuromelanin [16]. In adults with PD, DA neurons of SNc fire autonomously due to Ca^{++} ion influx and so they generate action potentials in the absence of synaptic input (like cardiac pacemakers). With increased intracellular Ca^{++}, there will be an increased demand on mitochondrial oxidative phosphorylation, leading to increased production of ROS and eventually cellular damage. As the cells undergo more stress over time, they "age faster". So, blocking Ca^{2+} channels in adult neurons induces a reversion of these changes. Thus, CCBs when taken as anti-hypertensive are associated with a lower prevalence of PD.

Interconnected Pathogenetic Mechanismsof PD

The best ever correlation of PD symptoms with continuing loss of the striatal dopamine are of bradykinesia and rigidity, that relate to DA deficiency in striatum and SNc. It can even be correlated well with a worsening decrease of DA imaging by scans such as PET/SPECT. Similar mechanisms may involve other mono-aminergic systems (NE and 5HT), the loss of which are thought to be instrumental in depression and anxiety in PD patients.

Multiple pathogenetic mechanisms are uncovered for loss of DA neurons, and probably much more are on their way. With the growing evidence of genetic causes for developing PD, a multiple-hit hypothesis was being proposed by Mr Sulzer (in 2007),taking into consideration, oxidative stress, excito-toxicity, mitochondrial dysfunction, inflammation and apoptosis happening in SNc. Abnormal protein aggregation and Lewy neuritis have placed a great emphasis on the accumulation of a toxic protein as the most significant pathogenetic factor. Each of the factors cross-interact with others in order to contribute to the mechanism of cell death. The toxic proteins will accumulate due to insufficient degradation or an excess synthesis.

i. **Oxidative stress (OS):** Free radical induced injury might underlie neuronal degeneration occurring in Parkinson's disease,

according to "Oxidant-stress hypothesis" or "Endogenous-toxin hypothesis". This occurs prior to nerve cell loss via mono-amine metabolism and auto-oxidation. Reduced form of glutathione (GSH) is decreased in SNc of PD patients, reflecting its excessive utilization (due to high OS). Iron and the oxidized products of proteins, lipids and DNA are also found in Substantia Nigra of Parkinson's patients. Neuro-melanin in DA neurons is formed from condensation of the oxidized DA products and so represents a fair protective mechanism against OS. Out of those mutant genes which can cause the PD, DJ-1 functions usually to defend against OS.16The Oxidant Stress Hypothesis is actually appealing because many aspects of neurochemistry of dopaminergic neurons and their local milieu within SN makes the concept reasonable. It says that SNr and Neuro-melanin granules, which are iron-rich (ferrous), may catalyze formation of hydroxyl (OH) radicals from H_2O_2. Two neurotoxins implicated to be the causative agents in few sporadic cases of PD are 6-OHDA and MPTP.6-OHDA reacts with O2 to produce superoxide radical, hydrogen peroxide and hydroxyl radical. Complex I's inhibition by MPTP and MPP+ not just interferes with synthesis of ATP, but results also in the augmented release of superoxide radical. Further, MPP+ is known to have a greater affinity for VMAT2 and can be imbibed into the dopaminergic vesicles. Further, like MPP+, amphetamines and pesticides (rotenone and paraquat) exert their effects in causing features similar to PD. In SN of brains of PD, we find a reduction in PUFA levels and a rise in MDA and lipid hydroperoxide levels. ROS lead to functional alterations in proteins, DNA and lipids. Lipid damage can in turn lead to the loss of membrane's integrity and enhanced permeability to Ca^{++} ions, which further promotes excitotoxicity. As cytosolic DA can rapidly form ROS, dopamine whether synthesized or transported into cell needs to be made harmless by its rapid storage in the synaptic vesicles. By virtue of low pH and lack of MAO enzyme, these structures provide a secured environment for DA, but this entire protective mechanism is altered in PD. It remains the first, foremost and most widely examined hypothesis of neuronal degeneration in Parkinson's disease. Nevertheless, it just remains as a hypothesis, with its own shortcomings: (a) There is no specific aspect regarding free-radical hypothesis to explain the vulnerability of DA neurons of ventral tier in PD (b) Non-aminergic neuronal groups ex. cholinergic (as in NBM) also tend to degenerate in Parkinson's, which remains unexplained by free-radical hypothesis [16].

ii. **Mitochondrial dysfunction:** It can both be a cause and a consequence of oxidative stress. A defect in mitochondrial genome can be transferred through numerous passages. And, such defect is probably because of: an inherent mutation or some sort of toxic insult may be secondary to OS. Certain factors such as MPTP, rotenone and the genes parkin and PINK1, are known to facilitate the development of PD by inducing mitochondrial dysfunction, which can impair ATP production and so hinder energy-dependent degradation of mis-folded proteins by UPP. Further, deregulation of mitochondrial respiration leads also to the generation of ROS, affects Ca^{++} homeostasis and instigates cell death pathways via apoptosis [2].

iii. **Excitotoxicity:** This results from excessive activity of glutamate, resulting in increase of Ca^{++} and can injure mitochondria and this is implicated in the pathogenesis of PD. Nitrosative stress induced by peroxy-nitrite, leads to protein nitration - a pathogenetic factor. Inflammation seen in SN of PD appears to augment the continuing degeneration [2].

iv. **Role of inflammatory mediators:** An apoptogenic-transduction pathway, mediated by oxidants may play an important role in neuronal death. A newly detected class of agents known as immunophilins tends to share its properties with so called trophic factors and immune-modulating molecules. They act by modulating cytokine production. However, it still remains unclear whether: an immune/inflammatory component is primary or secondary event in Parkinson's and whether changes noted are because of an autoimmune etiology or due to a natural response from microglia and astroglia for the neuronal damage. Thus, an in-depth understanding of role of the glial cells and its regulation of neuro-active molecules might however contribute to design of novel therapies that can protect or even repair degenerating neurons [2].

v. **Apoptosis:** It is considered to play an important role in the causation of Parkinson's disease. In this, the cell death occurs by the way of apoptosis (instead of necrosis). In general, a low concentration of a toxin can induce apoptosis especially when exposed slowly, while the higher levels or even its rapid delivery can induce necrosis. Apoptosis acts as a counter balance for excess replication of cells. Neuronal apoptosis could result from a multitude of insults, relevant to PD pathogenesis, such as: dopamine, levodopa, iron, glutathione depletion, amino acids (excitatory), MPTP, MPP+, 6-OH-dopamine, inhibitors of complex I and pro-oxidants. Quite a number of genes with their products have been known to influence apoptosis. In neuronal apoptosis, bax, bcl-2 and bcl-xL (the bax/bcl family) and the interleukin-1β converting enzyme family (ICE, ICH-1L and ICH-1S) or the caspases have gained particular attention. Increased expression of caspase or bax promotes apoptosis, while increased expression of bcl-2, bcl-xL and ich-1S promote survival [2].

vi. **Apoptosis and PD:** Apoptotic nuclei can be found in approximately 2% of SN melanin-containing neurons in patients with PD. This may reflect accelerated apoptosis due to agonal events happening in the susceptible neurons or even in those neurons which were already committed for apoptosis. Misfolded, damaged or even altered proteins can either be repaired (through chaperone-mediated mechanisms) or removed from cell (by ubiquitin proteasomal system or lysosomal autophagy), which otherwise would accumulate as toxins, damaging the cell. Autophagy is thought to play an important role in the removal of α-synuclein and other such unwanted proteins [2].

vii. **Apoptosis and Mitochondria:** Mitochondria are decisive to few forms of apoptosis. A fall in Mitochondrial Membrane Potential (MMP) together with a raise in intra-mitochondrial Ca^{++} is associated with mitochondrial mega-pore opening, known as the Permeability-Transition-Pore (PTP), and the release of AIFs (apoptosis initiating factors), such as Cyt C or ICE like protease. Factors like ADP, glutathione and ROS in mitochondrial matrix alter gating voltages needed to induce opening of PTP. Opening of PTP pore allows free interchange of solutes and small peptides between cytosol and mitochondrial matrix. AIFs from mitochondria may be directly released through PTP or may be through fractures developing in mitochondrial membrane. Agents such as cyclosporine-A or BCL-2, which are known to maintain the closure of PTP, prevent fall in MMP and release of AIF factors and are thereby anti-apoptotic. MMP will be decreased soon in the process of apoptosis, prior to onset of DNA fragmentation (nuclear) and chromatin condensation [2].

A multitude of etiological factors trigger n number of pathogenetic mechanisms just discussed, with environmental, endogenous and genetic factors being the major players. An intense interaction between the genetics and the remaining ones seem important.

"Multiple hit hypothesis" with a pivotal role of α-Synuclein

With many etiologic and pathogenetic factors, all leading to one common denominator i.e., loss of DA cells in SNc, it is challenging to blame a solitary unifying concept. Sulzer attempted to do it with multiple-hit hypothesis. He suggested that "multiple hits" combining toxic stress, to illustrate, from DA oxidation or even mitochondrial dysfunction, together with the inhibition of a neuroprotective response (like loss of parkin's function) or the autophagic degradation (stress induced), underlie the selective nerve cell death. He classifies PD causing genetic mutations into various categories: (a) proteins affecting mitochondria e.g., DJ-1, PINK1, Omi/HtrA2 and POLG(b) proteins involved in "organelle trafficking" and "vesicular fusion" eg., α-synuclein, Tau (c) proteins of ubiquitination or such other degradation pathways eg., Parkin and DJ-1 and lysosomal function e.g., β-Glucocerebrosidase (d) proteins modifying antioxidant function or oxidative stress e.g., Sepiapterin, DJ-1 and FGF-20.9 Despite the multiple hits that may be necessary for sporadic PD, α-synuclein appears to actually play a pivotal role. Further, this theory is even supported by (a) its presence in LBs (b) because of our knowledge that excess α-synuclein of wild-type can cause Parkinson's and (c) the indication that it mostly has prion like property. This implies that preventing accumulation of α-synuclein or finding ways to eliminate the same can be a very effective approach in order to arrest or even reverse disease process.

Controlling PD

Based on the existing knowledge on etiopathogenesis and mechanism of cellular death in Parkinson's, various neuro-protective methods can be devised, such as: (a) Eliminating a primary-etiology by avoiding agents interfering with excitotoxicity (or) by preventing an increase in cytoplasmic free calcium, and by the use of antioxidants, bioenergetic agents like Coenzyme Q, trophic factors and anti-inflammatory drugs (b) the selective MAO-B-inhibitor selegiline might delay emergence of the disability and slows progression of signs of PD (c) other drugs are also proved to be beneficial: Remacemide (antagonist of NMDA receptor), Riluzole (glutamate antagonist), the bioenergetic agent coenzyme-Q and DA agonists are thought to lessen the ROS formed by dopamine (d) agents that upregulate antioxidant synthesis and synthesis of anti-apoptotic chemicals, such as GSH, SOD-1 and BCL-2 might prove useful in all patients of PD regardless of their etiology. These drugs might help maintain PTP closure, preserve ΔΨM (Mitochondrial Membrane Potential) and prevent the release of AIF factors.2There are few studies which even say that neuroprotection offered by selegiline is related mainly to the inhibition of apoptotic mechanisms and not to inhibition by MAO-B, by upregulation of quite a good number of anti-apoptotic molecules, including glutathione, SOD-1, BCL2 and BCLXL. Another drug Desmethyl Selegiline (DMS) is better tolerated (even in high doses) in PD. It preferentially maintains GAP dehydrogenase as a dimer, (since in this form, it will not accumulate and so doesn't promote apoptosis). Hopefully, delineation of association between GAPDH, DMS and apoptosis will provide a path to the exploration and development of novel yet more potent therapeutic agents which can slow progression of Parkinson's disease by protecting susceptible neurons and thereby reducing death of DA neurons. Overall, it might be paving way for combined approaches, which interfere with the components of pathogenic as well as apoptotic pathways and this might eventually be needed in order to provide neuroprotection. The recent identification of a certain gene causing a PD phenotype is thought to provide an indispensable clue in determining those factors which are very much relevant to the cell death in Parkinson's and a model to test alleged neuro-protective agents [2].

Extensive neuropathology of the brainstem and cortex are thus responsible for numerous motor and non-motor indicators of PD. Though DA replacement therapy does improve the functional prognosis associated with PD, currently there is no treatment which prevents progression of the disease.

"Protective factors" associated with "lowering the risk of development of PD"

Smoking is now associated with 60% decreased risk to develop PD. Nicotine stimulates dopamine release and so, smoking cigarettes might suppress free radical formation via MAO-B-associated dopamine metabolism. Coffee consumption might be associated with 30% reduced risk for PD (estrogen use in women may eliminate this benefit). Caffeine has effects similar to A2A antagonists and suppresses MPTP toxicity through blockade of A2A receptors. A higher serum urate level is correlated well with a decline in the incidence of PD. In men, higher urate levels are associated negatively with a slow rate of PD progression, probably due to its antioxidant properties. Use of anti-inflammatory drugs is another putative protective factor [9].

Discussion and Conclusion

Although PD was first elaborately described almost nearly two hundred years ago, it's only in recent times that we started to understand intricate nature of functional deficits entailed in it or its neurobiological causes. Yet, the speed of its discovery is quite alarming. With the research pacing up on genetic basis of few familiar forms of this disorder, the discovery of the trophic factors which influence dopaminergic neurons, and the development of novel technologies including using of stem cells and viral vectors, we have every reason to believe strongly that within next generation, this crippling disease will surely become a forgotten chapter in the history of ancient diseases. Although basic chemistry and recent research dramatically escalated our knowledge and understanding of PD patho-physiology over last two centuries since its first description, various motor and non-motor PD symptoms still continue to constitute a deadly challenge for patients treated pharmacologically or even with DBS. Till date, no such treatment is yet available, which would cure or even slow the progression of synucleinopathy than just acting symptomatically. We wish that further research during the coming years makes it possible for such therapies, which will benefit PD patients and their families.

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