REVIEW

The Centrality of Mitochondria in the Pathogenesis and Treatment of Parkinson's Disease

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SUMMARY

Parkinson's disease (PD) is an incurable neurodegenerative disorder leading to progressive motor impairment and for which there is no cure. From the first postmortem account describing a lack of mitochondrial complex I in the substantia nigra of PD sufferers, the direct association between mitochondrial dysfunction and death of dopaminergic neurons has ever since been consistently corroborated. In this review, we outline common pathways shared by both sporadic and familial PD that remarkably and consistently converge at the level of mitochondrial integrity. Furthermore, such knowledge has incontrovertibly established mitochondria as a valid therapeutic target in neurodegeneration. We discuss several mitochondria-directed therapies that promote the preservation, rescue, or restoration of dopaminergic neurons and which have been identified in the laboratory and in preclinical studies. Some of these have progressed to clinical trials, albeit the identification of an unequivocal disease-modifying neurotherapeutic is still elusive. The challenge is therefore to improve further, not least by more research on the molecular mechanisms and pathophysiological consequences of mitochondrial dysfunction in PD.

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Introduction

Mitochondria are unique and complex organelles intimately involved in several key cellular processes that are critical to ensure neuronal survival. In addition to their prominent role in energy metabolism, mitochondria perform other essential functions, including the regulation of calcium homeostasis, oxidative stress response, and activation of cell death pathways. Consequently, mitochondrial pathophysiology aggressively promotes neuronal dysfunction and loss of synaptic viability, leading ultimately to neurodegeneration [1]. The most common motor neurodegenerative disease is Parkinson's disease (PD), which is generally prevalent in the elderly population and affects brain centers involved in the control and regulation of voluntary movement. The neuronal demise that contributes to the classic motor triad of the disease (bradykinesia, rigidity, and resting tremor) involves the substantia nigra (SN) and leads to a dopaminergic deficit in the corpus striatum [2]. On the basis of considerable evidence derived from genetic and toxin-induced cellular and animal models of PD, a critical role for mitochondrial dysfunction in the pathophysiology of PD is now regarded as being established [3]. Therefore, endeavors at developing mitochondria-targeted therapies that support and enhance mitochondrial function may have great potential in the amelioration of PD.

Mitochondrial Pathophysiology in Parkinson's Disease**Bioenergetic Defects and Oxidative Stress**

The vulnerability of neurons to mitochondrial dysfunction can be pinned down to their high metabolic activity and energetic requirement, especially to maintain ionic gradients across membranes and for neurotransmission [4]. The electron transport chain (ETC) of mitochondria is composed of four multiprotein complexes, named complexes I–IV, which couple the transport of electrons to the creation of a proton gradient across the inner mitochondrial membrane. Dissipation of the gradient by movement of protons through complex V drives the synthesis of adenosine triphosphate (ATP). Inefficiency in ETC function is recognized as one of the major cellular generators of reactive oxygen species (ROS) [5].

The most commonly observed bioenergetic deficit in PD is an impaired activity of mitochondrial complex I (NADH:ubiquinone oxidoreductase), the main site of entry of electrons into the respiratory chain. Complex I deficiency is linked to increased ROS accumulation, depletion of ATP, and dopaminergic neuron death [1]. Evidence obtained from postmortem samples revealed a 30–40% reduced activity of complex I in the SN of patients with

PD [6], as well as decreased activity and impaired assembly of complex I in the frontal cortex [7]. Outside the brain, inhibition of complex I has been observed in platelets and skeletal muscles of PD patients, although there is no consensus on such data [8,9].

To specifically address the question of whether complex I deficiency is etiological in PD, transgenic mice were recently generated that lack expression of a subunit of complex I (NADH: ubiquinone oxidoreductase Fe-S protein, *Ndufs4*) in midbrain dopaminergic neurons. Surprisingly, no significant neuronal loss was observed, and animals had no overt symptoms of parkinsonism [10]. However, the *Ndufs4*-knockout neurons were more vulnerable to complex I inhibition, and dopamine release was reduced from striatal axon terminals, implying that disruption of striatal dopamine homeostasis may be directly correlated with mitochondrial dysfunction [10].

Toxin-based Models of Mitochondrial Dysfunction in PD

The involvement of mitochondrial complex I, and of ETC dysfunction in general, in the pathogenesis of PD is strongly suggested by the fact that toxin-induced disruption of the ETC triggers degeneration of dopaminergic neurons and causes a PD-like phenotype in flies, rodents, and humans. Such toxins typically inhibit activity of the ETC while increasing ROS and mitochondrial permeability transition (mPT), thus impairing ATP synthesis and inducing a bioenergetic crisis.

Evidence implicating mitochondrial dysfunction in the pathogenesis of PD historically emerged with the discovery that humans accidentally exposed to the recreational drug 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) developed an acute form of parkinsonism. It was subsequently found that administration of MPTP to mice, rats, and primates also reproduced the major clinical and neuropathological hallmarks of PD [11–13]. MPTP rapidly crosses the blood–brain barrier into the brain, where it is metabolized into its toxic metabolite 1-methyl-4-phenylpyridinium ion (MPP^+). MPP^+ is subsequently taken up by dopamine transporters into SN neurons and actively concentrated in mitochondria [14]; mice lacking the dopamine transporter are resistant to MPTP toxicity [15]. At the mitochondrial level, MPP^+ blocks the transfer of electrons through complex I and inhibits the Krebs cycle enzyme α -ketoglutarate dehydrogenase, thereby eliciting a decrease in mitochondrial ATP production [16]. A more crucial effect might be the increased free radical generation which leads to significant oxidative stress and activation of pro-apoptotic pathways. The neurotoxin MPTP has been used extensively for reproducing the motor symptoms of PD in animal models and for unraveling the mechanisms and consequences of nigrostriatal neuron loss *in vivo* [17].

A complex I inhibitor closely related in chemical structure to MPP^+ is the ammonium herbicide, paraquat. Paraquat (1,1'-dimethyl-4,4'-bipyridinium) is an even more powerful inducer of ROS generation than MPTP, although the binding affinity to complex I is lower [18]. In humans, exposure to paraquat has been associated with a higher incidence of PD [19], while administration to rodents reproduces a selective degeneration of dopaminergic neurons, increased oxidative stress, and aggregation of the synaptic protein α -synuclein [20]. Of note, induction of ROS and

consequent lipid peroxidation by paraquat in mouse striatum involves nitric oxide and the increased expression of nitric oxide synthase [21].

The pesticide rotenone, another well-known inhibitor of mitochondrial complex I, impairs ATP synthesis and stimulates increased ROS formation by mitochondria [22]. Epidemiological studies have reported a significantly increased risk of developing PD for humans exposed to rotenone [23]. Indeed, rotenone rat models of PD accurately reproduce the cardinal features of PD, including selective dopaminergic neuron degeneration, loss of the nigrostriatal pathway [24,25] and motor symptoms such as ataxia, bradykinesia, and trembling in the forelimbs [26,27]. These rats also display Lewy body-like cytoplasmic inclusions [28]. Collectively, these features have made the rotenone rat model one of the most widely utilized to study neuroprotective modalities in PD. More recently, other mitochondrial ETC inhibitors have been identified that induce parkinsonism in humans or animal models. These include trichloroethylene, tetrahydro-beta-carbolines, pyridaben, tebufenpyrad, fenazaquin, and fenpyroximate. They all result in a similar loss of nigrostriatal dopaminergic neurons and a PD-like motor phenotype [13,29–31].

Genetic-based Models of Mitochondrial Dysfunction in PD

There is a striking convergence of the biochemical abnormalities from several inherited genetic defects with those found in sporadic cases. This denotes that there may be common pathways to dopaminergic cell loss and dysfunction, and thus common target areas for pharmacological treatment which may slow disease progression.

α -Synuclein

Alpha-synuclein (α -synuclein) is a 140-amino acid protein found enriched in presynaptic terminals and associated with synaptic vesicle membranes [32]. α -Synuclein is encoded by the *SCNA* gene, and missense mutations in *SCNA* were the first autosomal-dominant PD-associated mutations to be identified [33]. The three most common mutations segregating with familial PD are Ala53Thr, Ala30Pro, and Glu46Lys [33,34]. Patients harboring mutations in *SCNA* cannot be clinically distinguished from sporadic PD, although onset is typically earlier and the disease more aggressive. Neuropathological findings are also similar, with nigrostriatal degeneration and the defining deposition of amorphous Lewy bodies and Lewy neurites in the brain. Genetic studies prompted the subsequent finding of fibrillar α -synuclein as the main structural component of Lewy inclusions [35]. The α -synuclein protein displays an increased propensity for aggregation due to the presence of a central hydrophobic domain [36]. Aggregation propensity is augmented by increased protein concentration; thus, a higher dosage of wild-type *SCNA*, as in gene duplication or triplication, is associated with a more aggressive form of familial PD [37,38]. All this brought the α -synuclein protein to the fore in the investigation of PD pathogenesis.

Accumulated evidence from both *in vitro* and *in vivo* studies postulates a major pathogenic role for α -synuclein in mitochondrial dysfunction, thereby providing a link between protein

aggregation, mitochondrial damage, and neurodegeneration. Transgenic mice expressing human A53T α -synuclein developed mitochondrial DNA (mtDNA) damage and degeneration [39], with mitochondrial degeneration preceding the onset of motor symptoms [40]. A53T was found to directly localize to mitochondrial membranes and selectively inhibit mitochondrial complex I, thus suggesting a common mechanism with ETC inhibitors [41]. In primary cortical neurons that overexpress mutant A53T α -synuclein, there is massive mitochondrial destruction and loss that is associated with a bioenergetic deficit and neuronal degeneration [42]. Similarly, α -synuclein-overexpressing transgenic mice and neuronal cells exhibit mitochondrial dysfunction, increased mitochondrial DNA damage, and impaired ETC activity [43,44]. Interestingly, α -synuclein-knockout (*SNCA*^{-/-}) mice also showed reductions in ETC activity, although not in complex I. These alterations in mitochondrial function were associated with abnormalities in mitochondrial membrane properties, in particular a reduction in content of the phospholipid cardiolipin [45]. Furthermore, *SNCA*^{-/-} mice have been shown to be less sensitive to administration of mitochondrial toxins, such as MPTP, 3-nitropropionic acid, and malonate [46]. As mitochondrial toxins also promote α -synuclein aggregation, these findings suggest a functional link between mitochondrial dysfunction and accumulation of the misfolded α -synuclein protein [47]. Interestingly, also in the eukaryotic yeast *Saccharomyces cerevisiae*, there was a requirement for functional mitochondria in mediating α -synuclein-induced cell death [48].

A plausible mechanism for explaining mitochondrial dysfunction is the direct interaction of α -synuclein with mitochondrial components, especially mitochondrial membranes. Various *in vitro* investigations have correlated localization of α -synuclein aggregates to mitochondria with increased mitochondrial membrane damage and dysfunction [49–51]. Intriguingly, a cryptic mitochondrial-targeting signal has been identified in the N-terminal amino acid sequence of α -synuclein, effectively targeting the protein to the inner mitochondrial membrane, whereupon it inhibits complex I and increases ROS formation [49,52]. Another mechanism contributing to mitochondrial degeneration might involve the fragmentation of mitochondria upon direct association of α -synuclein to mitochondrial membranes [53,54].

LRRK2

Point mutations in the leucine-rich repeat kinase 2 (*LRRK2*) gene have been found in around 7% of familial PD cases with late-onset autosomal-dominant parkinsonism; mutations have also been reported in a few sporadic cases [55,56]. Patients with mutations in *LRRK2* have a clinical phenotype that closely resembles the sporadic form, and neuropathological findings typically include intracellular Lewy bodies and nigrostriatal neuronal loss [57]. *LRRK2* codes for a large protein of 2527 amino acids, with all of the identified mutations occurring in its multiple functional domains [58]. Most pathogenic mutations, including the most frequent Gly2019-Ser point mutation, increase the kinase activity of LRRK2 and mediate neuronal toxicity, although its substrates have yet to be determined [59]. The main site of action of LRRK is again the mitochondrion. Transient expression of mutant LRRK2 in neuronal cells activates mitochondrial-mediated apoptotic mechanisms

of cell death [60]. *Caenorhabditis elegans* nematodes expressing mutant LRRK2 showed selective vulnerability of dopaminergic neurons to the mitochondrial inhibitor rotenone [61]. Although predominantly cytosolic, LRRK2 can associate directly with the outer mitochondrial membrane, as demonstrated using synaptosomal cytosolic fractions from mammalian brain [62]. LRRK2 may also induce mitochondrial dysfunction by disturbing mitochondrial dynamics, particularly as it controls the expression of the mitochondrial fission factor dynamin-like protein 1 (DLP1) [63].

Parkin

Genetic mutations of the *Parkin* gene contribute to around 50% of familial PD cases having an autosomal-recessive mode of inheritance and disease onset before the age of 45 years [64]. Clinical manifestations closely resemble those of idiopathic PD, accompanied by loss of nigral neurons in the brain, although Lewy body deposition is typically absent [65]. Numerous *in vitro* and *in vivo* studies suggest that parkin function is intimately related to mitochondrial protection, especially from oxidative damage. Thus, parkin inhibited swelling of mitochondria and associated cytochrome *c* release in ceramide-treated cells and in cell-free assays [66,67]. Localization of parkin at the outer mitochondrial membrane has been observed in parkin-overexpressing cells and adult mouse brain [66,68]. Although the precise mechanism is not yet established, the ability of parkin to protect mitochondria from insult by chemical toxins has been demonstrated in a wide range of cellular and animal models [69,70]. Transgenic mice knocked out for *parkin* manifest impaired oxidative phosphorylation in striatal mitochondria, which are swollen and have fragmented cristae [71]. *Drosophila* models representing loss of parkin function have a shortened life span while showing degeneration of dopaminergic neurons and severe mitochondrial pathology [72,73]. Even mitochondria isolated from nonnervous tissues of parkin-mutant patients, such as leukocytes and fibroblasts, have various morphological abnormalities and functional deficits [74,75].

Parkin may additionally have an inherent role in regulating mitochondrial dynamics. Under normal physiological conditions, parkin is recruited selectively to impaired mitochondria, promotes mitophagy, and mediates mitochondrial elimination by catalyzing ubiquitination of targeted mitochondria [76]. Disease-associated parkin mutations, on the other hand, induce defective recognition and ubiquitination of dysfunctional mitochondria [77].

PINK-1

Another strong piece of evidence for the etiological role of mitochondria in PD comes from the discovery of families with early-onset parkinsonism caused by mutations in *PINK1* [78]. PINK1 is a putative mitochondrial serine/threonine kinase that plays an important neuroprotective role. In cell culture studies, wild-type, but not mutant, PINK1 protects against apoptotic cell death induced by MPTP, oxidative stress, and activation of the mPT pore [79–81]. PINK1 import into mitochondria occurs via an N-terminal mitochondrial-targeting sequence. Most investigations have shown that PINK1 attaches to the inner mitochondrial membrane, while others propose that PINK1 localizes to the intermembrane space [82,83]. Conversely, PINK1 suppression or knockdown in

human dopaminergic neurons increases the cellular susceptibility to apoptosis via the mitochondrial pathway, with increased oxidative stress markers, defective mitochondrial respiration, and abnormal mitochondrial morphology [84–87]. SN dopaminergic neurons prepared from *PINK1* null mice exhibited fragmented mitochondria, significant ROS generation, and a depolarized mitochondrial membrane potential [88]. Intriguingly, a functional link between parkin and PINK1 has also been found. First, the pathological phenotype of *parkin*-mutant flies closely resembles those of *PINK1* mutant flies [89]. Second, and more significantly, the effects of loss of PINK1 function in flies, including ATP depletion, shortened life span, and degeneration of dopaminergic neurons, could be rescued by means of transgenic expression of parkin [89,90].

DJ-1

Mutations in the *DJ-1* gene have been linked with rare cases of early-onset autosomal-recessive PD and are responsible for 1–2% of early-onset forms of the disease [91]. The overarching functional role of DJ-1 appears to be as an antioxidative stress sensor, particularly against oxidative stress in association with mitochondrial dysfunction [92]. Accordingly, downregulation of DJ-1 in neuronal cell models causes increased vulnerability to cell death by oxidative stress and mitochondrial toxins [92]. DJ-1 loss-of-function cellular models have a defect in the assembly of complex I and hence in mitochondrial respiration and morphology [93,94]. Likewise, *DJ-1* knockout mice suffered increased dopaminergic neuronal degeneration upon challenge by MPTP and paraquat [95,96], while *DJ-1*-deficient *Drosophila* flies were extremely sensitive to paraquat and rotenone [97]. Under basal conditions, DJ-1 is mostly a cytosolic protein, with a limited endogenous pool in the mitochondrial matrix and intermembrane space [98]. Oxidant exposure, including exposure to the complex I inhibitor rotenone, triggers relocalization of DJ-1 to mitochondria, correlating with neuroprotection [99,100]. Overexpression of DJ-1 resulted in an increased capacity to withstand these insults and reduced intracellular ROS [101]. It has been further demonstrated that DJ-1 function can be linked to other proteins implicated in PD pathogenesis, namely α -synuclein, PINK1, and parkin. In cellular models, wild-type DJ-1 inhibited the aggregation of wild-type α -synuclein, in part by demonstrating chaperoning activity [102,103]. DJ-1 also works in parallel to the PINK1/parkin pathway to maintain mitochondrial function and regulate mitophagy [104].

Genetic Defects in Mitochondrial DNA

Various somatic mtDNA deletions or rearrangements have been reported in patients with PD [105,106]. Cytoplasmic hybrid (cybrid) models, in which platelet mtDNA from PD patients is expressed in neural cell lines, have provided particularly convincing evidence [107]. For example, defects in complex I activity could be transferred from patients with sporadic PD to mitochondrial-deficient cybrid cell lines, which exhibited a decrease in mitochondrial membrane potential, impaired respiratory capacity, and abnormal Ca^{2+} sequestration by

mitochondria [108,109]. Significantly, PD cybrid cells were rescued by delivery of normal mtDNA to the defective mitochondria [110]. Conditional knockout mice have been generated with reduced or defective mtDNA expression. These mice develop a progressive motor phenotype associated with loss of midbrain dopamine neurons [111,112].

Therapeutic Approaches Targeting Mitochondria-induced Oxidative Stress

Current therapies for PD provide symptomatic control of motor impairments, but the beneficial effects wear off over time and clinical efficacy declines as the disease progresses. This is because available therapies are unable to modify the relentless nigral degeneration that underlies PD pathology [113]. The centrality of mitochondria in the pathogenesis of PD, as amply discussed above, indicates that mitochondria-directed therapeutics may offer scope for the discovery of novel disease-modifying drugs [114]. Hereunder, we discuss and evaluate the principal lines of therapies aimed at ameliorating mitochondrial dysfunction in PD. These embrace a wide range of antioxidant therapies, including small-molecule compounds, pharmacological therapies that restore mitochondrial calcium homeostasis, and peptides designed specifically to target mitochondria.

Antioxidant Therapies

Creatine

Creatine is a nitrogenous compound that is generated endogenously in muscle and nerve cells or acquired exogenously through the diet. Intracellular phosphorylation of creatine by creatine kinase generates phosphocreatine, which can be utilized in turn to generate ATP. Hence, the creatine kinase/phosphocreatine tandem functions as an energy reserve pool with consequent neuroprotection [115]. In view of the fact that PD is fundamentally characterized by a decline in cellular bioenergetics and metabolism, supplementation by exogenous creatine has been tested as a valid therapeutic intervention [116].

As MPTP models of PD are primarily based on a mechanism involving impaired energy production, creatine was administered to mice receiving MPTP. Indeed, oral supplementation with creatine protected against MPTP-induced striatal dopamine depletion as well as loss of SN tyrosine hydroxylase-positive neurons [117,118]. *In vitro*, creatine also improved the survival of tyrosine hydroxylase-positive rat embryonic mesencephalic neurons against MPP⁺ insult [119]. In 2006, data were published from a phase II clinical trial following 1 year of 10 g/day creatine administration to early-phase PD patients. Encouragingly, a 30% reduction in the Unified Parkinson's Disease Rating Scale was reported, compared to placebo [120]. A follow-up study confirmed that long-term creatine supplementation was safe and tolerable in early PD patients [121]. On the basis of these results, creatine has been selected for a larger, multicenter, phase III clinical trial initiated by the National Institutes of Health. Patients with early-stage symptomatic PD will be given 10 g creatine/day and evaluated for at least 5 years [122].

Polyphenols

Polyphenols represent a large group of low-molecular-weight secondary plant metabolites widely consumed by humans in the forms of fruits, vegetables, and beverages (e.g., tea, coffee, and red wine). As such, they are considered as an integral part of human diet with multiple health benefits, not least as nutraceutical agents in neurodegenerative diseases [123,124]. Habitual intake of polyphenol-rich foods (tea, berries, apples, red wine) was associated with up to 40% lower risk of developing PD in men [125].

The chemical structure of a polyphenol molecule typically has several hydroxyl groups attached to its aromatic rings, enabling them to function as effective ROS scavengers. Beyond their direct antioxidant and metal-chelating activities, however, polyphenols are known to modulate key cell signaling pathways involved in ROS regulation [126,127]. Moreover, increasing evidence over the last years has firmly established an important role for polyphenolic compounds as potential inhibitors of amyloid aggregation. Consistent with the “ π -stacking” hypothesis for the self-assembly of amyloid aggregates, the aromatic rings of polyphenols may themselves interact with aromatic residues in amyloidogenic proteins, thereby competitively hindering the aggregation mechanism [128]. As already mentioned, the aggregation of α -synuclein is considered to be central to the pathogenesis of PD and is intrinsically linked to mitochondrial dysfunction. A recent study on the effects of 14 natural polyphenols and black tea extract on the formation of toxic multimeric structures by α -synuclein identified the most effective flavonoid-based molecular scaffold [129]. Other than the aromatic elements required to bind α -synuclein, the study pointed to the importance of vicinal hydroxyl groups on the aromatic moiety of the polyphenol molecule. The most potent polyphenols able to interfere with α -synuclein aggregation were as follows: baicalein, epigallocatechin gallate (EGCG), myricetin, morin, and nordihydroguaiaretic acid (NDGA), apart from black tea extract [129].

The mouse model of PD induced by administration of the mitochondrial neurotoxin MPTP has been extensively utilized to demonstrate the therapeutic efficacy of a wide range of polyphenols, including EGCG, baicalein, resveratrol, kaempferol, and genistein. The neuroprotective effects variously involved preservation of tyrosine hydroxylase-positive neurons in the SN, raised striatal dopamine, increased striatal antioxidant activity, and better performance of motor tasks by the treated mice [130–134]. Extended treatment with EGCG also prolonged the life span and restored climbing ability in *Drosophila* flies chronically treated with another well-known mitochondrial toxin, paraquat [135]. Similarly, polyphenol-rich extract from whole grape (*Vitis vinifera*) fed to transgenic *Drosophila* expressing human α -synuclein improved significantly their climbing ability compared to controls. *In vitro*, the grape extract acted as a powerful ROS scavenger and maintained the activities of complexes I and II of the mitochondrial electron transport chain [136]. Myricetin, a polyphenol component of red wine, was reported to protect MPP-treated dopaminergic neuronal cells; the underlying mechanism involved suppression of ROS production by mitochondria and maintenance of the mitochondrial transmembrane potential [137].

Yet another, novel, neuroprotective mechanism which came to the fore in recent years concerns the ability of polyphenols to

interfere with disruption of phospholipid membranes induced by toxic amyloid aggregates. For instance, black tea extract was extremely effective in protecting against permeabilization of neuronal-like membranes by amyloid-beta and α -synuclein oligomeric aggregates [138,139]. Membranes of intracellular organelles can also provide targets for destabilization by amyloid aggregate species; this is especially the case for mitochondria, which are abundantly present in neuronal soma and synapses. Interestingly, black tea extract, rosmarinic acid, morin, and baicalein all proved effective in enhancing the resilience of the mitochondrial membrane barrier against insult by amyloid aggregates, including α -synuclein [51].

Vitamin E

MPTP toxicity in the mouse brain was significantly enhanced in vitamin E (α -tocopherol)-deficient mice [140], while supplementation protected against oxidative stress and SN degeneration [141]. Deficiency of vitamin E *in vivo* has also been modeled by the generation of mice lacking α -tocopherol transfer protein; these mice develop a delayed-onset ataxia on a background on chronic oxidative stress [142]. Brain mitochondrial oxidative phosphorylation in vitamin E-deficient rats was impaired, suggesting a physiological role for vitamin E in sustaining mitochondrial respiration [143]. In support of this argument, long-term treatment of organotypic SN cultures with vitamin E blocked oxidative damage and loss of tyrosine hydroxylase-immunoreactive neurons subjected to chronic complex I inhibition by rotenone [144]. Vitamin E significantly alleviated apoptosis of cerebellar granule neurons exposed to paraquat, another strong complex I inhibitor and free radical generator [145]. Furthermore, mitochondrial dysfunction and oxidative stress associated with intracellular α -synuclein accumulation were also attenuated by pretreatment with vitamin E [43].

Notwithstanding the compelling experimental data outlined above, the clinical benefit of chronic, high-dose vitamin E supplementation is still controversial. Of note, a large prospective examination on the association between vitamin E intake and risk of PD failed to find a protective effect of vitamin E supplements. A 32% reduction in risk, however, was found in association with a high intake of vitamin E from foods, suggesting that other constituents of foods rich in vitamin E may be protective [146]. A meta-analysis of observational studies published between 1966 and 2005 also found that a moderate-to-high dietary intake of vitamin E attenuates the risk of developing PD [147]. Finally, a relatively new role for vitamin E in the prevention or treatment of PD is now emerging from the use of mitochondrially targeted α -tocopherol, in which vitamin E is concentrated in mitochondria [148].

Coenzyme Q10

Coenzyme Q10 (CoQ10; ubiquinone) is an essential cofactor in the mitochondrial electron transport chain and accepts electrons from complexes I and II. CoQ10 is found in virtually all cellular membranes, including mitochondrial membranes where in its reduced form (ubiquinol) it may additionally function as an antioxidant [149]. Dopaminergic neuronal cell death mediated by the complex I inhibitor rotenone was reduced upon pretreatment

with CoQ10. The antiapoptotic mechanism of action of CoQ10 involved mitochondria, as it prevented collapse of the mitochondrial membrane potential and decreased mitochondrial production of ROS [150]. Dietary CoQ supplementation attenuated the MPTP-induced loss of striatal dopamine in aged mice [151] and in primates [152]. An initial clinical trial indicated that benefit from high-dose CoQ10 in early PD was present, with an apparent decline in disease progression [153]. A follow-up randomized, double-blind clinical trial in patients with midstage PD did not, however, result in changes from placebo group [154]. Therefore, current data from controlled clinical trials are not sufficient to answer conclusively whether CoQ10 is neuroprotective in PD.

In view of the convergence of CoQ and creatine action on mitochondrial energetics and energy pools, combination therapy was attempted in an MPTP mouse model of Parkinson's disease. Additive neuroprotective effects against striatal dopamine depletion and degeneration of SN neurons were in fact observed, which led to improved performance in motor tasks and extended survival [155,156].

Idebenone

Idebenone is a synthetic analog of coenzyme Q10 and a highly effective redox-cycling antioxidant currently used in the treatment of Friedreich's ataxia, a rare inherited neurodegenerative disorder [157,158]. Whether idebenone can be used clinically to treat PD has not yet been fully explored and is still in the initial stages. Paradoxically, dopaminergic neuroblastoma SH-SY5Y cells exposed to idebenone underwent oxidative stress-induced apoptosis [159].

Urate

Uric acid (urate) is the end product of purine metabolism and a recognized endogenous free radical scavenger and powerful antioxidant [160]. Not surprisingly, therefore, it partially rescued mid-brain dopaminergic neurons from cell death [161] and reduced oxidative stress, mitochondrial deficits, and apoptosis in human dopaminergic cells exposed to rotenone [162]. In humans, high plasma levels of urate were associated with a decreased risk of developing PD [163] and correlated with a substantially slower rate of clinical progression [164]. Such findings strengthen the rationale for urate dietary supplementation as a potential strategy to slow PD progression. Indeed, a higher dietary urate intake was associated with a lower risk of PD [165]. Nevertheless, the use of urate as a neuroprotective therapy in PD remains limited as increasing urate in the serum increases the risk of developing gout and cardiovascular disease [166].

Rasagiline

Monoamine oxidase B (MAO-B) enzymes are primarily responsible for the metabolic breakdown of synaptic dopamine; therefore, their inhibition results in enhanced availability and activity of endogenous striatal dopamine. Rasagiline is a prototype MAO-B inhibitor [167] that forms part of the established treatment for symptomatic relief in PD and used to treat motor fluctuations related to levodopa in patients with advanced disease [168]. The

ADAGIO study demonstrated that rasagiline delayed the need for symptomatic parkinsonian drugs when assigned to early PD patients [169]. Furthermore, a disease-modifying effect of rasagiline was suggested by rasagiline 1 mg per day, but not 2 mg per day [170]. These discordant effects prompted the Food and Drug Administration, in the final analysis, not to approve the drug label claiming a disease-modifying effect [171]. Evidence indicates that putative disease-modifying activities of rasagiline may be contingent on mitochondrial-linked mechanisms. It is thought that MAO-B located in the outer mitochondrial membrane [172] may be the site of action of rasagiline. Mito-protectant properties of rasagiline include the inhibition of the mitochondrial-dependent apoptotic cascade by preventing opening of the mPT pore, translocation of cytochrome *c* from the mitochondrial inner membrane to the cytosol, and caspase-3 activation [173,174]. Rasagiline stabilized the mitochondrial transmembrane potential even in isolated mitochondria [175].

Antioxidant Therapies Targeted to Mitochondria

As most small-molecule antioxidants are distributed throughout the body and only a small fraction are taken up by mitochondria, considerable progress has been made in developing chemically engineered forms of antioxidants that selectively accumulate in mitochondria, thereby achieving high local drug concentrations inside the organelle [176].

Mitoquinone

Mitoquinone (MitoQ) consists of the lipophilic triphenylphosphonium (TPP) cation covalently bound to a ubiquinone moiety of CoQ10. The strongly negative mitochondrial membrane potential results in the accumulation of MitoQ within mitochondria, where the ubiquinone moiety inserts into the mitochondrial membrane and is reduced to ubiquinol by the respiratory chain. It is therefore an antioxidant that has the ability to target mitochondrial dysfunction, especially oxidant stress [177]. Thus, MitoQ inhibits mitochondrial ROS generation, maintains glutathione pools, and preserves mitochondria function, independently of the presence of mitochondrial DNA [178]. In cellular models of PD, pretreatment with MitoQ prevented mitochondrial fragmentation due to oxidative stress [179] and protected cultured dopaminergic neurons from mitochondrial apoptosis [180]. In MPTP-treated mice, MitoQ inhibited the loss of nigrostriatal neurons and maintained striatal dopamine levels, in association with improved locomotor ability of the mice [180]. Importantly, chronic administration of MitoQ indicated no evidence of toxicity in wild-type mice, demonstrating that MitoQ can be safely administered [181]. Nevertheless, despite the fact that MitoQ is a potent mitochondrial antioxidant, a double-blind clinical trial failed to demonstrate that MitoQ could slow the clinical progression of PD over a 1-year period [182,183].

Mitotocopherol and MitoTEMPO

The TPP cation has been covalently coupled to vitamin E to form mitotocopherol (MitoVitE) and to the redox-cycling nitroxide TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl)

to form MitoTEMPO. As expected, both achieve high concentration levels in energized, respiring mitochondria, driven by the organelle's large membrane potential [184,185]. The protective efficacy of MitoVitE against cellular oxidative stress was several 100-fold times better than water-soluble analog Trolox [186] and significantly ameliorated ethanol-induced toxicity of cerebellar granule neurons [187]. Peroxide-induced oxidative stress, inactivation of complex I, cytochrome *c* release, caspase-3 activation, and apoptosis in endothelial cells were inhibited by MitoVitE, but not by untargeted antioxidants [188]. When administered to mice, MitoVitE rapidly accumulated in tissues which are most compromised by mitochondrial impairment and oxidative stress, such as heart, brain, muscle, liver, and kidneys [189]. Moreover, MitoVitE significantly bettered systemic oxidative stress parameters in a mouse model of obesity [190]. With regard to MitoTEMPO, its efficacy in protecting against mitochondrial oxidative stress, mPT opening, and apoptosis has been documented in several *in vitro* studies [191,192]. Interestingly, a recent report describes a new method for visualization of superoxide generation in the dopaminergic area in mice, using MitoTEMPO. The technique enabled direct imaging of superoxide generation in intact animals treated with MPTP [193]. To date, the efficacy of MitoTEMPO and MitoVitE in animal models of PD has been hardly attempted, even less so their potential therapeutic use in humans.

Szeto-Schiller Peptides

Szeto-Schiller (SS) peptides are cell-permeable, aromatic-cationic peptides directed to the inner mitochondrial membrane which are able to concentrate >1000-fold in mitochondria [194]. Dimethyltyrosine residues provide free radical-scavenging activity; therefore, these peptide antioxidants offer mitochondrial protection from oxidative stress and prevent mitochondrial swelling, cytochrome *c* release, and apoptosis [195]. SS peptides afforded strong protection in cellular models of ROS generation and also in isolated mitochondria [196,197]. With regard to Parkinson's disease, two SS peptides (SS-20 and SS-31) have been evaluated for protection against MPTP neurotoxicity in mice. Both peptides demonstrated significant neuroprotective effects on dopaminergic neurons of MPTP-treated mice and prevented loss of dopamine in the striatum [198]. In isolated mitochondria, they were shown to prevent MPP⁺-induced inhibition of mitochondrial respiration and ATP synthesis. Of note, although the SS-20 peptide lacks intrinsic antioxidant properties, it also had significant neuroprotective effects, implying that the mechanism of action extends beyond mere ROS scavenging [198].

Daily intraperitoneal injections of SS-31 to transgenic mice in an animal model of amyotrophic lateral sclerosis resulted in improved survival and better performance of motor tasks [199]. Hence, one would expect that SS peptides may offer a realistic treatment approach in PD, which has yet to be explored.

Pharmacological Therapies Aimed at Stabilizing Mitochondrial Calcium

There is a very tight relationship between oxidative phosphorylation, ROS generation, and Ca²⁺ homeostasis in the dopaminergic neuron. Mitochondria maintain a large Ca²⁺ gradient across the

inner membrane, supporting the notion that Ca²⁺ signaling within mitochondria couples ATP production to neuronal activity. Studies on isolated mitochondria and cells have documented the role of Ca²⁺ ions in the stimulation of several matrix dehydrogenase enzymes, such as pyruvate dehydrogenase, isocitrate dehydrogenase and aconitase, and stimulation of complex V (ATP synthase) [200]. The bioenergetic crisis in PD is accompanied by a disturbance in intracellular Ca²⁺ homeostasis, in particular impaired Ca²⁺ handling by mitochondria, which in turn implies that manipulating mitochondrial Ca²⁺ homeostasis might represent another important therapeutic strategy in PD.

For instance, in MPP⁺-triggered cell apoptosis, there is release of Ca²⁺ from endoplasmic reticulum stores, thereby activating the execution of the mitochondrial pathway of apoptosis. Mitochondrial peroxiredoxin-5, an antioxidant enzyme, blocked intracellular Ca²⁺ increase and prevented cell death [201]. Aberrations in mitochondrial Ca²⁺ handling were also found in other cellular models of PD, variously involving exposure to α -synuclein [202], silencing of *parkin* expression [203], and mutant LRRK2 expression [204]. In most cases, the application of voltage-gated calcium channel (VGCC) blockers restored cell viability.

Administration of isradipine, a dihydropyridine Ca²⁺ channel blocker, attenuated loss of dopamine neurons and nigrostriatal degeneration *in vivo* using the MPTP animal model of PD [205]. The safety and tolerability of controlled-release isradipine have recently been evaluated in patients with early-stage PD in a clinical trial [206]. Now, a large placebo-controlled trial (STEADY-PD) is planned for systematic assessment of its efficacy as a disease-modifying agent [207].

Conclusions and Future Perspectives

A large body of experimental evidence now indicates that multiple genetic and toxin-based mechanisms of dopaminergic neurodegeneration converge on mitochondria, both in sporadic and familial PD. Mitochondrial alterations associated with PD include the following: defects in electron transport and oxidative phosphorylation, free radical generation, abnormal calcium handling, mitochondrial DNA mutations, damage to mitochondrial lipid membranes, activation of pro-apoptotic machinery, dysfunctional mitochondrial dynamics, and impaired mitophagy. Collectively, these pathophysiological features of mitochondrial biology appear to correlate with the unique sensitivity of adult dopaminergic neurons to the degeneration. Therapies that help restore mitochondrial function and physiology therefore should offer the prospect of slowing or stopping the otherwise relentless progression of the disease (Table 1). Metabolic antioxidants, polyphenolic compounds, mitochondria-targeted antioxidants, and SS peptides with remarkable neuroprotective and neurorestorative properties have been identified in the laboratory and in preclinical studies.

Nevertheless, to date, none has proved to have an unequivocal disease-modifying effect in the human clinical trials so far completed [113]. A major limitation is that most trials are conducted on patients who have already been diagnosed with PD, by which time, the neurodegenerative process is already sufficiently advanced (50% of dopaminergic neurons and 80% of striatal dopamine may have already been lost). Ideally, therefore,

Table 1 Therapies that modulate mitochondrial function and oxidative stress in PD—summary of animal and human studies

Compound	Study	Summary of results	References
Creatine	<i>Animal model</i> MPTP—mouse	Neuroprotective effect against dopamine depletion	(117,118)
	<i>Human</i> Phase II trial	Reduced UPDRS scores (after 12 months); well-tolerated up to 10 g/day	(121)
	Phase III trial	On-going: 10 g creatine/day for 5–7 years	(122)
Vitamin E	<i>Animal model</i> MPTP—mouse	Increased toxicity in vitamin E-deficient mice	(140)
	MPTP—mouse	Neuroprotective effect against dopamine depletion	(141)
Coenzyme Q10	<i>Animal model</i> MPTP—mouse	Neuroprotective effect against dopamine depletion	(151,155)
	MPTP—primate	Neuroprotective effect against dopamine depletion	(152)
	<i>Human</i> Clinical trial	Reduced UPDRS scores (after 16 months); well-tolerated up to 1.2 g/day	(153)
	Clinical trial	No change in UPDRS scores (after 3 months)	(154)
Urate	<i>Human</i> DATATOP trial	Slower rate of clinical decline with high plasma urate levels	(163, 164)
Rasagiline	<i>Human</i> ADAGIO trial	Reduced UPDRS scores (after 18 months) and suggested disease-modifying effect with 1 mg/day	(173)
Mitoquinone	<i>Animal model</i> MPTP—mouse	Neuroprotective effect against dopamine depletion and improved locomotor ability	(180)
	<i>Human</i> Clinical trial	No change in UPDRS scores (after 12 months)	(183)
Szeto-Schiller peptides	<i>Animal model</i> MPTP—mouse	Neuroprotective effect against dopamine depletion	(198)
Isradipine	<i>Animal model</i> MPTP—mouse	Neuroprotective effect against dopamine depletion	(205)
	<i>Human</i> STEADY-PD trial	Well-tolerated up to 10 mg/day; large trial planned to assess disease-modifying effect in early PD	(206,207)
Polyphenols	<i>Animal model</i> MPTP—mouse	EGCG, baicalein, resveratrol, kaempferol, and genistein all had neuroprotective effects against dopamine depletion	(130–134)
	α -synuclein— <i>Drosophila</i>	Grape seed polyphenolic extract extended life span, improved locomotor function, and protected mitochondria	(136)
	Paraquat— <i>Drosophila</i>	Grape seed polyphenolic extract extended life span and improved locomotor function	(135)
	<i>Human</i> Health Professional Follow-up Study; Nurses' Health Study	Data analysis revealed that flavonoid intake reduces PD risk in men by up to 40%	(125)

ADAGIO, attenuation of disease progression with azilect given once daily; DATATOP, deprenyl and tocopherol antioxidant therapy of parkinsonism; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; STEADY-PD, safety, tolerability, and efficacy assessment of dynacirc CR for PD; UPDRS, unified Parkinson's disease rating scale.

putative disease-modifying compounds are used as prophylactic treatment in the population. Other important reasons for lack of efficacy include a short duration of treatment (under 1 year) or modest group sizes (<500 patients). Lastly, preclinical experimental studies of mitochondria-targeted compounds in animal models

of PD may be largely incomplete, with inadequate information on dose–response relationships, pharmacokinetic profiles, therapeutic windows, and dosage regimens.

Clearly, therefore, more preclinical studies and larger clinical trials with larger numbers of participants are needed to finally

arrive at the identification and development of an effective neurotherapeutic for PD in the not-too-distant future.

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Conflict of Interest

The authors declare no conflict of interest.

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