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MITOCHONDRIA-TARGETED ANTIOXIDANTS FOR TREATMENT OF PARKINSON'S DISEASE: PRECLINICAL AND CLINICAL OUTCOMES

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Abstract

Parkinson's disease (PD) is a progressive neurodegenerative disease in the elderly, and no cure or disease-modifying therapies exist. Several lines of evidence suggest that mitochondrial dysfunction and oxidative stress have a central role in the dopaminergic neurodegeneration of PD. In this context, mitochondria-targeted therapies that improve mitochondrial function may have great promise in the prevention and treatment of PD. In this review, we discuss the recent developments in mitochondria-targeted antioxidants and their potential beneficial effects as a therapy for ameliorating mitochondrial dysfunction in PD.

Keywords

mitochondrial dysfunction; mitochondria-targeted antioxidant; neuroprotection; oxidative stress; Parkinson's disease

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder that affects more than 1 million individuals over the age of 60 years in the United States [1]. According to a recent article, about 50,000 new cases are diagnosed annually, and this figure is expected to increase substantially as the median age of the population continues to rise in the coming decades [2]. Epidemiological studies suggest that sporadic late-onset PD accounts for 90% of cases, whereas the remaining 10% are early onset cases that mainly occur in familial

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clusters [3, 4]. Pathologically, PD is characterized by the loss of dopaminergic neurons within the substantia nigra pars compacta (SNc) and the ensuing depletion of dopamine in the striatum. This loss of dopaminergic neurons causes most of the motor symptoms of PD. By the time PD motor symptoms are clinically recognized, 60% of dopaminergic neurons and 80% of putamen dopamine have been lost [5]. PD is also associated with the presence of ubiquitin- and α-synuclein-positive cytoplasmic inclusions known as Lewy bodies within surviving dopaminergic neurons [6]. In addition to the nigrostriatal dopaminergic defects, emerging clinical evidence suggests that extranigral degeneration and non-motor symptoms are key features of early stages of PD pathogenesis [7-10].

Currently, the available therapies for PD only treat the symptoms; none slow or prevent progressive neuronal degeneration in the dopaminergic system [11, 12]. Dopamine replacement therapy, i.e., levodopa administered orally or stimulation of dopamine receptors, has been the most widely used treatment option for PD, but the beneficial effects of dopaminergic therapy wear off over time and its clinical efficacy gradually declines as the disease advances [13].

Despite extensive research, the precise cause of sporadic PD or non-familial PD remains unknown, but several pathogenic mechanisms have been proposed, including oxidative stress, mitochondrial dysfunction, impairment of the ubiquitin-proteasome system, and neuroinflammation. Convincing evidence from postmortem brain tissue, cell culture and animal models of PD and the analysis of human genetics support the involvement of oxidative stress and mitochondrial dysfunction in PD pathogenesis [14, 15]. Mitochondrial dysfunction due to oxidative stress, mitochondrial DNA deletions, altered mitochondrial morphology and the interaction of pathogenic proteins with mitochondria all result in dopaminergic neurodegeneration. Thus, therapeutic approaches targeting mitochondrial dysfunction and related oxidative stress may hold great promise of a cure for PD. One potential approach to ameliorating complications arising from PD is to suppress mitochondrial reactive oxygen species (ROS) generation with specific antioxidants. Several small antioxidant molecules, such as ubiquinol and creatine, have shown promising neuroprotective effects in different models of PD [16, 17]. However, a major limitation of using these compounds to treat PD is their failure to accumulate preferentially in the target organelle mitochondria. For this reason, several strategies to identify antioxidants with therapeutic potential that specifically target mitochondria have been developed. In this review, we will describe cellular changes in the progression of PD, and in our discussion of promising PD therapeutic strategies, we will focus on the mitochondrially targeted antioxidants as potential therapies for PD.

2. Production of mitochondrial ROS

Mitochondria play a central role in the life and death of cells. Physiologically, mitochondria perform a variety of fundamental regulatory processes in the cell, including ATP production [18], calcium homeostasis and modulation [19], amino acid and nitrogen metabolism [20], apoptotic cell death [21], ROS generation and detoxification [22, 23], and heme and iron-sulfur center biosynthesis [24]. They supply the vast majority of cellular energy in the form of ATP through oxidative phosphorylation. During oxidative phosphorylation, electrons

from reduced cofactors are transferred through a series of respiratory chain complexes (Complexes I-IV) located in the mitochondrial inner membrane to oxygen, the ultimate electron acceptor. The flow of electrons simultaneously leads to the pumping of protons out of the mitochondrial matrix. This electrochemical reaction generates a transmembrane potential ($\Delta\Psi m$) yielding the energy for ATP synthesis from ADP and inorganic phosphate.

ROS can be generated within mitochondria in several sites of mitochondrial electron transport chains (ETC), in particular on Complexes I and III, where electrons occasionally leak to oxygen and form a superoxide anion ($O_2^{\bullet -}$), the predominant ROS in mitochondria (Fig. 1) [25, 26]. In fact, mitochondria are the major sites of cellular ROS production, with approximately 1-3% of mitochondrial oxygen consumption being converted to ROS [27]. In addition to formation from incomplete reduction of oxygen in ETC, a number of enzyme systems also generate superoxide, including the tricarboxylic acid cycle (TCA) enzymes α-ketoglutarate dehydrogenase [28] and aconitase [29], the non-TCA cycle enzymes pyruvate dehydrogenase, dihydroorotate dehydrogenase [30] and glycerol-3-phosphate dehydrogenase [31], and the mitochondrial outer membrane proteins such as methemoglobin reductase [32]. Because of its negative charge and poor membrane permeability, superoxide is relatively unreactive, but it can react rapidly with nitric oxide (NO) to form the potent oxidant and nitrating agent peroxynitrite ($ONOO^-$) and subsequently other reactive nitrogen species (RNS). Moreover, it is able to damage some mitochondrial iron-sulfur cluster-containing proteins [33]. Most cellular superoxide is rapidly converted to hydrogen peroxide (H_2O_2) either through spontaneous dismutation or dismutation reactions catalyzed by superoxide dismutase (SOD) [34]. Hydrogen peroxide itself is a reactive free radical that is stable, membrane permeable and has a relatively long half-life enabling diffusion within the cell. As a redox active species, H_2O_2 can inactivate some enzymes by oxidizing their thiol groups [35], although it is unable to oxidize DNA or lipids directly [33]. Hydrogen peroxide can be decomposed by cytosolic and mitochondrial antioxidant systems such as glutathione peroxidase (GPx), catalase (CAT), and thioredoxin reductase (TPx). However, if not removed, it can further produce the highly reactive hydroxyl radical ($OH\bullet$) in the presence of Fe^{2+} cations via the Fenton reaction [36]. The $OH\bullet$ has a strong oxidizing potential and can damage virtually every type of macromolecule close to their site of origin, making it an extremely dangerous compound to the organism. Furthermore, unlike superoxide and hydrogen peroxide, which can be detoxified by an enzymatic conversion, no enzymatic routes are known for eliminating hydroxyl radicals. Nonenzymatic mechanisms for scavenging peroxyl radicals include several antioxidants such as vitamin E and glutathione. Other radicals derived from oxygen include peroxyl radical ($RO_2\bullet$), hypochlorous acid (HOCl), alkoxyl radical ($RO\bullet$), and hydroperoxyl radical ($HO_2\bullet$), which are high-energy species and exhibit a broad array of biological actions. Additional endogenous sources of cellular ROS are macrophages, neutrophils, and eosinophils. ROS generation can also occur through a host of exogenous sources including ultraviolet and high-energy irradiation, redox-cycling of quinones, xenobiotics, ions, metals, aging, and environmental toxins [37-41].

It is worth pointing out that ROS production can be significant in both physiological and pathological situations depending on the environment. For example, it has been demonstrated that at lower physiological levels, mitochondrially generated H_2O_2 acts as an

intracellular signaling molecule, affecting multiple cellular functions [42-44]. Thus, it is possible that an overuse of antioxidants may be detrimental. In contrast, at high concentrations, ROS may cause extensive damage to cells and the whole organism, consisting of the peroxidation of lipids, particularly phospholipids of biological membranes, the carbonylation of proteins, or oxidative damage to mtDNA [23, 26, 36, 45]. These noxious actions, often referred to as “oxidative stress”, either individually or collectively can disrupt mitochondrial function and cause ROS to flow to the cytosol, which in turn results in a further increase in ROS production, thus forming a vicious cycle [46]. In this sense, mitochondria are believed to have developed an excessive network of antioxidant defenses. Therefore, a delicate balance between ROS and antioxidants within mitochondria is essential for the functions of cells, tissues, and organs.

3. Scavenging of mitochondrial ROS

Mitochondria contain a series of well-defined and tightly controlled antioxidant defense systems, which work synergistically to intercept ROS, thereby minimizing oxidative damage. Disruption of these antioxidant defenses may result in extensive oxidative damage to mitochondria. There are two main types of mitochondrial antioxidant defense systems: enzymatic and nonenzymatic. Enzymatic antioxidants involve SOD, GPx, catalase, and TPx, all of which are encoded by the nuclear genome and are subsequently imported into the mitochondria. SOD is one of the most effective intracellular enzymatic antioxidants, and as mentioned in the previous section, SOD catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. In mammals, three forms of SOD exist depending on the nature of the active metal center. SOD1 or Cu/Zn SOD, binds copper and zinc and is a cytoplasmic and mitochondrial inter-membrane space protein [47]. SOD2 (Mn-SOD) has manganese ion at its reactive center and is only expressed in the mitochondrial matrix, and SOD3 is secreted into the extracellular space after its protein translation [48]. Mn-SOD null mice die shortly after birth, confirming the importance of keeping superoxide in check in mammals [49]. GPx mainly detoxifies free H_2O_2 and lipid peroxides using reduced glutathione (GSH) as a cofactor. In this process, GSH is converted to its oxidized form, glutathione disulfide (GSSG). Once oxidized, GSH can be regenerated by the action of glutathione reductase using NADPH as its electron donor. In addition, GSH serves as a nonenzymatic antioxidant, scavenging hydroxyl radical and singlet oxygen directly and maintaining vitamin C and E in their reduced forms.

Other nonenzymatic antioxidants include α-tocopherol (vitamin E), ascorbic acid (vitamin C), coenzyme Q_{10} (CoQ_{10}), β-carotene, flavonoids, and α-lipid acid [50]. Vitamin E is lipid-soluble, and therefore, is widely distributed in mammalian cell membranes. Of the different forms of vitamin E, α-tocopherol is the most biologically active form. As a lipid-soluble antioxidant, vitamin E acts as a scavenger of peroxyl radical, peroxynitrite, and hydroxyl radical by preventing the propagation of the radical chain, thus effectively inhibiting lipid peroxidation. During the antioxidant reaction, α-tocopherol is converted to α-tocopherol radical, which gets reduced to its original α-tocopherol form. Another vitamin antioxidant is vitamin C, which is water-soluble, making it able to work in the body’s aqueous environments. Vitamin C functions in association with vitamin E to regenerate α-tocopherol from α-tocopherol radical. CoQ_{10} is an essential component of the ETC, where it accepts

electrons from Complexes I and II. It is also a coenzyme for Complex III and acts as a lipid-soluble antioxidant as well. The antioxidant activity of CoQ_{10} arises from its capacity to exchange two electrons in a redox cycle between its oxidized (ubiquinone) and its reduced form (ubiquinol). Ubiquinol is a very effective chain-breaking antioxidant that not only inhibits lipid peroxidation and protein and DNA oxidation, but also directly quenches oxidants such as superoxide and peroxyl radicals.

Despite the presence of elaborate defense systems to counteract oxidative damage, it seems that cellular ROS levels are not perfectly under control. Indeed, oxidative damage due to excessive ROS production may accumulate during both normal and stressful conditions. Oxidative stress accumulation and concomitant mitochondrial dysfunction have been implicated in PD pathogenesis.

4. Oxidative stress in PD

Oxidative stress has long been implicated in the process of neurodegeneration in PD pathogenesis. Oxidative stress, arising from excessive production of ROS and/or defective ROS removal, can potentially damage cellular lipids, proteins, and DNA. Postmortem studies have consistently shown high levels of oxidation of lipids, proteins, and nucleic acids in the SNc of sporadic PD brains [51-56]. Also, significant alterations of the antioxidant defense system, in particular reduced glutathione, were found in the SNc of PD patients [57]. As mentioned above, ETC is the major source of ROS, in particular the hydrogen peroxide and superoxide anions [58]. In the presence of ferrous iron, these ROS can be converted to even more potent ROS, such as the hydroxyl radical and hydroxyl anion [59, 60]. Not surprisingly, the level of iron was significantly increased in the SNc of PD brains [61-64].

Apart from being the main source of increased oxidative stress in PD brains, mitochondrial function itself can be affected by oxidative stress [26, 65, 66], which further contributes to the accumulation of ROS and mitochondrial damage in a vicious cycle. This feed-forward mechanism is what commonly underlies neuronal cell death in neurodegenerative diseases. In addition to mitochondria, the auto-oxidation of dopamine, a reaction known to generate superoxide and hydrogen peroxide, as well as reactive dopamine quinones, specifically contribute to cellular ROS in dopaminergic neurons [67, 68]. This dopamine-dependent oxidative stress is believed to partially explain the selective vulnerability of dopaminergic neurons in PD. Another important contributor to oxidative stress is NO, which is generated by nitric oxide synthase (NOS) [52]. Reaction of ROS with NO produces highly toxic RNS, such as the peroxynitrite and nitro-tyrosyl radicals [69]. Besides damaging cellular proteins, lipids, and DNA, oxidative stress also activates a variety of cell death pathways [70-79].

5. Mitochondrial dysfunction in PD

Considerable evidence exists suggesting a role for mitochondrial dysfunction in PD pathogenesis [38, 80]. Mutations in mitochondrial DNA (mtDNA) play a role in the demise of dopaminergic neurons. Indeed, high levels of somatic mtDNA point mutations in elderly PD patients have also been reported [81]. However, the most definitive evidence of mitochondrial dysfunction in PD has come from studies using MPTP, a Parkinsonian

toxicant which causes Parkinsonian syndromes in humans, rodents and primates by inhibiting the mitochondrial complex-I of the electron transport chain [82]. Similar to MPTP, other complex-I inhibitors such as rotenone, maneb, paraquat, fenzaquin and trichloroethylene result in the loss of nigral dopaminergic neurons in the mouse model of PD, implicating mitochondrial dysfunction in its pathogenesis [83, 84]. Additionally, impairment of complex-I activity has been reported in the substantia nigra, platelets and skeletal muscles of PD patients [85, 86]. Moreover, reduced complex-I activity and an increased susceptibility to MPP^+, the toxic metabolite of MPTP, were also observed in mitochondrial DNA from PD patients, clearly demonstrating mtDNA-encoded defects in PD [87, 88].

Recently, more convincing evidence of mitochondrial dysfunction in PD has been reported in conditional knockout ‘MitoPark’ mice, which have a disrupted mitochondrial transcription factor A (Tfam) gene in dopaminergic neurons [89]. These mice exhibit reduced mtDNA expression, attenuated expression of respiratory chain function in dopaminergic neurons in nigra along with behavioral impairments and striatal dopamine depletion, mimicking progressive PD phenotypes starting from 18 weeks of age [89].

Pathogenic mutations in several genes including α-synuclein, LRRK2, parkin, DJ-1 and PINK-1 also play an important role in mitochondrial dysfunction in PD patients [2, 90]. Impaired mitochondrial function in the MPTP mouse model of PD has been reported in cells overexpressing α-synuclein and in transgenic mice [91, 92]. Based on all these findings, it can be inferred that intervening in one or more of these processes could alleviate the harmful effects of mitochondrial dysfunction.

6. Antioxidant therapy

Since PD is a complex, multifactorial disease with oxidative stress and mitochondrial dysfunction playing central roles in nigrostriatal dopaminergic neurodegeneration, antioxidants targeting these factors have become attractive therapeutic agents in the treatment of PD. In this section, we will summarize the current evidence for preventing or slowing the development of PD by neuroprotective antioxidant agents.

6.1 Vitamin antioxidants

Various forms of vitamin antioxidants (Fig. 2A-B) have been tested for their neuroprotective potential. Vitamin E deficiency increased MPTP-induced dopaminergic neurotoxicity [93]. In another study, researchers found that administering vitamin E ameliorated oxidative stress induced by iron accumulation in the mouse brain [94], suggesting that vitamin E may be neuroprotective against PD. Additionally, vitamin E was neuroprotective in 6-OHDA-treated animals [95]. The observational data in humans suggest that the combined administration of high-dose vitamin E and vitamin C supplements was associated with a reduced progression of PD [96]. However, the results of double-blind, randomized controlled trials have been disappointing, where vitamin E showed no benefits in PD [97, 98]. More recently, a large cohort study demonstrated that high dietary vitamin E intake, but not vitamin C or carotenoids, reduced the risk of PD [99]. But in contrast, other studies contradict results regarding food intake of vitamin E and its efficacy to prevent PD

progression [100-102]. The protective role of vitamin C in PD remains controversial since one epidemiological study found a decreased risk of PD in individuals consuming diets rich in vitamin C, whereas other studies showed no effects or even an increased risk of PD with consumption of vitamin C [99, 100, 103].

6.2 Creatine

Creatine (Fig. 2C) is a nitrogenous guanidine compound that forms high energy phosphate bonds, occurs naturally in vertebrates and supplies energy to muscle and nerve cells. Creatine also possesses antioxidant properties and can be an effective inhibitor of mitochondrial permeability transition pore opening and mitochondrial iron accumulation. Preclinical studies in various models have demonstrated its potential role as a neuroprotective agent [104]. Recently, a neuroprotective effect of creatine was demonstrated in MPP^+ and 6-OHDA-treated dopaminergic neuronal cell cultures, where it protected tyrosine hydroxylase immunoreactive dopaminergic neurons and their fibers [105]. In the MPTP mouse model of PD, creatine restored the MPTP-induced loss of dopamine and protected dopaminergic neurons [106]. In early clinical studies, two grams daily creatine administration improved behavioral difficulties in a clinical trial of 200 subjects who were within 5 years of a PD diagnosis [16]. In an additional follow-up study, creatine continued to show neuroprotective efficacy 18 months after creatine administration [107]. Although a 2-year placebo-controlled study of 60 subjects demonstrated that creatine had no effect on PD scores or dopamine transporter imaging, improved mood behavior (a non-motor symptom of PD) was noticed in those patients [108]. Recently, a phase III clinical trial has been started by the NIH, where creatine is administered with a dose of 10 g in a large long-term study of PD targeting 1720 participants with the disease [109, 110]. This study involving 52 medical facilities is expected to be completed in 5-7 years.

6.3 CoQ_{10}

CoQ_{10} also shows promise as a neuroprotective agent in PD (Fig. 2D). Postmortem studies have shown that CoQ_{10} levels in the plasma and platelets of PD patients were significantly lower compared to age-matched controls, and that the oxidized form of CoQ_{10} was elevated in PD patients, suggesting that taking CoQ_{10} supplements may be beneficial. Neuroprotective properties of CoQ_{10} have since been demonstrated in various *in vivo* and *in vitro* models of PD. More specifically, paraquat- and rotenone-induced mitochondrial dysfunction and neurodegeneration in rat mesencephalic primary neurons were inhibited by CoQ_{10} [111]. Also, a neuroprotective role for CoQ_{10} was shown in iron-induced apoptosis in dopaminergic neurons [112]. Pretreatment of neuronal cells with CoQ_{10} has maintained the mitochondrial membrane potential during oxidative stress and reduced the mitochondrial generation of ROS [113]. In the MPTP mouse model, CoQ_{10} protected against MPTP-induced dopamine depletion and the loss of dopaminergic neurons in aged mice [114]. Similarly, CoQ_{10} protected against MPTP toxicity in the chronic MPTP model, which mimics the progressive nature of PD [114].

A combination of creatine and CoQ_{10} exhibited a significant neuroprotective effect in chronic MPTP-treated mice [115]. Oral administration of CoQ_{10} also slowed the progression of PD in a primate model of PD [116]. Evidence from clinical trials suggests that high doses

of CoQ_{10} are needed for beneficial results. A double-blind, placebo-controlled phase II study of CoQ_{10} with three different doses, such as 300, 600 and 1200 mg daily, in 80 early untreated patients for 16 months was conducted recently, resulting in a statistically improved PD rating only at the highest dose [117]. Next, this phase II trial was extended to test a higher dose (2400 mg/daily) of CoQ_{10} in early PD patients. Although a trend emerged, the improvement in clinical scores, unfortunately, was not statistically significant. One possible reason could be the lower number of patients [118]. Recently, an NINDS-funded phase III, multicenter, randomized, placebo-controlled, double-blind trial of CoQ_{10} at doses of 1200 and 2400 mg/daily was initiated on 600 early, non-medicated PD patients. Hopefully, some positive outcome will come from this clinical trial.

6.4 Urate

Urate (uric acid; Fig. 2E) plays a role in the regulation of oxidative stress by acting as a scavenger of superoxide, hydroxyl radical, and singlet oxygen and as an iron chelator [119-121]. Urate inhibits oxidative stress and prevents dopaminergic cell death in both cell culture and animal models of PD [122, 123]. In humans, several prospective cohort studies demonstrated that higher blood levels of urate were associated with a significantly reduced risk of developing PD [124-127]. In another prospective study, PD patients with high urate intake progressed slower toward the disability endpoint requiring dopamine treatment [128]. Although these findings indicate that urate intake could protect individuals from PD progression, the use of urate in PD treatment remains limited because elevating urate in the serum increases the risk of gout, a risk that is further compounded by the consumption of alcohol and fructose [129].

6.5 Apocynin and its derivative

Apocynin (4-hydroxy-3-methoxyacetophenone; Fig. 2F) is a plant-derived antioxidant widely used as an effective NADPH oxidase inhibitor, which interferes with the assembly of cytosolic NADPH oxidase components with the membrane components. NADPH is one of the major sources of cellular ROS during neurodegeneration. Apocynin protects dopaminergic neurons against MPP^+-induced oxidative stress and cell death in cell cultures [130]. In contrast, no beneficial efficacy [131] or pro-oxidant property [132] of apocynin has been reported. Apocynin can be converted to diapocynin (Fig. 2G) *in vivo*, which is a more efficient inhibitor of NADPH oxidase than apocynin itself [133]. Additionally, diapocynin has higher lipophilicity than apocynin [134]. Interestingly, we recently demonstrated in a mouse MPTP model of PD, that the compound diapocynin protected against nigrostriatal damage and inhibited inflammatory and oxidative stress processes [135]. In LRRK2-R1441G transgenic mice, which are reported to develop PD-like symptoms at ~10 months of age, administration of diapocynin at an oral dose of 200 mg/kg three times per week protected against neurobehavioral dysfunction [136]. These findings suggest that diapocynin can be neuroprotective against PD, although additional animal and human studies are needed.

In summary, although a range of antioxidant moieties has been used with partial success in experimental cellular and animal models of PD, no human clinical studies to date have provided conclusive evidence of antioxidants benefitting PD patients. Evidence from these

clinical trials, particularly with vitamin E, vitamin C, and creatine, is largely inconclusive. Furthermore, clinical trials involving CoQ_{10} have demonstrated that supplementation with very high doses of CoQ_{10} is necessary to show some benefit in PD subjects. This may be due to the fact that most small-molecule antioxidants get distributed throughout the body, with only a small fraction being taken up by mitochondria. Consequently, approaches selectively targeting mitochondria with antioxidants have been developed.

7. Mitochondria-targeted antioxidant therapy

During the past decade, considerable progress in developing mitochondrially targeted antioxidants has been made. A well-established approach is conjugation to a lipophilic cation, such as triphenylphosphonium (TPP) [137-139]. Phosphonium derivatives have been traditionally used to determine mitochondrial inner membrane potential. Triphenyl-phosphonium cations consist of a positively charged phosphorus atom surrounded by a large hydrophobic surface (Fig. 3A), thereby giving it the ability to directly and rapidly permeate lipid bilayers while retaining the positive charge. This positive charge is used to facilitate TPP cation accumulation within the mitochondrial matrix driven by the large mitochondrial membrane potential ($\Delta\Psi m$) of 150-180 mV (negative inside), which was generated by the proton gradient during the transfer of an electron to oxygen. As shown in Fig. 3B, the plasma membrane potential (30-60 mV, negative inside) enables up to a 10-fold accumulation of TPP in the cytoplasm. Subsequently, the large, negative potential gradient across the mitochondrial inner membrane potentiates the redistribution of TPP from the intracellular space into the mitochondria, leading to a 100- to 500-fold higher concentration of TPP inside mitochondria [140, 141]. Based on this theory, Murphy and coworkers [139] discovered a series of orally bioavailable mitochondria-targeted antioxidants (MTAs), including MitoQ, MitoVitE, and MitoTEMPOL. These compounds are known to pass through all biological membranes and accumulate within mitochondria more easily than their non-targeted parent antioxidants, rendering them far more effective in protecting against mitochondrial oxidative damage. This section is dedicated to the most promising MTAs for treating neurodegenerative diseases, with emphasis on the evidence for managing PD.

7.1 MitoQ

MitoQ (mitoquinone) is the most studied and widely used antioxidant targeted to mitochondria. It consists of TPP covalently attached to the ubiquinone moiety of the endogenous antioxidant CoQ_{10} through a ten-carbon aliphatic carbon chain (Fig. 4A). Multiple lines of evidence indicate that MitoQ predominantly accumulates within the mitochondria, where it is primarily absorbed to the matrix-facing surface of the inner mitochondrial membrane with the ubiquinone component penetrating deeply into the hydrophobic interior of the membrane [138]. MitoQ is a promising neuroprotective compound due to its direct antioxidant action. Like its parent antioxidant CoQ_{10}, MitoQ continuously scavenges peroxyl, peroxynitrite, and superoxide, and thus can protect mitochondria against lipid peroxidation. After detoxifying oxidants, MitoQ is recycled back to the active ubiquinol antioxidant form by the respiratory chain Complex II [142]. MitoQ may also display concomitant anti-inflammatory and anti-hypoxic properties. Under certain

conditions, however, it may become pro-oxidant and proapoptotic due to redox cycling of quinone and generation of superoxide [143, 144]. MitoQ was protective in a number of animal models of diseases involving oxidative stress, including neurodegenerative diseases [145], ischemia-reperfusion [146, 147], hypertension [148], sepsis [149, 150], fatty liver disease [151], alcoholic fatty liver disease [152], and kidney damage in type I diabetes [153].

Although the therapeutic efficacy of MitoQ in PD needs to be further confirmed, several *in vitro* and *in vivo* studies have demonstrated beneficial effects (Table I). When used in SH-SY5Y cells, MitoQ markedly inhibited 6-OHDA-induced mitochondrial fragmentation [154]. In another study, we recently demonstrated the neuroprotective efficacy of MitoQ both in cell culture and in a pre-clinical animal model of PD [155]. MitoQ protected the MPP^+-induced loss of neurons and neurites in a dopaminergic cell culture model of PD. At a dose of 4 mg/kg/day, MitoQ protected against the MPTP-induced loss of dopaminergic neurons and terminals in the nigrostriatum and it reversed both the MPTP-induced loss of dopamine and its metabolites as well as the MPTP-induced loss of behavioral activities [155]. Electron paramagnetic resonance analysis revealed that MitoQ inhibited the MPTP-mediated mitochondrial aconitase inactivation [155], suggesting that MitoQ indeed exerts its neuroprotective action at the intended target mitochondria. Administering MitoQ at a dose up to 500 μM through drinking water for 28 weeks showed no evidence of toxicity, indicating that MitoQ can be safely administered long-term in rodents [156]. MitoQ has now been developed as a pharmaceutical by Antipodean Pharmaceuticals Inc., and it has undergone Phase I and II clinical trials [157, 158]. In one clinical study, MitoQ at an oral dose of 40 - 80 mg/kg prevented liver damage in hepatitis C patients [157]. In contrast to this, another recent double-blind clinical trial failed to show any benefit from the oral intake of MitoQ in slowing the clinical progression of PD over the course of one year [158]. One explanation for the conflicting results, as put forward by the authors, is that MitoQ may not work for PD clinical remission, since nearly 50% of the dopaminergic neurons and 80% of striatal dopamine have been lost by the time PD is diagnosed. Hence, further studies are required to clarify the therapeutic effects of MitoQ in PD subjects.

7.2 MitoVitE

MitoVitE (mitotocopherol) was the first MTA to be discovered, and consists of TPP conjugated to the α-tocopherol moiety of vitamin E through a two-carbon chain (Fig. 4B). Internalized MitoVitE is immobilized by insertion in the lipid bilayer of the mitochondrial inner membrane. Like MitoQ, MitoVitE appears to protect mitochondria and cells from oxidative damage by inhibiting lipid peroxidation. MitoVitE is taken up rapidly by isolated mitochondria and cells in culture [140]. When administered to mice by intravenous injection, it rapidly accumulated in the tissues most affected by mitochondrial dysfunction and oxidative damage, including heart, brain, muscle, liver and kidney [141].

MitoVitE is protective in a number of cellular models of mitochondrial oxidative stress. MitoVitE was reported to reduce peroxide-mediated oxidative stress and to maintain proteasomal function in endothelial cells [159]. Likewise, another cell culture study demonstrated that MitoVitE protected against peroxide-induced caspase activation [160].

MitoVitE was protective against oxidative stress-induced cell death in fibroblasts from Friedrich ataxia (FRDA) patients [161]. However, it should be noted that higher levels of MitoVitE are cytotoxic in Jurkat cells [37]. There is also *in vivo* evidence for the beneficial effects of MitoVitE in several animal models of human diseases. Administering MitoVitE to rats 30 min post-induction of sepsis by pneumonia led to protection against cardiac damage [162]. MitoVitE was reported to decrease oxidative stress and reduce fat deposition in a mouse model of obesity [163]. In contrast, continuous striatal infusion of MitoVitE in rats was not protective against the hypoxia-ischemia-induced cell death of striatal medium-spiny neurons; moreover, MitoVitE at the higher dose of 435 μM was neurotoxic [164]. To date, neither the efficacy of MitoVitE in PD nor its therapeutic potential in humans has been investigated.

7.3 MitoTEMPOL

MitoTEMPOL is another TPP^+ derivative, but one with the stable piperidine nitroxide radical TEMPOL (4-hydroxy-2,2,6,6,-tetramethyl-piperidine-1-oxyl; Fig. 4C), which accepts an electron from the potent radical scavenger hydroxylamine. MitoTEMPOL may also act as a cytosolic SOD mimetic, which converts superoxide molecules into water, and is able to detoxify ferrous iron by oxidizing it to ferric iron. The conjugated compound also accumulated inside energized, isolated mitochondria [165].

MitoTEMPOL has shown beneficial effects in several *in vitro* settings of mitochondrial oxidative stress. It has been demonstrated that MitoTEMPOL protected pancreatic β-cells against oxidative stress [166]. In a cellular model of ischemia-reperfusion, the related amide linked TPP, MitoTEMPO [167], partially inhibited ATP depletion-recovery mediated mitochondrial permeability transition pore opening and cell death [168]. In another cellular study, treatment with MitoTEMPOL significantly improved arteriolar endothelial function in vessels from type 2 diabetes mellitus patients [169]. Co-administering MitoTEMPO to rats simultaneously with angiotensin II (Ang II) infusion significantly protected them against Ang II-induced hypertension [170]. In animal models of diabetes, MitoTEMPOL prevented mitochondrial and cytosolic ROS production in the aorta and restored coronary collateral growth [171]. MitoTEMPOL has not yet been tested in animal models of PD.

7.4 “Sk” compounds

Skulachev et al. developed an alternative series of mitochondria-targeted antioxidants, termed “SkQs”, by using palstoquinone to replace the ubiquinone antioxidant moiety of MitoQ [172]. The compound SkQ1, which is a TPP^+ derivative conjugated with palstoquinone itself (Fig. 4D), is the most studied “Sk” compound. SkQ1 performed as a potent antioxidant in isolated mitochondria [172]. Additional cell culture studies have confirmed that very low concentrations of SkQ1 and its analogs inhibited cell death induced by hydrogen peroxide [172, 173]. Extensive animal studies have demonstrated beneficial roles of SkQ1 and related compounds in a number of diseases associated with elevated oxidative stress [174-177], although little information regarding the effectiveness of “Sk” compounds against PD is currently available. Nevertheless, current evidence regarding “Sk” compounds is promising, and further exploration of these antioxidants in models of PD is warranted.

7.5 Other Mito compounds

Currently, other mitochondrially targeted antioxidants incorporating the TPP function have been developed. For instance, apocynin, a plant derived antioxidant and NADPH oxidase inhibitor, has been conjugated to TPP to form MitoApocynin. Recently, we observed significant beneficial effects of MitoApocynin in a preclinical MPTP mouse model of PD (unpublished data; Table I). MitoPBN is a similar TPP^+ derivative with phenoxy-butyl-nitrone (Fig. 4E). The spin trap PBN was chosen based on PBN's well-known reactivity with carbon-centered radicals [178]. MitoPBN was rapidly taken up by mitochondria and can block the oxygen-induced activation of uncoupled proteins [178]. MitoPeroxidase (Fig. 4F), a TPP-based mitochondria-targeted analog of ebselen, which has the peroxidase-like activity, was also prepared [179]. Growing evidence has indicated a class of SOD mimetics with neuroprotective effects in experimental models of PD [180-182]. Accordingly, more recently, Kelso et al. developed a mitochondrially targeted macrocyclic SOD mimetic, MitoSOD (Fig. 4G) [167]. In preliminary experiments, it rapidly accumulated within mitochondria and possessed SOD activity. The efficacy of MitoPeroxidase, MitoPBN and MitoSOD *in vivo*, particularly in the preclinical models of PD, however, remains to be determined.

7.6 SS tetrapeptides and alternative targeting approaches

To circumvent adverse effects of TPP^+, alternate strategies have been explored for effective targeting of antioxidant to mitochondria. For example, small antioxidant molecules have been successfully targeted to mitochondria by incorporating mitochondria-targeted peptides. The Szeto-Schiller (SS) tetrapeptides contain an aromatic-cationic sequence motif that specifically enables them to be delivered to mitochondria, where they localize to the inner mitochondrial membrane with an approximate 1000-5000-fold accumulation [183, 184]. The mechanism behind the specific mitochondrial uptake of SS peptides is not fully clear, but it does not seem to depend on mitochondrial potential. Delivery of these peptides to tissues *in vivo* through intravenous, intraperitoneal or subcutaneous injection has been documented [142]. Currently, a number of SS tetrapeptides have been developed, of which SS-20 and SS-31 (Fig. 5A) are the most studied. Both of them comprise a dimethyltyrosine (Dmt) residue, which reacts with a variety of free radicals and inhibits lipid peroxidation [185].

In vitro studies have reported that the peptide antioxidants potently protected neurons against toxicity induced by amyloid beta (Aβ) [186, 187], herbicides [188], t-butyl hydroperoxide (tBHP) and 3-nitropropionic acid (3NP) [184, 189]. Interestingly, SS tetrapeptides have also shown promising benefits in animal models of PD (Table I). In the MPTP mouse model, administering SS-31 via intraperitoneal injection protected mice against nigrostriatal dopaminergic neurodegeneration and the loss of striatal dopamine and its metabolites [190]. Surprisingly, the SS-20 peptide, which lacks the Dmt residue and thus lacks antioxidant properties, also demonstrated significant protection in MPTP-treated mice [190], suggesting that the neuroprotective actions of SS peptides may not be attributable to the mechanism of scavenging ROS. Hence, additional preclinical studies on SS tetrapeptides are required to develop an effective drug capable of intervening in the progression of PD in humans.

In addition to SS peptides, several novel XJB peptides, including XJB-5-131 (Fig. 5B), have been invented. These peptides consist of an electron and ROS scavenger (4-NH_2-TEMPO) conjugated to the Leu-D-Phe-Pro-Val-Orn fragment of gramicidin S. This pentapeptide fragment can specifically target the XJB peptides to mitochondria. XJB-5-131 improved mitochondrial function and enhanced the survival of neurons in a mouse model of Huntington's disease [191].

Another approach to targeting mitochondria with small bioactive molecules is through a polymer based nano-carrier. This method involves biodegradable poly-lactide-co-gylcolide (PLGA) nanoparticles, which includes for example the PLGA-CoQ_{10} nanoparticles [192]. However, the biological efficacies of these CoQ_{10} nanoparticles remain to be investigated.

8. Conclusion and perspectives

PD is a complex, multifactorial disease triggered by both genetic and environmental factors. Substantial evidence has implicated mitochondrial dysfunction and oxidative damage as important components of PD pathogenesis. This has led to the enthusiastic use of mitochondria-targeted interventions as a tool for modulating mitochondrial function in the prevention and treatment of PD. Within the past 15 years, a class of compounds, referred to as mitochondria-targeted antioxidants, has been developed by conjugating the lipophilic triphenylphosphonium cation to an antioxidant moiety. These compounds have shown some promise in experimental models of neurodegenerative diseases against mitochondrial oxidative damage. Most notably, MitoQ and MitoApocynin have been efficacious in experimental models of PD, supporting the concept that mitochondria-targeted interventions would be effective in treating PD. However, a more recent, 1-year clinical study failed to show a link between MitoQ supplementation and prevention of PD progression. Although many explanations for the negative results may be postulated, one reason could be that the pharmacological effects of these Mito compounds are not completely known. For instance, MitoQ can act as a pro-oxidant and promote cell death due to redox cycling and generation of the superoxide anion. Therefore, an intriguing perspective would be to fine-tune the chemical biology of these compounds to remove the undesirable effects, as well as identify new compounds with bioactivity against mitochondrial oxidative damage. A second reason for the lack of efficacy may be that the preclinical assessment of these compounds in animal models of PD is woefully inadequate. Prior to entering the clinical development phase, a substantial preclinical data set should be developed to better define neuroprotective dose-response relationships, pharmacokinetic-pharmacodynamic correlations, therapeutic windows and optimum dosing regimens and treatment durations. Clearly, more preclinical studies in rodent and other mammalian models are necessary. Preclinical research in this area should not be limited to the traditional MPTP models of PD. Perhaps a more promising animal model for preclinical studies of mitochondria-targeted antioxidants is the MitoPark mouse, which exhibits spontaneous progressive PD-like pathology and behavior. Another potential explanation for the failure of MitoQ in the clinical trial could be due to the heterogeneous nature of PD, which will render any single therapeutic agent less beneficial than it appeared in simpler pre-clinical models. A more rational strategy would be to interfere at multiple points in the mitochondrial dysfunction by combining the mechanistic strategies offered by two or three antioxidants to achieve clinically demonstrable

neuroprotection. Additionally, it should be noted that the tetraphenylphosphonium-based method changes mitochondrial potentials, which could be associated with toxic side effects. Thus, the future of mitochondria-targeted antioxidant therapies for PD will also depend on our developing better and more effective strategies targeting mitochondria with bioactive molecules. On the other hand, the mitochondria-targeted peptides (SS-31 and SS-20) have been effective in the MPTP models of PD, although their potential to treat or prevent PD needs further investigation. Finally, delivery of antioxidants using novel mitochondrially targeted nanomaterials may also be an attractive strategy for development of disease modifying drugs for treatment of neurodegenerative diseases such as PD.

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Abbreviations

MTA	mitochondria-targeted antioxidant
PD	Parkinson's disease
SNc	substantia nigra pars compacta
ETC	electron transport chains
$O_2^{\bullet -}$	superoxide anion
ROS	reactive oxygen species
NO	nitric oxide
TCA	tricarboxylic acid cycle
$ONOO^-$	peroxynitrite
RNS	reactive nitrogen species
H_2O_2	hydrogen peroxide
SOD	superoxide dismutase
GPx	glutathione peroxidase
TPx	thioredoxin reductase
OH•	hydroxyl radical
RO_2•	peroxyl radical

HOCl	hypochlorous acid
RO•	alkoxyl radical
HO_2•	hydroperoxyl radical
GSH	glutathione
GSSG	glutathione disulfide
NOS	nitric oxide synthase
CoQ_{10}	coenzyme Q_{10}
mtDNA	mitochondrial DNA
apocynin	4-hydroxy-3-methoxyacetophenone
TPP	triphenylphosphonium
MitoQ	mitoquinone
MitoVitE	mitotocopherol
TEMPOL	4-hydroxy-2,2,6,6,-tetramethyl-piperidine-1-oxyl
Ang II	angiotensin II
DMT	dimethyltyrosine
PLGA	poly-lactide-co-gylcolide
CAT	catalase
tBHP	t-butyl hydroperoxide
3NP	3-nitropropionic acid
Aβ	amyloid beta

Highlights

- MTAs are a novel class of antioxidant molecules targeted to the mitochondria
- MTAs attenuate oxidative damage underlying mitochondrial dysfunction
- MTAs show promise for treatment of many neurodegenerative diseases including PD
- Recent advances and alternative strategies of MTA therapy in PD are discussed

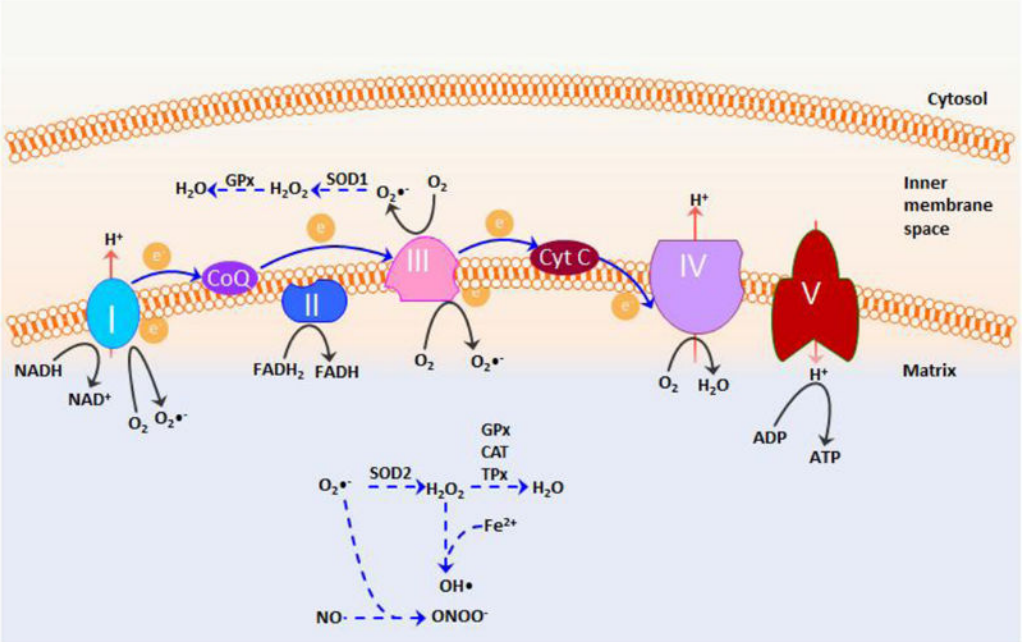

Figure 1.

Schematic presentation of the generation of ROS in mitochondria. ROS are generated from the transfer of electrons (e^-) to molecular oxygen to form superoxide ($O_2^{\bullet -}$) at the mitochondrial electron transport chain complex I and III. Once generated, superoxide is decomposed enzymatically by superoxide dismutase 1 (SOD1) in the intermembrane space and by SOD2 (MnSOD) in the matrix to form hydrogen peroxide, which is further catabolized to water by the action of enzymes such as catalase (CAT), glutathione peroxidases (GPx), and thioredoxin reductase (TPx) to avoid possible buildup of oxidative stress. However, under mitochondrial stress, superoxide may react with nitric oxide to form the potent oxidant and nitrating agent peroxynitrite ($ONOO^-$). Hydrogen peroxide can also form the highly reactive hydroxyl radical (OH•) in the presence of Fe^{2+} cations. These highly reactive radicals may cause damage to proteins, lipids, and nucleic acids. CoQ, coenzyme Q; Cyt C, cytochrome C.

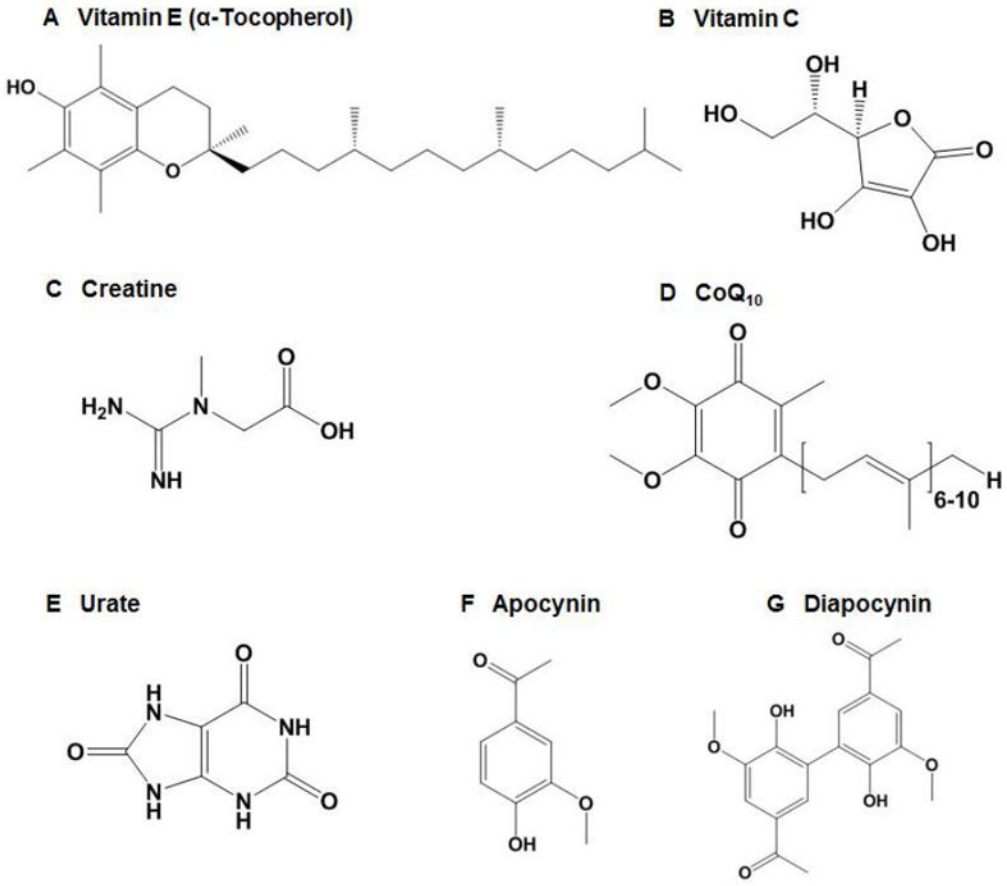

Figure 2.

Structures of a series of small-molecule antioxidants showing protection in PD models, including (*A*) vitamin E, (*B*) vitamin C, (*C*) creatine, (*D*) CoQ_{10}, (*E*) urate, (*F*) apocynin, and (*G*) diapocynin.

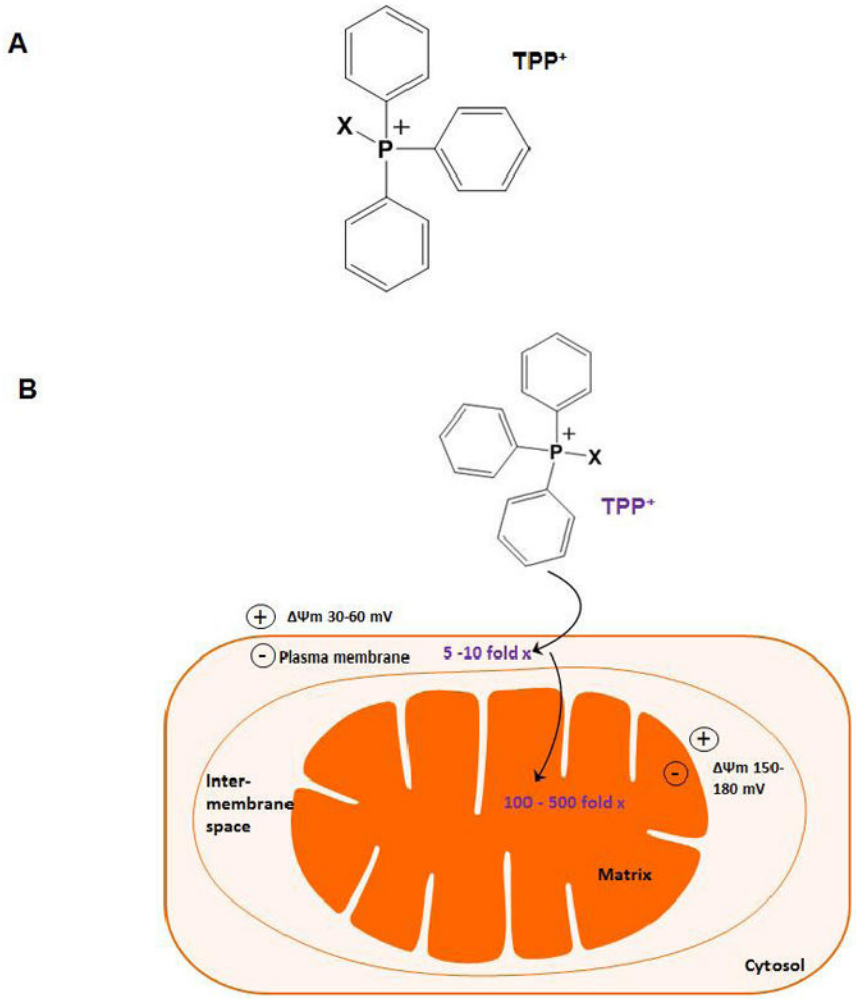

Figure 3.

Mitochondrial accumulation of targeted lipophilic cationic antioxidants. *A*, Structure of TPP (triphenylphosphonium). *B*, Conjugation to a lipophilic cation such as TPP specifically targets the attached bioactive moiety (X) into the mitochondrial matrix in a ΔΨm-dependent fashion. This strategy leads to a 100-500 fold accumulation of the bioactive compound within mitochondria. TPP, triphenylphosphonium.

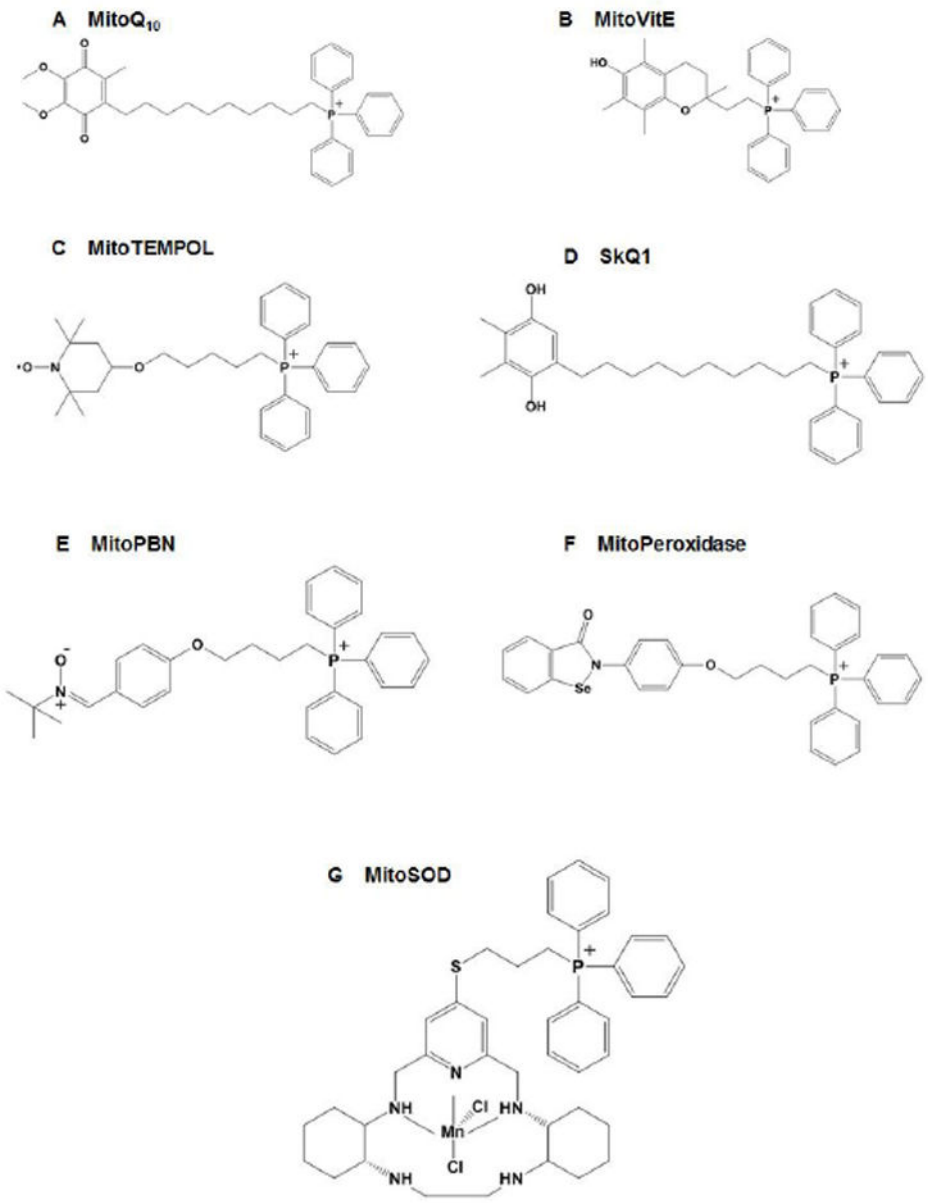

Figure 4.

Structures of a range of TPP-based mitochondria-targeted antioxidants, including (*A*) MitoQ, (*B*) MitoVitE, (*C*) MitoTEMPOL, (*D*) SkQ1, (*E*) MitoPBN, (*F*) MitoPeroxidase, and (*G*) MitoSOD.

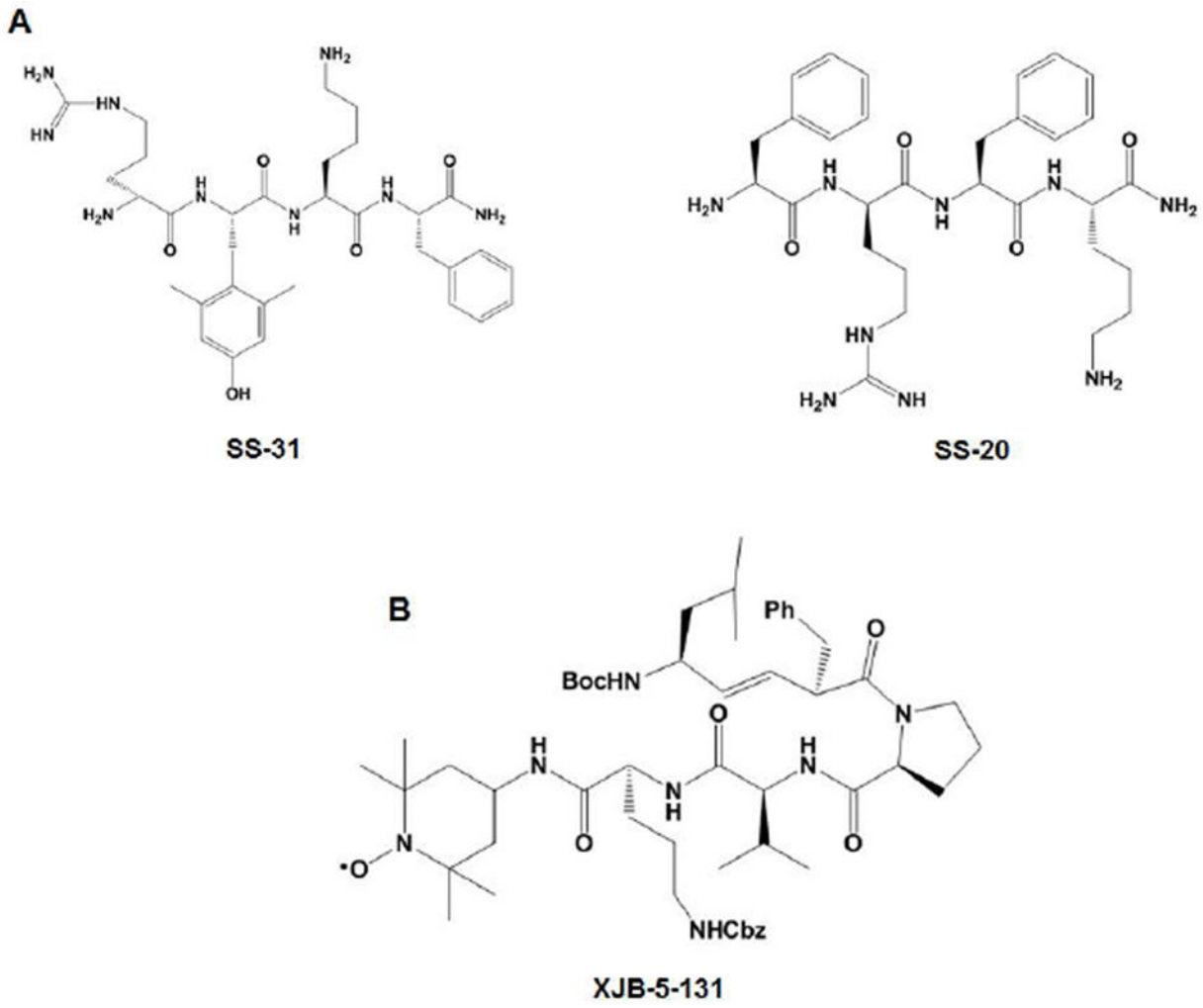

Figure 5.
Structures of (*A*) SS peptides (SS-20 and SS-31) and (*B*) XJB peptide XJB-5-131.

Table I

Mitochondria-targeted antioxidants in PD models

In vitro Compounds	Cellular Models	Results	References[a]
MitoQ	MPP^+-induced cellular model	Reduced MPP^+ toxicity in N27 cells and primary mesencephalic cultures	*[1]*
	6-OHDA-induced cellular model	Blocked 6-OHDA-induced mitochondrial fragmentation in SH-SY5Y cells	*[2]*
SS-20	MPP^+-induced cellular model	Reduced MPP^+ toxicity in SN4741 cells	*[3]*
SS-31	MPP^+-induced cellular model	Reduced MPP^+ toxicity in SN4741 cells	*[3]*
In vivo Compounds	Model Systems	Results	
MitoQ	MPTP mouse model	Protected dopaminergic neurodegeneration, preserved striatal dopamine, and improved motor functions	*[1]*
MitoApocynin	MPTP mouse model	Showed neuroprotection in MPTP-treated mouse	*[4]*
SS-31	MPTP mouse model	Decreased dopaminergic neuronal loss and restored dopamine levels	*[3]*
SS-20	MPTP mouse model	Decreased dopaminergic neuronal loss and restored dopamine levels	*[3]*
Human studies Compounds	Mode Systems	Results	
MitoQ	Phase II clinical trial	Showed no neuroprotective benefit of taking MitoQ	*[5]*

[a]References:

*[1]*A. Ghosh, K. Chandran, S.V. Kalivendi, J. Joseph, W.E. Antholine, C.J. Hillard, A. Kanthasamy, B. Kalyanaraman, Neuroprotection by a mitochondria-targeted drug in a Parkinson's disease model, Free radical biology & medicine, 49 (2010) 1674-1684.

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*[4]*A. Ghosh, C. Hogan, A. Kanthasamy, V. Anantharam, B. Kalyanaraman, A.G. Kanthasamy, unpublished data.

*[5]*B.J. Snow, F.L. Rolfe, M.M. Lockhart, C.M. Frampton, J.D. O'Sullivan, V. Fung, R.A. Smith, M.P. Murphy, K.M. Taylor, A double-blind, placebo-controlled study to assess the mitochondria-targeted antioxidant MitoQ as a disease-modifying therapy in Parkinson's disease, Movement disorders : official journal of the Movement Disorder Society, 25 (2010) 1670-1674.