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Antioxidants as Therapy for Parkinson's Disease

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DURING THE PAST TWO DECADES, substantial data have accumulated indicating that excessive oxidative damage occurs in the nervous system in Parkinson's disease (PD) and that oxidative stress plays a role in the pathogenesis of the disorder. In the following review, I summarize the evidence that oxidative damage contributes to the pathogenesis of PD, the mechanisms for the generation of reactive oxygen species (ROS) in the brain, particularly in the nigrostriatal dopaminergic system, and the defenses against oxidative stress. Finally, I review the preclinical and clinical studies for antioxidant compounds proposed as therapies in PD.

EVIDENCE OF EXCESSIVE OXIDATIVE DAMAGE IN PD

ROS can damage proteins, lipids, and nucleic acids, and methods have been developed to measure the amount of oxidative damage to each of these cellular components. Protein carbonyls reflect oxidative damage (16), and widespread increases in protein carbonyls in postmortem PD brains have been reported (1). Lipid oxidation is reflected by increased levels of malondialdehyde and cholesterol lipid hydroperoxides (33), and both malondialdehyde (18) and cholesterol lipid hydroperoxides (20) have been found to be increased in parkinsonian brains. Oxidative damage to DNA is reflected in the amount of 8-hydroxy-2-deoxyguanosine and 8-hydroxyguanine, and increased levels have been reported in the brains (2, 61) and cerebrospinal fluid (CSF) and serum (37).

In addition to the direct action of oxygen free radicals on proteins, superoxide can react with nitric oxide to form peroxynitrite, which can result in nitration of proteins (32), and nitration of Lewy bodies has been reported in parkinsonian brains (22).

MECHANISMS FOR FORMATION OF ROS IN THE BRAIN

The most common ROS in the brain is superoxide, which is primarily produced in the mitochondria. Mitochondria are the main source of adenosine triphosphate (ATP) in cells through oxidative phosphorylation in the mitochondria, but, unfortunately, passage of high-energy electrons down the mitochondrial electron transport chain can be a source not only of ATP, but also of ROS, as the high-energy electrons can react with O_2 to form superoxide (26). Up to 2% of the O_2 consumed by healthy mitochondria is estimated to be converted to superoxide, and this amount is higher in damaged and aged mitochondria.

Other sources of superoxide include xanthine oxidase (27, 42), which not only can produce superoxide, but has also recently been reported to be able to catalyze the formation of nitric oxide and peroxynitrite. However, it is located primarily in vasculature. NADPH oxidase, which is the superoxide-generating enzyme in phagocytes, is also expressed in microglia, astrocytes, and neurons in the brain (32).

Superoxide and hydrogen peroxide are not particularly toxic themselves, but they can be converted to hydroxyl radicals, which are highly reactive, by the Haber–Weiss reaction and the Fenton reaction, respectively. The presence of transition metals, *e.g.*, iron and copper, is crucial to the formation of hydroxyl radicals (26), and iron has been reported to be increased in the substantia nigra in PD brains (19). However, Beckman (7) has reported that these reactions are too slow to be a major source of toxicity. Ischiropoulos and Beckman (32) posit that the toxicity of superoxide may be enhanced through reaction catalyzed by peroxidases to produce hypohalous acids (HOCl, HOBr, and HOI) or that the toxicity of superoxide may be due to its interaction with nitric oxide to form peroxynitrite.

Dopaminergic neurons are at additional risk for oxidative damage because metabolism of dopamine in the presence of iron, copper, or manganese results in the formation of superoxide and hydrogen peroxide (26), which can be converted to the hydroxyl radical, as described above. Also, metabolism of dopamine by monoamine oxidase results in the formation of hydrogen peroxide.

Although substantial data indicate a role of oxidative stress in the pathogenesis of PD, a number of other pathogenic mechanisms have been implicated in PD, including mitochondrial dysfunction, inflammation, protein aggregation,

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and impaired protein degradation. Obviously, certain of these mechanisms, *e.g.*, mitochondrial dysfunction and inflammation, can contribute to the formation of ROS, and ROS can accelerate certain of these mechanisms, *e.g.*, protein aggregation and mitochondrial dysfunction.

DEFENSES AGAINST OXIDATIVE DAMAGE

Cells have a number of mechanisms to deal with ROS; these include superoxide dismutase (SOD) 1 (Cu/Zn-SOD), which is located primarily in the cytosol, SOD 2 (Manganese SOD), which is located in the mitochondrial matrix, the glutathione system, catalase, and thioredoxin (26). SOD converts superoxide to hydrogen peroxide. Hydrogen peroxide is typically detoxified by glutathione peroxidase, thioredoxin, and catalase. In the presence of transition metals, hydrogen peroxide can be converted by the Fenton reaction to the highly reactive hydroxyl radical. Because of the potential toxicity of free iron and copper, they are sequestered by binding proteins. In plasma, iron is bound to transferrin, which is present in excess, and copper is bound to ceruloplasmin or other copper-binding proteins. In the cell, iron is bound by ferritin and copper by metallothionein. However, some iron and copper obviously must move between these binding proteins and enzymes that they are part of. The pool of "free" iron is increased by hydrogen peroxide and damage to cells.

The importance of SOD 2 is underscored by the fact that mice in which SOD 2 has been knocked out die several days after birth with severe mitochondrial damage in several tissues (40). Catalase converts hydrogen peroxide to H_2O and O_2. Hydrogen peroxide can also be converted to H_2O by the glutathione peroxidase system.

Other antioxidant defenses are obtained from the diet, for example, ascorbate (vitamin C) and α-tocopherol (vitamin E). Ascorbate can reduce a number of ROS, including superoxide, hydroxyl radical, and peroxynitrite. α-Tocopherol is especially important because it is a potent chain breaking antioxidant in lipids, reducing the $LO_2^{\bullet}$ with formation of the tocopherol radical, which has a lower capacity to propagate lipid peroxidation. The α-tocopherol radical can be reduced by ascorbate and reduced coenzyme Q_{10} (ubiqinol). Other dietary antioxidants include β-carotene and polyphenols, particularly the flavonoids.

POTENTIAL ANTIOXIDANT THERAPIES IN PD

Selegiline (deprenyl)

The "Deprenyl and Tocopherol Antioxidative Treatment of Parkinsonism" (DATATOP) trial, which was conducted by Shoulson and colleagues in the Parkinson Study Group, was the first large trial of potential neuroprotective therapies in PD (55). The rationale for use of deprenyl (selegiline), which is an irreversible inhibitor of monoamine oxidase B (MAO-B), was twofold. First, deprenyl reduces metabolism of dopamine and generation of hydrogen peroxide, a ROS, by MAO-B. Second, the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which is a protoxin, is converted to the neurotoxin 1-methyl-4-phenylpyridinium (MPP^+) by MAO-B (14, 28). Researchers speculated that exogenous and endogenous compounds similar to MPTP might require conversion by MAO-B to be toxic. α-Tocopherol (vitamin E) was hoped to be protective through its antioxidant properties. Eight hundred subjects were randomized to receive one of four treatments: deprenyl (10 mg/day) and α-tocopherol (2,000 IU/day), deprenyl and α-tocopherol placebo, deprenyl placebo and α-tocopherol, deprenyl placebo and α-tocopherol placebo. The study demonstrated that treatment with deprenyl (with or without α-tocopherol) postponed disability requiring treatment with levodopa by ~9 months. However, a benefit was noted at 1 month, raising the possibility that the benefit of deprenyl was due to a symptomatic effect, perhaps by inhibition of dopamine metabolism. Treatment with α-tocopherol had no effect on the progression of PD.

Vitamin E (α-tocopherol)

Vitamin E is the generic name for a group of compounds known as tocopherols and tocotrienols (70). α-tocopherol is the major form of vitamin E in tissues of animals, including humans, and has the highest biological activity of all of the vitamin E compounds. Vitamin E is the major lipid-soluble, chain-breaking antioxidant in biological membranes (31). Some epidemiological studies have reported that dietary intake of vitamin E is associated with a lower risk of PD (17, 23), but another study using friends as case controls did not find this (62). Interestingly, Zhang *et al.* (74) reported that dietary, but not supplemental, intake of vitamin E was associated with a lower risk of PD, suggesting that vitamin E and other factors in diets high in vitamin E might have an effect on the risk for PD.

Perry *et al.* (57) reported that α-tocopherol protected the nigrostriatal dopaminergic system in MPTP-treated mice. However, other studies have not demonstrated benefit in MPTP-treated mice and marmosets (45, 48, 58). The negative results should be interpreted with caution because data from the study of Vatassery *et al.* (69) indicated the long period over which oral α-tocopherol gradually increases levels in the CSF and presumably in the brain. Vatassery *et al.* (69) reported that, in the DATATOP study (55) of 18 randomly selected subjects treated with α-tocopherol (2,000 IU/day, 37–644 days), there was a gradual, linear increase in the level of α-tocopherol in the CSF with increasing duration of treatment and a mean net increase of 76%. The net increase in CSF α-tocopherol concentrations after treatment showed a significant positive correlation with the number of days of vitamin E ingestion, suggesting that high-dose vitamin E treatment results in the elevation of CSF vitamin E levels and possibly brain vitamin E levels. However, a study of five PD patients did not find that oral α-tocopherol raised CSF levels (54). However, there were a number of differences between the two studies. The study of Pappert *et al.* (54) had fewer subjects, the subjects received dosages of α-tocopherol greater than 2,000 IU/day for only 2 months, and sampling was from the lateral ventricle where the study of Vatassery *et al.* (69) used lumbar CSF.

In the 6-hydroxydopamine (6-OHDA) model of PD in the rat, α-tocopherol has been shown to protect the nigrostriatal system (10). Roghani and Behzadi (60) reported benefit using *d*-α-tocopherol succinate, which reportedly gives higher and longer elevation of plasma levels.

As mentioned above, the DATATOP study indicated that α-tocopherol did not delay the need for levodopa in patients with early PD (55).

In a small, open-label, pilot study, Fahn (21) reported that treatment with both α-tocopherol and ascorbate (vitamin C) delayed the time until PD subjects needed treatment with levodopa or a dopaminergic agonist. Such combinations might merit further investigation.

Coenzyme Q_{10}

Coenzyme Q_{10} is both the electron acceptor for complexes I and II of the mitochondrial electron transport chain and an antioxidant (66). The antioxidant effect of coenzyme Q_{10} may be due to its ability to work in concert with α-tocopherol and reduce oxidized tocopherol and regenerate the reduced, antioxidant form (39).

Reduced levels of coenzyme Q_{10} have been reported in PD. Matsubara *et al.* (46) reported that the serum level of coenzyme Q_{10} in parkinsonian patients was significantly lower than that in patients with stroke, who were of similar age. Similarly, Molina *et al.* (49) reported that the serum level of coenzyme Q_{10}, but not the coenzyme Q_{10}/cholesterol ratio, was reduced in patients with Lewy body disease. However, Jiménez-Jiménez *et al.* (34) did not find a reduction in the serum level in PD. Shults *et al.* (67) reported reduced levels of coenzyme Q_{10} in platelet mitochondria in patients with early untreated PD compared with age/gender-matched control subjects.

Coenzyme Q_{10} has been shown to reduce damage to the nigrostriatal dopaminergic system in MPTP-treated mice and monkeys (5, 29).

Shults *et al.* (68) reported in a Phase II trial of three dosages (300, 600, and 1,200 mg/day) of coenzyme Q_{10} versus placebo (all subjects also received α-tocopherol at 1,200 IU/day) in patients with early untreated PD a positive trend toward slowing the functional decline as measured by the Unified Parkinson Disease Rating Scale. However, the authors have stressed that the results need to be confirmed and extended in a definitive Phase III trial before the recommendation can be made for widespread use of coenzyme Q_{10} in PD.

Dopaminergic agonists

Dopaminergic agonists have been used in the treatment of PD for over two decades on the basis of their ability to mimic the action of dopamine through binding to dopaminergic receptors. In addition, a number of dopaminergic agonists have been reported to be antioxidants with protective effects in models of PD. These include apomorphine (25), bromocriptine (52), cabergoline (73), pramipexole (3), and ropinirole (30). Studies have reported that ropinirole (30) not only acts as a ROS scavenger, but also up-regulates endogenous ROS-scavenging systems, apparently through the D_2 receptor, and that pramipexole also acts as an inhibitor of the mitochondrial permeability transition pore (11).

The potential neuroprotective effects of dopaminergic agonists in PD have been studied in two clinical trials comparing ropinirole (71) or pramipexole (56) with levodopa in patients with early PD using [^{18}F]fluorodopa PET or [^{123}I]β-CIT SPECT, respectively, as biomarkers of the preservation of remaining nigrostriatal dopaminergic axons. Patients treated initially with ropinirole had greater [^{18}F]fluorodopa accumulation in the striatum than those treated with levodopa at 2 years after initiation of therapy, and patients treated initially with pramipexole had greater [^{123}I]β-CIT binding in the striatum than those treated with levodopa at 22, 34, and 46 months after initiation of therapy. Unfortunately, the effects of levodopa and dopaminergic agonists on accumulation of [^{18}F]fluorodopa PET and [^{123}I]β-CIT SPECT are not known, and these agents may cause adaptive changes in [^{18}F]fluorodopa metabolism and/or [^{123}I]β-CIT binding, so the results cannot be unequivocally interpreted as indicative of preservation of nigrostriatal dopaminergic axons (9).

Melatonin

Melatonin is an indole synthesized from serotonin by 5-methoxylation and *N*-acetylation in the pineal gland. In addition to its role in circadian rhythms, melatonin has been found to have antioxidant capabilities, both as an antioxidant itself and possibly through its ability to stimulate endogenous antioxidant systems, *e.g.*, SOD, glutathione peroxidase, and glutathione reductase (59). Melatonin has been reported to be protective of the nigrostriatal dopaminergic system in rats treated with 6-OHDA (15) and MPP^+ (35). In mice treated with chronic, low-dose MPTP treatment, melatonin provided substantial benefit (4), but the benefit was less in animals treated with an acute MPTP lesion model (43). Not all groups have found benefit. Willis and Armstrong (72) reported that intracerebroventricular implants of slow-release melatonin in rats undergoing central injection of 6-OHDA or intraperitoneal injection of MPTP worsened outcome on a number of behavioral tests, but levels of dopamine and number of dopaminergic neurons were not reported. Intraperitoneal injection of MPTP is typically an effective model of parkinsonism in mice, but not in rats. Also, Morgan and Nelson (50) did not find benefit of melatonin in mice chronically administered it in drinking water (raising the plasma level 20-fold) and later treated with MPTP.

Despite, the availability of melatonin and some evidence that it can be protective of the nigrostriatal dopaminergic system in models of PD, there has been relatively little research on its effects in patients with PD. Over 30 years ago, Shaw *et al.* (65) reported that in four PD patients it did not affect disability at doses up to 1 g per day.

Polyphenols

There has been increasing interest in polyphenols, particularly flavonoids and catechins, as potential treatments for a number of disorders, including PD (44). Sources of polyphenols include green tea and berries (8). Studies have indicated that treatment of mice with either green tea extract or (−)-epigallocatechin-3-gallate, a polyphenolic extract of green tea, prevented MPTP-induced injury to the nigrostriatal dopaminergic system (13, 41) and in PC12 cells treated with 6-OHDA (53). However, Levites *et al.* (41) reported protection at lower, but not higher, doses of green tea extract and that green tea extract and (−)-epigallocatechin-3-gallate

were weak inhibitors of MAO-B, but did not increase striatal levels of dopamine in mice not treated with MPTP.

A single study has found an association between consumption of tea and reduced prevalence of PD, but the effect may be through caffeine (12).

Further investigation of the usefulness of polyphenols, particularly flavonoids, as agents in PD seems warranted.

Miscellaneous agents

Nitrone spin traps. Molecules incorporating a nitrone moiety, which have been nicknamed spin traps and were first developed as tools for study of oxidative reactions, were noted to be able to protect biological systems from oxidative damage (6, 24). Beal and his colleagues have reported on protection of the nigrostriatal dopaminergic system in MPTP-treated mice by a series of nitrone compounds (38, 47, 63).

Iron-chelating agents. Because of the increase in iron in the substantia nigra in PD (19) and the role that it plays in the generation of hydroxyl radicals, research has been directed to development of therapies to reduce iron levels, particularly through the use of chelating agents. Kaur *et al.* (36) reported that oral administration of the bioavailable metal chelator clioquinol reduced damage to the nigrostriatal dopaminergic system in MPTP-treated mice. Shachar *et al.* (64) more recently reported that a brain permeable iron chelator (VK-28) protected against 6-OHDA administered intracerebroventricularly.

Ebselen. Ebselen, which has glutathione peroxidase-like activity, has been reported to ameliorate the behavioral impairment and attenuate the loss of nigral neurons in MPTP-treated marmosets (51).

CONCLUSION

Excessive oxidative damage in PD is now well established, and a number of pathogenic mechanisms that appear to be involved in PD, *e.g.*, mitochondrial dysfunction and inflammation, could contribute to oxidative stress. Certain potential antioxidant therapies have been demonstrated to be beneficial in animal models of PD. However, in a number of preclinical studies, the antioxidant tested has not been found to be protective of the nigrostriatal dopaminergic system, and careful consideration should be given to the dose, duration of treatment, and model used. One Phase II trial of a mitochondrial component with antioxidant properties, coenzyme $Q_{10,}$ with α-tocopherol showed a positive trend toward benefit in patients with early PD, but the one Phase III trial of an antioxidant treatment, high dosage of α-tocopherol, did not demonstrate benefit. Antioxidants warrant further preclinical studies in models of PD, and for promising compounds clinical studies in PD patients.

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Dr. Shults is listed as co-inventor in a pending patent application for the use of coenzyme Q_{10} in neurodegenerative diseases. The application is jointly owned by Enzymatic Therapy, Inc. and The Regents of the University of California.

ABBREVIATIONS

ATP, adenosine triphosphate; CSF, cerebrospinal fluid; DATATOP, Deprenyl and Tocopherol Antioxidative Treatment of Parkinsonism; MAO-B, monoamine oxidase B; MPP+, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; 6-OHDA, 6-hydroxydopamine; PD, Parkinson's disease; ROS, reactive oxygen species; SOD, superoxide dismutase.

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