

## Review

# Multiple Antioxidants in the Prevention and Treatment of Parkinson's Disease

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Kedar N. Prasad, PhD, William C. Cole, PhD, Bipin Kumar, MD

*Center for Vitamins and Cancer Research, Department of Radiology, University of Colorado Health Sciences Center, Denver, Colorado*

**Key words:** oxidative stress, antioxidants, neurodegeneration, prevention, Parkinson's disease

Parkinson's disease (PD) is one of the major progressive neurological disorders for which no preventative or long-term effective treatment strategies are available. Epidemiologic studies have failed to identify specific environmental, dietary or lifestyle risk factors for PD except for toxic exposure to manganese, meperidine (Demerol®), the "designer drug" version of which often contains a toxic byproduct of the synthesis, 1-methyl-4-phenyl 1,2,3,6 tetrahydropyridine [MPTP]), and some herbicides and pesticides. The search for genetic risk factors such as mutation, overexpression or underexpression of nuclear genes in DA neurons in idiopathic PD has not been successful as yet. Polymorphism in certain genes appears to be a risk factor, but there is no direct evidence for the causal relationship between polymorphism and increased risk of PD. In familial PD, mutation in the  $\alpha$ -synuclein gene is associated with the disease, but a direct role of this gene in degeneration of DA neurons remains to be established. Although mutations in the Parkin gene has been associated with autosomal recessive juvenile Parkinson's disease, the role of this gene mutation in causing degeneration of DA neurons has not been defined. We have reported that in hereditary PD, a mutation in the  $\alpha$ -synuclein gene may increase the sensitivity of DA neurons to neurotoxins. We hypothesize that, in idiopathic PD, epigenetic (mitochondria, membranes, protein modifications) rather than genetic events are primary targets which, when impaired, initiate degeneration in DA neurons, eventually leading to cell death. Although the nature of neurotoxins that cause degeneration in DA neurons in PD is not well understood, oxidative stress is one of the intermediary risk factors that could initiate and/or promote degeneration of DA neurons. Therefore, supplementation with antioxidants may prevent or reduce the rate of progression of this disease. Supplementation with multiple antioxidants at appropriate doses is essential because various types of free radicals are produced, antioxidants vary in their ability to quench different free radicals and cellular environments vary with respect to their lipid and aqueous phases. L-dihydroxyphenylalanine (L-dopa) is one of the agents used in the treatment of PD. Since L-dopa is known to produce free radicals during its normal metabolism, the combination of L-dopa with high levels of multiple antioxidants may improve the efficacy of L-dopa therapy.

### Key teaching points:

- Free radicals from diverse groups of neurotoxins are a common intermediary risk factor for Parkinson's disease.
- Multiple antioxidants can reduce the risk of Parkinson's disease among a high risk population.
- Multiple antioxidants can enhance the efficacy of standard therapy in the treatment of Parkinson's disease.
- Epigenetic components of neurons (mitochondria, membrane and membrane structures, protein modifications) are primary targets for the action of neurotoxins.

## INTRODUCTION

Parkinson's disease (PD) is considered one of the major neurological disorders of the population over 65 years of age [1], and about 3% of the population over the age of 65 have PD

[2]. This is a slow, progressive disease and is characterized by the loss of dopaminergic neurons in the pars compacta of the substantia nigra (SN) and intraneuronal cytoplasmic inclusions called Lewy bodies. The causes for degeneration of DA neurons are not well understood. However, it can be assumed that

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Address reprint requests to: Kedar N. Prasad, Ph.D., Campus Box C-278, UCHSC, 4200 E. 9th Ave., Denver, Colorado, 80262.

interactions between external toxins (which arise from environmental, dietary and lifestyle factors), internal toxins arising from normal metabolism and the genetic (nuclear genes) and epigenetic (mitochondria, membranes, and proteins) components of neurons occur continuously. Any sustained adverse interaction between toxins and genetic or epigenetic components could initiate degeneration in DA neurons. The nature of this adverse reaction is not well understood, and external or internal risk factors have not been fully identified except in two cases. For example, an exposure to excessive amounts of manganese such as observed among manganese miners increases the incidence of PD-like disease [3]. This is because increased brain levels of free manganese can enhance production of free radicals, which then cause damage to DA neurons. Increased incidence of PD-like disease is also seen among users of the designer drug, meperidene, which contains 1-methyl-4-phenyl 1,2,3,6 tetrahydropyridine (MPTP), a neurotoxic byproduct formed during the synthesis of this drug [4]. At least one of the harmful effects of MPTP on DA neurons is mediated by free radicals [4]. In an effort to identify other external agents as risk factors, several epidemiological studies [5–9] that primarily focused on environmental and dietary agents have been performed. Some potential risk factors such as rural living, well-water consumption, exposure to herbicides and pesticides (for example, dieldrin and dithiocarbamates) and certain dietary toxins have been suggested. Although no particular dietary risk factors for PD have been found, the consumption of nuts and salad oil (pressed from seeds) has been found to be of protective value [6]. A large community-based study in the Netherlands has reported that vitamin E consumption was significantly lower among patients with PD than among controls [9]. Most of these investigations have not been confirmed by other independent studies. In addition, the above studies treated environmental, dietary and lifestyle factors separately. In reality, external risk factors can arise from all of these three sources, and neuroprotective agents may be present in the diet. Therefore, a well-designed epidemiologic study that simultaneously takes into account environmental, dietary and lifestyle risk and protective factors must be performed in order to identify potential neurotoxic and neuroprotective agents that may have impact on the incidence of PD.

Neurons are constantly exposed to external and internal toxins in the brain. One of the common mechanisms of action of these neurotoxins is mediated by oxidative stress due to an excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS). It may be reasonable to assume that they can cause point mutation and/or overexpression of certain genes which may initiate degeneration and eventually death of DA neurons in idiopathic PD. However, no such genetic defects in idiopathic PD have been demonstrated as yet. Polymorphism in certain genes, such as those that code for dopamine-transporter protein [10],  $\alpha$ -1-antichymotrypsin [11], monoamine oxidase B [12] and cytochrome P4501A1 (CYP1A1) [13], have been associated with increased risk of

idiopathic PD (Table 1). Polymorphism in DA neurons could lead to increased accumulation of neurotoxins in these cells. Since polymorphism in these genes was measured in peripheral cells, it is difficult to suggest that it also occurs in DA neurons. In addition, there is no direct evidence that polymorphic genes are either neurotoxic or increase the sensitivity of DA neurons to neurotoxic agents.

Point mutations in the  $\alpha$ -synuclein gene [14], which codes for a presynaptic nerve terminal protein and which has been identified as a component of Alzheimer's disease  $\beta$ -amyloid plaques [15], are associated with familial PD. This association can be causally related only when at least one of the following criteria is met: (a) products of mutated  $\alpha$ -synuclein are neurotoxic, (b) dividing DA neurons, when transfected with mutated  $\alpha$ -synuclein gene, undergo rapid degeneration upon differentiation, and this degeneration can be prevented by treatment with antisense RNA of the mutated  $\alpha$ -synuclein gene and (c) DA neurons expressing high levels of mutated  $\alpha$ -synuclein become more sensitive to neurotoxins. The last condition has been demonstrated in our recent study in which immortalized DA neurons expressing mutated  $\alpha$ -synuclein gene showed increased sensitivity to 6-hydroxydopamine [16]. However, point mutations in the coding region of  $\alpha$ -synuclein gene could not be demonstrated in autopsy samples of idiopathic PD [17]. A recent study [18] has reported that mutations in the Parkin gene are associated with autosomal recessive juvenile parkinsonism (AR-JP). The normal protein product of this gene may be related to the ubiquitin family of proteins, disturbances of which have been associated with many neurodegenerative disorders including Alzheimer's disease. It is interesting to note that no Lewy body inclusions occur in AR-JP, wherein a defective Parkin gene is present. Since Lewy body formation is a hallmark of idiopathic Parkinson's disease, the role of the Parkin gene mutation as a cause of PD remains to be established.

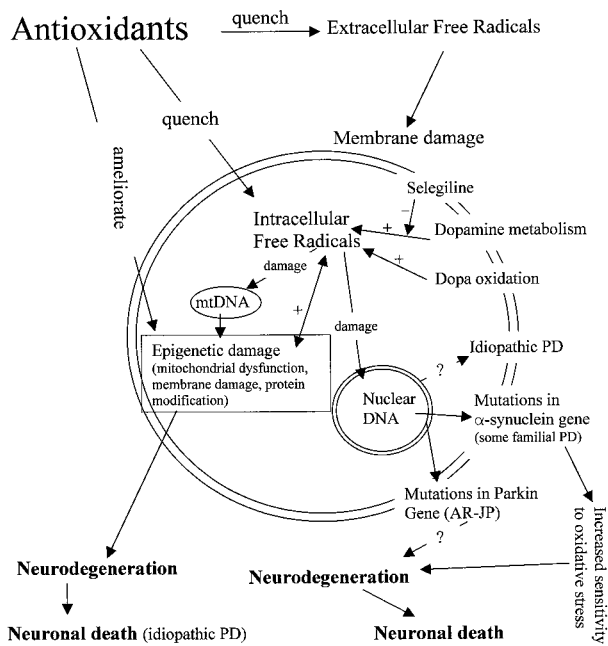
We propose an hypothesis that epigenetic components (cytoplasmic organelles such as mitochondria, cellular and nuclear membranes and proteins) rather than nuclear genes of DA neurons may be the primary targets of neurotoxins. Therefore, abnormalities in epigenetic components of DA neurons could initiate degeneration in idiopathic PD (Fig. 1).

In spite of the fact that external risk factors for PD incidence

**Table 1.** Increased Susceptibility to PD Associated with Gene Polymorphism

Gene Polymorphism	Consequences
3'-untranslated regions of DAT	Increased susceptibility to PD
$\alpha$ -1-antichymotrypsin	Increased susceptibility to PD
Monoamine oxidase B	Increased susceptibility to PD
Cytochrome P4501A1 (CYP1A1)	Increased susceptibility to PD

Data were taken from previously published work (10–13).  
 DAT = Dopamine transporter proteins.



**Fig. 1.** A diagrammatic view of free radical involvement as a common intermediary risk factor for many neurotoxic events in the pathogenesis of Parkinson's Disease. mtDNA=mitochondrial DNA, AR-JP=Autosomal Recessive Juvenile Parkinsonism +=increases, -=decreases, ?=Evidence lacking for point mutations or altered gene expression.

have not been specifically identified, it is certain that potentially damaging free radicals are constantly formed extracellularly and intracellularly as a part of normal metabolism in DA neurons. Antioxidant defense systems represented by antioxidant enzymes, synthesized antioxidants and dietary antioxidants can quench these radicals; however, if excessive numbers of free radicals are produced or endogenous antioxidant levels are reduced, damage to neurons can occur. Therefore, an appropriate balance between levels of free radicals and antioxidants is essential for the viability of neurons. Any imbalance in favor of free radicals could initiate degeneration by epigenetic and, subsequently, genetic alterations in DA neurons.

Several hypotheses have been proposed to explain the mechanisms of neuronal degeneration in PD. They include (a) production of increased levels of free radicals during oxidative metabolism of dopamine (DA) [19], (b) mitochondrial dysfunction [20–23], which can cause leakage of free radicals from mitochondria into the cytoplasm and nucleus, (c) increased accumulation of free iron [24,25] (Table 2), which could increase the production of free radicals and (d) reduced levels of antioxidant nutrients (glutathione, ascorbic acid) (Table 2) and antioxidant enzymes (catalase and glutathione peroxidase) [26–30]. These studies revealed that oxidative stress could play a critical role in the degeneration of DA neurons in PD. Evidence of increased oxidative stress in autopsy samples of substantia nigra in PD includes increased levels of malonyldialdehyde, lipid and cholesterol hydroperoxide [31,32] and increased levels of 8-hydroxy-2-deoxyguanosine, a marker of

**Table 2.** Levels of Ferritin, Fe+3, Glutathione and Ascorbic Acid in Substantia Nigra from Control and Parkinson's Disease (PD)

	Control (µg/g fresh tissue)	Parkinson's Disease (µg/g fresh tissue)
Ferritin	223±22	288±27
Fe+3	16±4	42±5
Glutathione	57±13	30±12
Ascorbic acid	309±47	271±15

Values are the means ± S.E.M.

Data were summarized from the work of Riederer, *et al.* (26).

DNA oxidative damage [33]. These studies suggest that supplementation with antioxidants may be useful in the prevention and treatment of PD. However, the results of clinical studies using a single antioxidant have been controversial [34,35].

One of the standard treatments of advanced PD includes L-dihydroxyphenylalanine (L-dopa), a precursor of dopamine (DA). L-dopa administration can increase the generation of free radicals in vivo [36–39], although this has not been demonstrated in PD patients. Severe side-effects, which are observed within five years of L-dopa therapy [40–43], may be due to increased production of free radicals during oxidative metabolism of L-dopa and dopamine. Therefore, the use of multiple antioxidants in combination with L-dopa may reduce certain side-effects of L-dopa therapy such as off-on phenomena and movement disorders. However, no clinical studies have been performed to test the efficacy of antioxidants in combination with L-dopa therapy. Selegiline, an inhibitor of monoamine oxidase, appears to be neuroprotective in some studies [34,43–45], but not in others [46]. The exact reasons for this discrepancy are unknown. Free radicals are produced during oxidative metabolism of dopamine. Since selegiline reduces this process, the levels of free radicals available from the oxidation of dopamine would be less. It has been estimated that about 70% of dopamine neurons are lost by the time clinical symptoms of PD occur [47]; therefore, oxidation of dopamine in the remaining DA neurons may not contribute greatly to the progression of disease compared to the cumulative effects of neuronal degeneration already suffered. Consequently, selegiline alone is not expected to prevent the progression of PD. However, when levodopa is used, dopamine levels in surviving DA neurons are increased. Thus, increased amounts of free radicals would be expected due to the oxidative metabolism of dopamine by monoamine oxidase and from non-enzymatic oxidation of levodopa. In this case, selegiline used in combination with L-dopa could be expected to reduce the increased levels of free radicals arising from the oxidation of dopamine, but not those free radicals arising from the oxidation of L-dopa. Therefore, treatment of PD with levodopa in combination with selegiline may have marginal beneficial effects, assuming the proper dosages are used. A rational treatment approach would be administration of a combination of multiple antioxidants, selegiline and L-dopa.

Two excellent recent reviews on the etiology, pathogenesis and treatment of PD have been published [48–50]. Although previous reviews have discussed the role of oxidative stress in the pathogenesis of PD, no unified hypothesis for the primary cellular targets of neurotoxins in DA neurons has been developed.

The purpose of this review is to critically examine the following: (a) the pro-oxidative and anti-oxidative status in normal and PD brains, (b) clinical studies that address the role of antioxidants in the prevention and treatment of PD and (c) scientific rationales for using multiple antioxidant supplements in the prevention of PD and, in combination with L-dopa therapy, in the treatment of PD. We also propose a novel hypothesis that epigenetic components of DA neurons represent primary targets for neurotoxins, and any defects in epigenetic components of cells could initiate slow neurodegeneration, leading eventually to cell death. Before discussing these issues, it is essential to describe briefly the processes by which oxidative stress could be generated in the brain.

### Processes of Generating Oxidative Stress in Brain

Free radicals are generated during the normal intake of oxygen, during infection and during normal oxidative metabolism of certain substrates. During normal aerobic respiration, the mitochondria of each rat nerve cell process about  $10^{12}$  oxygen molecules and reduce them to water. During this process,  $O_2^{\bullet-}$ ,  $H_2O_2$  and  $OH^{\bullet}$  are produced. In addition, partially reduced oxygen, which represents about 2% of consumed oxygen, leaks out and generates about  $2 \times 10^{10}$  molecules of  $O_2^{\bullet-}$  and  $H_2O_2$  per cell per day [51,52]. During bacterial or viral infection, phagocytic cells generate high levels of NO,  $O_2^{\bullet-}$  and  $H_2O_2$  in order to kill infective agents; however, these radicals can also damage normal cells [52]. During degradation of fatty acids and other molecules by peroxisome,  $H_2O_2$  is produced as a byproduct. During oxidative metabolism of ingested toxins, free radicals are also generated. Some brain enzymes such as monoamine oxidase (MAO), tyrosine hydroxylase and L-amino acid oxidase produce  $H_2O_2$  as a normal byproduct of their activity [19]. Furthermore, oxidation of ascorbate and catecholamines generate  $H_2O_2$  [53]. Oxidative stress can also be generated by  $Ca^{2+}$ -mediated activation of glutamate receptors. The  $Ca^{2+}$ -dependent activation of phospholipase  $A_2$  by N-methyl-D-aspartate (NMDA) releases arachidonic acid, which then liberates  $O_2^{\bullet-}$  during the biosynthesis of eicosanoid [53]. Another radical, NO, is formed by nitric oxide synthase stimulated by  $Ca^{2+}$ . NO can react with  $O_2^{\bullet-}$  to form peroxynitrite anions which can form  $OH^{\bullet}$  [54], the highly reactive hydroxyl radical. NMDA receptor stimulation produces marked elevations in  $O_2^{\bullet-}$  and  $OH^{\bullet}$  levels [55]. Some enzymes such as xanthine oxidase and flavoprotein oxidase (for example, aldehyde oxidase) also form superoxide anions during metabolism of their respective substrates. In addition, oxidation

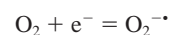
of hydroquinone and thiols and the synthesis of uric acid from purines form superoxide anions.

Certain external agents can increase oxidative stress. For example, cigarette smoking increases the level of nitric oxide (NO) by about 1000 ppm [56,57] and depletes antioxidant levels [58,59]. Free iron and copper can increase the levels of free radicals [60]. Plant foods contain large amounts of phenolic compounds such as chlorogenic and caffeic acid, which can be oxidized to form radicals [61,62]. Both reactive oxygen species (ROS) and reactive nitrogen species (RNS) contribute to neurodegeneration in the brain.

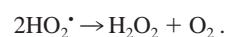
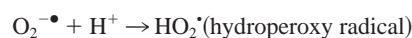
### Reactive Oxygen Species (ROS)

About 15% of the resting cardiac output is directed to the brain, although it represents only 5% of the total body weight. This corresponds to an amazingly constant oxygen utilization of 3.5 mL oxygen/100 grams of brain tissue/minute [63]. About 2% of the oxygen consumed becomes reactive oxygen species (ROS) [64], which include many types of free radicals that are described below.

When molecular oxygen ( $O_2$ ) acquires an electron, the superoxide anion ( $O_2^{\bullet-}$ ) is formed:



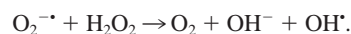
Superoxide dismutase (SOD) and  $H^+$  can react with  $O_2^{\bullet-}$  to form hydrogen peroxide,  $H_2O_2$ :



Both ferric and ferrous forms of iron can react with the superoxide anion and hydrogen peroxide to produce molecular oxygen and the hydroxyl radical ( $OH^{\bullet}$ ), respectively:



The hydroxyl radical can also be formed from superoxide anion by the Haber-Weiss reaction:

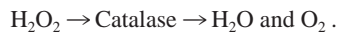


Both the Fenton and Haber-Weiss reactions require a transition metal such as copper or iron. Among ROS,  $OH^{\bullet}$  is the most damaging and is very short-lived.

The hydroxyl radical is very reactive with a variety of organic compounds, leading to the production of more radical compounds:



Catalase detoxifies hydrogen peroxide to form water and molecular oxygen:

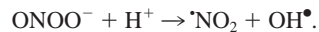


### Reactive Nitrogen Species (RNS)

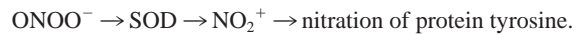
Non-oxygen reactive species (RNS) are represented by nitric oxide ( $\text{NO}^\bullet$ ), the quinone moiety of xenobiotics, the neurotoxin MPTP (N-methyl-4-phenyl-1,2,5,6-tetrahydropyridine) and the herbicide, paraquat.  $\text{NO}^\bullet$  is synthesized by the enzyme nitric oxide synthase from L-arginine, and, in brain, it acts both as a neurotransmitter and, in excessive amounts, acts as a neurotoxin.  $\text{NO}^\bullet$  can combine with the superoxide anion to form peroxynitrite, a powerful oxidant:



When protonated (likely at physiological pH), peroxynitrite spontaneously decomposes to reactive nitroso and hydroxyl radicals:



Superoxide dismutase can also enhance the peroxynitrite-mediated nitration of tyrosine residues on critical proteins, presumably via species similar to the nitronium cation ( $\text{NO}_2^+$ ):



These data reveal that several different types of radicals are constantly formed in the brain. Their levels can be increased by enhanced turnover of dopamine, increased levels of free iron, impaired mitochondrial functions, decreased glutathione levels and so on. Antioxidant enzymes which can protect cells against the damaging effects of these free radicals include catalase, superoxide dismutase and glutathione peroxidase. Reduced levels of these enzymes may increase the levels of free radicals. The levels of catalase and glutathione peroxidase are decreased in the substantia nigra of PD brains [29], but the level of superoxide dismutase is enhanced [65,66]. The latter can be considered as a transient adaptive response to oxidation stress. Natural dietary antioxidants include vitamins A, C and E, carotenoids, flavanoids and polyphenols. Some biosynthetic antioxidants include co-enzyme Q<sub>10</sub>, lipoic acid, reduced glutathione, reduced NADH and urates. The consumption of a diet low in antioxidants can also increase the levels of free radicals. Therefore, maintenance of a balance in favor of antioxidants is essential for optimal brain function. When this balance is shifted in favor of oxidants, epigenetic components of neurons suffer damage, and slow accumulation of such damage could initiate degeneration and eventually cause cell death.

### Analysis of the Status of Oxidation and Antioxidation in PD Brain

The normal brain has the highest concentration of unsaturated fatty acids of all organs, and these fatty acids are very

susceptible to lipid peroxidation [31]. The substantia nigra (SN) is the primary area of the brain that undergoes degeneration in PD. Autopsy samples of SN from PD brains revealed increased oxidant levels and decreased antioxidant levels. Increased levels of free iron (Table 2) were demonstrated in autopsy samples [24,25], as well as in brains of living PD patients, by iron-mediated contrast magnetic resonance imaging (MRI) [25]. The presence of free iron could increase the production of free radicals, especially hydroxyl radicals, which are considered the most reactive and damaging *in vivo*. One study shows that levels of ferritin, an iron-binding protein, decrease in the SN of PD brains [67], but another study shows that it slightly increases (about 30%) [26]. The reasons for this discrepancy are unknown.

SN samples from PD brains have reduced levels of antioxidant enzymes [29,30] and reduced levels of antioxidants [26–28]. These changes increase the balance in favor of a prooxidant environment, which can increase oxidative damage. Indeed, evidence of oxidative damage in the brain of PD has been observed [31–33]. It is not certain whether the above oxidative damage is a consequence or the cause of PD. Nevertheless, several studies [68–71] have emphasized the importance of oxidative stress in neurodegeneration. Therefore, it is rational to propose that supplementation with appropriate multiple antioxidants may reduce the rate of neurodegeneration. A few animal and clinical studies have been performed on this issue.

### Analysis of Animal Studies

In order to investigate the effect of antioxidants in an animal model of Parkinsonism, it is essential to study whether dietary antioxidant supplementation can increase brain levels of antioxidants. It has been reported that dietary supplementation with *dl*- $\alpha$ -tocopherol (1000 IU/day) for four months increased rat brain levels of vitamin E by about onefold and a quarter [72]. The brain and cerebrospinal fluid levels of vitamin E also increased twofold in dogs treated with vitamin E supplements for two years [73]. These studies suggested that dietary supplementation with vitamin E could be of protective value in an animal model of PD. Indeed, dietary supplementation with vitamin E and vitamin C [74] protected rats against 6-hydroxydopamine-induced striatal damage.

### Analysis of Clinical Studies

A few clinical studies have been performed using antioxidants in the treatment of PD. The results of these studies have been controversial. A large clinical, double-blind, placebo-controlled study involving 800 patients in the early stages of untreated PD was initiated to evaluate the efficacy of  $\alpha$ -tocopherol and deprenyl on the rate of progression of PD [34]. This study was referred to as "Deprenyl and Tocopherol Antioxidant Therapy of Parkinsonism (DATATOP)." The major endpoint

of this study was the time interval between initiation of experimental treatment and need for L-dopa treatment. Synthetic *dl*- $\alpha$ -tocopherol at a daily dose of 2,000 IU was given orally. This study failed to show any significant improvements based on the proposed endpoint [34]. This could imply that the rate of degeneration of DA neurons was not effected by synthetic vitamin E. The study proved beyond reasonable doubt that a daily dose of synthetic *dl*- $\alpha$ -tocopherol at a dose of 2,000 IU a day was ineffective in the treatment of early PD. The question of brain bioavailability of oral vitamin E is an important issue. One study has shown that oral supplementation with  $\alpha$ -tocopherol (400 IU–4,000 IU/day) for 30 days did not increase vitamin E levels in ventricular cerebral spinal fluid (CSF) of PD patients [75]. However, in the DATATOP study, the level of vitamin E in the lumbar CSF of patients receiving high doses of vitamin E was found to be about 74% higher than that found in the CSF of control patients [76]. The reasons for this discrepancy cannot be explained except for the fact that the sampling origin of CSF was different in the two studies. It is also uncertain whether the CSF levels of vitamin E reflect brain levels. In a preliminary study, supplemental vitamin E (3,000 IU/day) and vitamin C (3,000 mg/day) increased the time interval for requiring L-dopa therapy by about 2 to 4 years in 75% of patients when compared to historical controls; however, 16% of patients on vitamin therapy did not require L-dopa at the time of writing of the manuscript (about eight years) [35]. It is interesting to note that a combination of vitamin E and vitamin C produced some beneficial effect in patients with early PD, whereas vitamin E alone was ineffective [34]. Because of the contradictory results of these trials, we recommend antioxidant supplements based on scientific rationale for three population groups: (a) persons at high risk for PD, (b) patients with early stage PD and (c) patients on L-dopa therapy alone or in combination with selegiline. These recommendations should be assessed for efficacy in a well-designed clinical trial.

## RATIONALE FOR USING MULTIPLE ANTIOXIDANTS FOR PD PREVENTION AMONG HIGH-RISK

### Populations

High-risk populations include persons over 65 years of age, those who have suffered brain trauma or have been exposed to high levels of pesticides or herbicides or have a family history of PD. Because of potentially increased oxidative stress in the brains of members of these population groups, oral supplementation with appropriate antioxidants appears to be one of the rational choices for the prevention of PD among these high-risk populations. Conventional experimental designs for prevention use one or two antioxidants. These designs are not suitable for maximal efficacy of antioxidant therapy due to the varied actions of antioxidants, varied environments at the cellular and

organ levels and the varied nature of free radicals. We propose the use of multiple antioxidants for PD prevention trials. The biological rationale for using multiple antioxidants is described below.

Almost all antioxidants, when oxidized, can act as free radicals; therefore, the use of a single antioxidant in any clinical trial can not be considered a rationale for improving the disease outcome.  $\beta$ -carotene ( $\beta$ C) is more effective in quenching oxygen radicals than most other antioxidants [77].  $\beta$ C can produce certain effects that cannot be produced by its metabolite vitamin A and vice versa [78,79]. It has been reported that  $\beta$ C treatment enhances the expression of the connexin gene, a gap junction protein in mammalian fibroblasts in culture, whereas vitamin A treatment does not produce such an effect [78]. Vitamin A can induce cell differentiation in certain normal and cancer cells, whereas  $\beta$ C does not [80,81]. Thus  $\beta$ C and vitamin A can have different biological functions. The gradient of atmospheric (oxygen) pressure varies within cells. Some antioxidants such as vitamin E are more effective quenchers of free radicals in reduced oxygen pressure, whereas  $\beta$ C and vitamin A are more effective at higher atmospheric pressures [82]. Vitamin C is necessary to protect cellular components in aqueous environments, whereas carotenoids and vitamins A and E protect cellular components in non-aqueous environments. In addition, vitamin C is necessary for the activity of tyrosine hydroxylase, which is the rate-limiting enzyme in the synthesis of catecholamines. Oxidized forms of vitamin C and vitamin E can act as radicals; therefore, excessive amounts of any one of these forms, when used as a single agent, could be harmful over a long period of time. Vitamin C also plays an important role in maintaining cellular levels of vitamin E by recycling vitamin E radical (oxidized) to the reduced (antioxidant) form [83]. Also, oxidative damage produced by vitamin C (oxidized adenine nucleotides) [84] could be protected by vitamin E. The form of vitamin E is also important in any clinical trial. It is now known that, at least in rats, various organs selectively pick up the natural form of vitamin E [85]. It is also now established that  $\alpha$ -tocopheryl succinate ( $\alpha$ -TS) is the most effective form of vitamin E *in vitro* [86,87] and *in vivo* [88,89] by the criteria of growth inhibition of tumor cells and antioxidation. This form of vitamin E is more soluble than  $\alpha$ -tocopherol and enters cells readily [86]. Therefore, it is expected to cross the blood-brain barrier in greater amounts than  $\alpha$ -tocopherol. However, this has not been demonstrated in animals or humans. We have reported that oral ingestion of  $\alpha$ -TS (800 IU/day) in humans increased plasma levels of not only  $\alpha$ -tocopherol, but also  $\alpha$ -TS, suggesting that  $\alpha$ -TS can be absorbed from the intestinal tract before hydrolysis to  $\alpha$ -tocopherol [86]. This observation is important because the conventional assumption using rodents has been that esterified forms of vitamin E such as  $\alpha$ -tocopheryl acetate,  $\alpha$ -tocopheryl nicotinate and  $\alpha$ -TS can be absorbed from the intestinal tract only after they are hydrolyzed to  $\alpha$ -tocopherol. Our data show this assumption may not be true for the absorption of  $\alpha$ -TS in humans. Levels of reduced glutathione decrease

in PD [26–28]. Glutathione is effective in catabolizing  $H_2O_2$  and anions. However, oral supplementation with glutathione failed to significantly increase plasma levels of glutathione in human subjects [90], suggesting that this tripeptide is completely hydrolyzed in the G.I. tract. N-acetylcysteine, a glutathione precursor that is absorbed from the G.I. tract and increases the intracellular levels of glutathione, can be used as one of the dietary supplements. Since mitochondrial dysfunctions are associated with PD and since coenzyme  $Q_{10}$  and nicotinamide adenine dinucleotide (reduced form, NADH) are needed for generation of ATP by mitochondria, it is essential to use these antioxidants among the high-risk populations. A study has shown [91] that ubiquinol (coenzyme  $Q_{10}$ ) scavenges peroxy radicals faster than  $\alpha$ -tocopherol, but it is rapidly oxidized to give hydroperoxy radicals and/or superoxide. Therefore, it is a weaker antioxidant than  $\alpha$ -tocopherol. However, ubiquinol, like vitamin C [92], can regenerate vitamin E in a redox cycle [93,94]. Coenzyme  $Q_{10}$  administration improves clinical symptoms in patients with mitochondrial encephalomyopathies [95]. NADH administration (1.4 mg/Kg) has been useful in 415 PD patients [96]. In addition to acting as an antioxidant, it can stimulate the production of L-dopa *in vivo* [97] and dopamine in PC-12 cells, a dopaminergic cell line [98] as well as ATP. Selenium is a cofactor of glutathione peroxidase, and Se-glutathione peroxidase acts as an antioxidant. Therefore, selenium supplementation is essential. These studies suggest that appropriate doses of multiple antioxidants together with selenium would be essential for a maximal effect on PD prevention among high-risk populations.

### Recommended Antioxidant Supplements for High Risk PD Populations

A multiple antioxidant preparation containing vitamin A (retinyl palmitate, 5,000 IU/day), natural  $\beta$ -carotene (15 mg/day), vitamin E (*d*- $\alpha$ -TS, 100 IU/day with *d*- $\alpha$ -tocopherol 100 IU/day), vitamin C (calcium ascorbate, 500 mg/day), vitamin D (400 IU/day), B vitamin doses twofold to threefold higher than RDA values, selenium (100  $\mu$ g/day), chromium (50  $\mu$ g/day), zinc (15 mg/day), but no iron, copper or manganese is recommended. A commercial preparation, SEVAK, containing the above formulation is available (Scientific Nutrition, Inc., Oakland, CA). The latter trace minerals were not added to SEVAK because they are known to interact with vitamin C to produce increased levels of free radicals. In addition, increased iron stores have been linked to increased risk of chronic diseases including PD. SEVAK has been recommended for maintaining general health as well as increasing the antioxidant capacity of the brain. The doses in this preparation have been selected based on safety rather than for tested efficacy. This could serve as base antioxidant supplement for all risk groups of PD.

Additional supplements include one gram of vitamin C in the form of calcium ascorbate, 200 IU of vitamin E in the form of *d*- $\alpha$ -TS and 250 mg of N-acetylcysteine. Calcium ascorbate

is recommended because it is a nonacidic form of vitamin C and unlikely to cause the gastric upset observed in some cases when high doses of ascorbic acid are used. These doses are based on safety, the efficacy of which can be tested in clinical trials.

## RATIONALES FOR USING MULTIPLE ANTIOXIDANTS IN PATIENTS WITH EARLY STAGE PD

Early stage PD is referred to as a condition where no L-dopa therapy is required. Therefore, any significant extension of this time interval would be an important contribution to the management of PD. The large DATATOP study using 2,000 IU of synthetic vitamin E in the form of  $\alpha$ -tocopherol failed to extend the above time interval. Based on the rationale for using multiple antioxidants in this review, it is not surprising that DATATOP did not yield positive results. There was another flaw in the DATATOP study design. A multiple vitamin preparation (One-A-Day™) was allowed for all individuals who wished to take it. It was argued that the effect of 30 IU of vitamin E, which was present in the above vitamin preparation, would not significantly contribute to the effect of 2,000 IU of vitamin E. This argument may not be valid, since it has been shown that the effect of multiple antioxidants is more pronounced than that produced by the same doses of antioxidants used individually [99,100]. Therefore, the impact of such a multiple vitamin on antioxidation activity would be more than that produced by 30 IU of vitamin E alone. The consumption of One-A-Day™ is likely to create an unacceptable variable while evaluating the efficacy of vitamin E supplementation in early PD, especially when both experimental and placebo groups were allowed to have this multiple vitamin/antioxidant preparation in an uncontrolled fashion. A combination of vitamin E and vitamin C was useful in extending the time needed for L-dopa therapy [35]. This study was not a placebo-controlled trial; therefore, the data remain to be confirmed.

### Recommended Antioxidant Supplements for Patients with Early-Stage PD

It is proposed that consumption of the following antioxidants may reduce the rate of progression of early PD: The multiple antioxidant preparation, SEVAK, as described earlier. In addition, natural  $\beta$ -carotene (15 mg/day), *d*- $\alpha$ -tocopheryl succinate (400 IU/day), vitamin C (calcium ascorbate, 2 g/day), selenium (200  $\mu$ g/day), coenzyme  $Q_{10}$  (100 mg/day), and reduced NADH (10 mg/day). The doses of coenzyme  $Q_{10}$ , NADH, vitamin E, and vitamin C at higher doses than proposed here have been used in patients with neurodegenerative diseases with some efficacy when used individually (coenzyme  $Q_{10}$  [95], and NADH [96]) or used in combination (vitamin E and vitamin C) [35]. Because of positive interactions between

these nutrients, we have proposed lower doses of antioxidants. Nevertheless, these doses should be considered arbitrary, but safe.

## **RATIONALE FOR USING MULTIPLE ANTIOXIDANTS IN PATIENTS REQUIRING L-DOPA THERAPY ALONE OR IN COMBINATION WITH SELEGILINE**

L-dopa therapy is one of the common therapies for advanced PD. However, the severe side-effects of this therapy appear in about five years [40–43]. The reasons for this are not known; however, L-dopa can generate free radicals during its own oxidation as well as during oxidative metabolism of its product, dopamine. Thus, it appears rational to propose that an excessive quantity of free radicals is generated, and this may be one of the factors which contribute to the side-effects of levodopa therapy. Selegiline used in combination with levodopa may reduce free radical levels by reducing the oxidative metabolism of dopamine; however, it would not affect the level of free radicals generated by the oxidation of L-dopa. Therefore, it is possible that supplementation with appropriate multiple antioxidants may improve the efficacy of L-dopa therapy alone or in combination with selegiline.

### **Recommended Antioxidant Supplements for Patients Requiring L-dopa Alone or In Combination With Selegiline**

In addition to the multiple antioxidants recommended for high risk populations, additional antioxidants include natural  $\beta$ -carotene (30 mg/day), *d*- $\alpha$ -tocopheryl succinate (600 IU/day), vitamin C (4 g/day), coenzyme Q<sub>10</sub> (200 mg/day), NADH (10 mg/day), N-acetylcysteine (500 mg/day), Zn (30 mg/day) and selenium (200  $\mu$ g/day). Doses of coenzyme Q<sub>10</sub>, NADH, vitamin E and vitamin C higher than proposed here have been used in patients with neurodegenerative diseases with some efficacy when used individually (coenzyme Q<sub>10</sub>, and NADH) or used in combination (vitamin E and vitamin C). N-acetylcysteine can also behave as a metal chelator; a dose of 800 mg/day of N-acetylcysteine for two weeks was shown to mobilize zinc stores and increase zinc's urinary excretion [101]. Therefore, additional Zn supplement is included. In contrast, N-acetylcysteine doses of 200 mg t.d.s. for two weeks produced no change in the plasma levels or urinary excretion of calcium, magnesium, iron, zinc or copper [102]. In advanced cases of PD in which a deficiency of glutathione exists, supplementation with N-acetylcysteine is necessary. Because of positive interactions between these nutrients, lower doses have been proposed. Nevertheless, these doses should be considered arbitrary, but safe.

The recommended antioxidant supplements for all risk

groups for PD should be taken orally and divided into two doses, half in the morning and the other half in the evening. This is because the biological half-life of most antioxidants varies from six to twelve hours. To sustain high levels of antioxidants in the brain, these antioxidants must be taken twice a day. Compliance rates should be determined by counting the pills and by determining the blood levels of antioxidants and appropriate minerals before supplementation, then, once a year, for the entire period of study. A well-designed placebo-controlled clinical trial should be initiated for each PD risk group.

In summary, in spite of the fact that specific risk factors for most idiopathic PD have not been identified, reactive oxygen species (ROS) and reactive nitrogen species (RNS) represent common intermediary agents arising from a diverse group of neurotoxins which initiate neuronal degeneration. We have proposed that in idiopathic PD, the primary targets for ROS and RNS are epigenetic components (mitochondria, membranes and proteins) rather than nuclear genes. We have provided scientific rationale for using multiple antioxidants in the prevention and, as an adjunct to standard therapy, in the treatment of PD.

## **ACKNOWLEDGMENT**

This study was supported by U.S. Public Health Service grants RO1 NS 29982 and RO1 NS 35348.

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*Received May 1999; revision accepted July 1999.*