Vitamin E therapy in Parkinson’s disease

Marc W. Fariss *, Jin-Gang Zhang

Departments of Pharmaceutical Sciences and Pharmacotherapy, College of Pharmacy, Washington State University, Pullman, WA 99164-6534, USA

Abstract

Though the etiology is not well understood, late-onset Parkinson’s disease (PD) appears to result from several key factors including exposure to unknown environmental toxicants, toxic endogenous compounds and genetic alterations. A plethora of scientific evidence suggest that these environmental and endogenous factors cause PD by producing mitochondrial (mito) oxidative stress and damage in the substantia nigra, leading to cell death. Thus assuming a critical role for mito oxidative stress in PD, therapies to treat or prevent PD must target these mito and protect them against oxidative damage. The focus of this article is to briefly review the experimental and clinical evidence for the role of environmental toxicants and mito oxidative stress/damage in PD as well as discuss the potential protective role of mito d-α-tocopherol (T) enrichment and vitamin E therapy in PD. New experimental data are presented that supports the enrichment of mito with T as a critical event in cytoprotection against toxic mito-derived oxidative stress. We propose that chronic, high dose vitamin E dietary supplementation or parenteral vitamin E administration (e.g. vitamin E succinate) may serve as a successful therapeutic strategy for the prevention or treatment of PD (by enriching substantia nigra mito with protective levels of T).

© 2003 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Vitamin E; Vitamin E succinate; Alpha-tocopherol; Mitochondria; Parkinson’s disease; Oxidative stress; Environment

1. Introduction

Parkinson’s disease (PD) is a chronic, late onset, progressive neurodegenerative disease that is characterized by the intraneuronal deposition of alpha synuclein proteins (Lewy bodies) and the irreversible loss (cell death) of dopaminergic neurons in the substantia nigra pars compacta. The etiology of PD is not well understood but appears to result from several factors, including exposure to unknown environmental toxicants (e.g. mitochondrial complex I inhibitors), toxic endogenous compounds (dopamine metabolism), and genetic alterations. An estimated 1% of the United States and European populations over the age of 60 have PD (Maher et al., 2002). Epidemiological evidence clearly implicates environmental factors in non-familial PD, which represents over 90% of all PD cases and onset typically occurs after 50 years of age (Tanner et al., 1999).

Fortunately, excellent experimental model systems are available to study non-familial or late-onset PD. For example the mitochondrial (mito) complex I inhibitors, 1-methyl-4-phenyl-1,2,3,6-
tetrahydropyridine (MPTP) and rotenone have been shown to induce a PD-like syndrome in humans, monkeys, and mice (MPTP) and in rats (rotenone) (Ebadi et al., 1996; Betarbet et al., 2000). Using these model systems, researchers have made considerable progress over the past decade in understanding the molecular, biochemical, and pathological processes underlying PD. However, to date, a successful therapeutic strategy for the prevention or treatment of this devastating disease has not been found. The focus of this article is to briefly review the experimental and clinical evidence for the role of environmental toxicants and mito oxidative stress in PD as well as discuss the potential protective role of mito d-α-tocopherol (T) enrichment and vitamin E therapy in PD.

2. Role of mitochondria and oxidative stress in Parkinson’s disease

Based on experimental and clinical data, it is well established that both mito and oxidative stress play critical roles in the pathogenesis of PD (Ebadi et al., 1996; Betarbet et al., 2000). Researchers have shown that both forms of cell death, apoptosis (Hartley et al., 1994; Duan et al., 1999; Gomez et al., 2001) and necrosis (Hartley et al., 1994; Maruyama et al., 2000; Hartmann et al., 2001) appear to be involved in the destruction of dopaminergic neurons in experimental and clinical PD, and that mito and oxidative stress play key roles in initiating these cell death processes. For example, numerous studies have now demonstrated that exposure of dopaminergic cell lines (e.g. PC12) to low levels of PD-inducing chemicals such as MPTP (use MPP+ in cell culture) and rotenone, result in modest mito complex I inhibition and dysfunction, enhanced reactive oxygen species (ROS) production, modest cellular ATP depletion and the translocation of mito cytochrome c to the cytosol leading to caspase-3 activation and apoptosis (Hartley et al., 1994; Duan et al., 1999; Gomez et al., 2001). Compelling evidence for the important role of mitochondria in PD comes from the in vivo studies of Vila et al. (2001), demonstrating that Bax- (a protein thought to permeabilize the mito outer membrane to release cytochrome c) null mice are completely protected against severe MPTP-induced neurotoxicity in a murine model of PD. Furthermore, evidence for caspase-3 and bax activation has also been found in postmortem substantia nigra samples from parkinsonian patients (Hartmann et al., 2000; Tatton, 2000). The induction of necrotic cell death by these same experimental neurotoxicants has been shown to occur at much higher concentrations and is characterized by the presence of enhanced ROS production, severe mito complex I inhibition, severe cellular ATP depletion, mito permeability transition and mito swelling (Hartley et al., 1994; Maruyama et al., 2000; Mayo et al., 1999; Hartmann et al., 2001; Vila et al., 2001).

Finally, recent studies by Lee et al. (2002), have demonstrated that alpha synuclein aggregates, a hallmark sign of PD, are formed in cells exposed to mito electron transport inhibitors (e.g. rotenone) and these protein inclusions are reversed by restoring normal mito metabolism. In conclusion, the experimental data, though unclear as to which form of cell death predominates in PD (current evidence favors apoptosis), strongly implicates pathological changes in mitochondria of dopaminergic neurons as a critical event that leads to alpha synuclein aggregation, cell death, and PD.

In addition to mito dysfunction, it is clear that oxidative stress plays an important role in the pathogenesis of PD. Reported evidence of ongoing oxidative stress in PD includes (1) enhanced ROS production, lipid peroxidation, mito DNA (mtDNA) damage and inactivation of alpha ketoglutarate dehydrogenase (KGDH), a key mito citric acid cycle enzyme, in dopaminergic cells or mice (striatal region) exposed to toxic levels of MTPP (Mizuno et al., 1987; Wei et al., 1996; Moussaoui et al., 2000; Mandavilli et al., 2000; Gibson et al., 2000) and (2) the identification of high levels of 4-hydroxynonenal-protein adducts (in Lewy bodies) and high levels of 8-hydroxyguanosine as well as the loss of KGDH immunostaining in the substantia nigra of PD patients but not in other brain regions (also observed in experimental animal models for PD) (Mizuno et al., 1994; Yoritaka et al., 1996; Alam et al., 1997; Selley, 1998; Zhang et al., 1999; Gibson et al., 2000). The selective expression of toxic
oxidative stress in mito of these dopaminergic cells has been explained by several pro-oxidant factors, namely (a) mito complex I inhibitors (such as MPTP and rotenone) enhance mitochondria electron transport chain generation of ROS (Hasegawa et al., 1990; Cleeter et al., 1992) and the cation MPP⁺ (a metabolite of MPTP) rapidly accumulates in dopaminergic cells (via dopamine transporter) and into their negatively charged mitochondria, (b) in dopaminergic cells, dopamine is metabolized by mito monoamine oxidase B and in the process generates ROS and toxic metabolites (Cohen and Kesler, 1999; Berman and Hastings, 1999; Kristal et al., 2001) and (c) iron levels in the substantia nigra are increased in PD patients and experimental animal models (Temlett et al., 1994; Graham et al., 2000). Iron is abundant in mitochondria and the release of free iron by oxidative stress catalyzes the production of toxic ROS species such as hydroxyl radicals (resulting in lipid, nucleic acid and protein oxidation) and lipid alkoxyl radicals (resulting in 4-hydroxynonenal formation). Additional evidence supporting a critical role for oxidative stress in PD includes the findings that cellular antioxidants such as glutathione (GSH) are depleted in PD patients (Cohen and Kesler, 1999) and treatment with antioxidants (including trolox, an anionic vitamin E derivative), with iron chelators, or with overexpression of Cu/Zn-superoxide dismutase can protect mice and cells from MPTP-induced cell death and neurotoxicity (Temlett et al., 1994; Beal et al., 1998; Ithayarasi and Shyamala, 1998; Berman and Hastings, 1999; Graham et al., 2000; Moussaoui et al., 2000; Kristal et al., 2001; Mansouri et al., 2001). In summary, there exists considerable data to support mitochondria-derived oxidative stress and mito dysfunction in the pathogenesis of PD.

3. Role of environmental toxicants in Parkinson’s disease

It is now widely believed that the etiology of late-onset PD has a strong environmental component, with the possibility of coexisting genetic susceptibility (Tanner et al. 1999; Maher et al., 2002; Payami et al., 2002). As briefly outlined below, clinical, epidemiological and experimental studies support the potential role of many different environmental toxicants in the development of PD such as pesticides and herbicides (rotenone, paraquat, heptachlor, dieldrin), metals (manganese, iron, copper), synthetic drug products (MPTP) and plant-related food and natural products (cycads, beta-carboline alkaloids, tropical plants). It is interesting to note that regardless of the toxicant category listed above, each agent’s mechanism of toxicity (except plants not studied) is related to the induction of mito oxidative stress and/or mito dysfunction (Hirai et al., 1992, 1999; Betarbet et al., 2000; Chun et al., 2001; Collins, 2002; Roth et al., 2002; Sherer et al., 2002). These consistent findings again (as described in the previous section) support a causative relationship between substantia nigra mito oxidative stress/damage and mito dysfunction (induced by environmental and endogenous factors) and the development of PD. Though no gene defects have been identified for late-onset PD, one can easily envision how genetic variations (e.g. polymorphisms) might influence this toxic mito oxidative stress in PD by affecting endogenous processes that are responsible for environmental toxicant bioactivation and detoxification, mito pro-oxidant production or mito antioxidant protection, to name a few (Payami et al., 2002; Maher, et al., 2002).

Critical to understanding the pathogenesis of PD is defining the environmental factors that are responsible for this chronic, late-onset progressive disease (see the reviews of Lockwood, 2000; Jenner, 2001; Sherer et al., 2002). The importance of the environment in the development of PD was clearly shown in the study of Tanner et al. (1999), in which approximately 20,000 pairs of twins were screened for PD. The authors concluded that the similarities in the concordance rates of monozygotic as compared with dizygotic twins after the age of 50 years (late-onset) indicated that genetic factors do not play a significant role in the development of PD. However, in the same study genetic factors appeared to play an important role when one of the twins developed PD at an earlier age. The findings of this study continue to have a strong influence on the direction of parkinsonism research with this topic given a research funding
emphasis by the National Institute of Environmental Health Sciences. In fact, there are numerous epidemiological studies, which support the role of environmental factors in PD. Past studies have reported an increased risk for developing PD associated with living in a rural area, drinking well water, farming and exposure to pesticides and herbicides, head trauma and industrial pollutants such as heavy metals (Gorell et al., 1999a,b; Engel et al., 2001; Priyadarshi et al., 2001; Maher et al., 2002). Unfortunately, epidemiological studies have had little success in identifying an association between long-term exposure to a specific environmental toxicant and the development of PD.

The most dramatic direct evidence for a specific environmental toxicant causing PD was reported by Langston et al. (1983), in which the administration of MPTP, a product from an attempt to synthesize meperidine, caused an acute and irreversible parkinsonism syndrome in these individuals. This finding has led to the use of MPTP as an experimental model for PD and to a better understanding of the mechanism of PD pathogenesis (as described in the previous section). The identification of MPTP-induced inhibition of complex I of the mito electron transport chain and mito dysfunction as important events in the development of experimental PD has prompted a search for other environmental toxicants that have structural and mito inhibition properties that are similar to MPTP. As a result, experimental or human exposure studies have identified several pesticides (such as rotenone and the organochlorine insecticides, dieldrin and heptachlor) that cause mito dysfunction and induce a parkinsonism syndrome when administered to experimental animals or nigral dopaminergic neurons (Betarbet et al., 2000; Lockwood, 2000; Chun et al., 2001; Kirby et al., 2001; Sherer et al., 2002). In addition, chemicals that are structurally similar to MPTP, such as the herbicide paraquat, have recently been shown to induce experimental PD in vivo and in vitro, presumably through a mito oxidative stress mechanism (Hirai et al., 1992, 1999; Manning-Bog et al., 2002; McCormack et al., 2002) and have been associated with clinical PD following dose-dependent lifetime paraquat exposure (Liou et al., 1997).

Prior to the industrial age and the widespread production and use of synthetic chemicals (such as the pesticides mentioned above), PD clearly existed as noted by James Parkinson in 1817. Thus assuming a strong role for environmental factors in the development of this chronic disease, human exposure to natural products including plant derived foods or products and heavy metals must be considered potential contributors to PD. Certainly, previous reports support such a role for natural products. For example, manganese miners are known to have developed PD-like symptoms that responded to L-dopa therapy (Huang et al., 1989) and manganese induces an oxidative stress, mito dysfunction, and apoptosis in rat PC 12 cells and human dopaminergic (SN4741) neuronal cells (Chun et al., 2001; Roth et al., 2002). The transition metals, iron and copper, are natural environmental toxicants that have also been reported to play a role in the etiology of PD (Gorell et al., 1999a; Perry et al., 2002). Since plant-based foods or products are known to contain numerous compounds that may have structures similar to MPTP (e.g. plant alkaloids or beta carboline; Collins, 2002) or have mito complex I inhibition activity (Sherer et al., 2002), these environmental-related substances represent the greatest unknown source of potential agents that may contribute to the development of PD. Several natural products that have been shown to induce parkinsonism symptoms in humans include cycad plant neurotoxins (ingested through the consumption of flying foxes that consume cycad seeds) and plant extracts consumed in Guam and the French West Indies (Spencer et al., 1987; Caparros-Lefebvre and Elbaz, 1999; Cox and Sacks, 2002).

As illustrated above, environmental factors most likely play an important role in late-onset PD. Unfortunately, based on our limited understanding of specific environmental agents that are responsible for clinical PD, reducing or eliminating our exposure to these unknown agents is not a viable therapeutic strategy for the prevention of PD. Instead, additional studies are required to identify and characterize PD-causing environmental agents, which will promote the development of effective protective strategies.
4. Use of vitamin E treatment in Parkinson’s disease

Once investigators realized that oxidative stress and lipid peroxidation play an important role in the etiology of PD, vitamin E was investigated as a potential treatment for PD, both clinically and in experimental models (Prasad et al., 1999). In terms of antioxidant protection, this is an excellent choice since T is the predominate membrane-bound antioxidant in mammalian cells that scavenges oxygen radicals and protects lipids, nucleic acids and proteins against oxidation (Tappel, 1962; Burton et al., 1983; Cheeseman et al., 1988; Ithayarasi and Shyamala, 1998; Mansouri et al., 2001). This chain-breaking antioxidant is not synthesized by mammalian cells, and once membrane T is consumed (oxidized to inactive form) during periods of oxidative stress, cellular macromolecules are subject to peroxidation that can result in toxic injury (Traber, 1996; Fariss, 1997). Thus the T enrichment of membranes appears to be important for cell viability during a toxic oxidative challenge (Vatassery, 1993; Fariss, 1997). Surprisingly, the T content of cellular membranes and especially mito (see Figs. 1 and 3) are maintained at low levels and close to the threshold concentration required to halt the propagation of lipid peroxidation and the oxidation of cellular proteins and nucleic acids (Kornburst and Mavis, 1980; Fry and Green, 1981; Liebler et al., 1986; Nomura et al., 2000; Lesnefsky et al., 2001). Since cells have a limited ability to store T, the replacement of oxidized T comes from extracellular sources (diet). Recent studies from our lab and others suggest that the ability of dietary or extracellular T to rapidly enrich cellular membranes with active T (as would be needed during an oxidative challenge) is, in most cases, limited (see Fig. 1; Kornburst and Mavis, 1980; Sukalski et al., 1993; Fariss et al., 2001; Umansky et al., 2001). This is especially true for the central nervous system (CNS). Machlin and Gabriel (1982) reported that rats given a 1 g TA/kg diet for 15 weeks showed a slow but continual accumulation of T in the brain at 0.5 μg/g tissue per day as compared with 3 μg/g tissue per day for the heart. This conclusion is also supported by clinical data from the studies of Vatassery et al. (1998). These investigators measured the T level in spinal fluid from 18 patients randomly selected from the DATATOP study, who consumed 2000 IU dl-α-tocopherol/day for 37–644 days. Their findings clearly showed that the net increase in spinal fluid T levels were significantly and positively correlated with the number of days of vitamin E ingestion.

![Fig. 1. Effect of the in vivo administration of T, TA or TS on T and T ester levels (A and B) and on the susceptibility to lipid peroxidation (C) in liver homogenate and pure mitochondria. Sprague/Dawley (S/D) male rats were administered a single equimolar i.p. dose of vitamin E (ca. 100 mg T/kg body wt.) and 18 h later, fractions were isolated as described by Fariss et al. (2001). Pure mitochondria were further purified by the modified method of Hovius et al. (1990). Vitamin E levels were measured by the HPLC method of Fariss et al. (1989) and the susceptibility to lipid peroxidation measurements were conducted according to Fariss et al. (2001). Data points are the mean ± S.E. of three to five separate animals per treatment. ND is not detected and *P < 0.05 as compared with control.](image-url)
and suggested that even after 644 days of treatment, the spinal fluid (and presumably the brain) continues to slowly accumulate T. Thus, this inability to rapidly accumulate T seems to place the CNS at considerable risk especially when you consider that this organ system has the highest level of peroxidizable polyunsaturated fatty acids as well as high mito activity and ROS generation (Ebadi et al., 1996). As a result, these cells are often dependent on the presence of additional cellular antioxidants such as GSH, ascorbate or ubiquinol to rapidly regenerate oxidized T (free radical form) to its reduced, active state, preventing toxic oxidative damage (Machlin and Gabriel, 1982; Liebler et al., 1986; Leedle and Aust, 1986; Lan and Jiang, 1997).

4.1. Experimental evidence for vitamin E-mediated protection in PD

In view of the limited ability of vitamin E administration to rapidly supplement cellular membranes with active and protective T (Machlin and Gabriel, 1982; Fariss et al., 2001), it is not surprising that the attempts to treat PD or experimental models of PD with vitamin E have resulted in inconsistent findings (especially when a variety of dosing concentrations and pre-treatment times for vitamin E and neurotoxicants are used). Using both in vitro and in vivo experimental model systems for PD, studies have demonstrated both vitamin E-mediated protection and lack of protection.

Review of published experimental PD studies indicates that there is a predominance of in vivo studies investigating the protective effect of vitamin E treatment. Clearly, using the MPTP-induced experimental PD model in C57/B1 mice, vitamin E deficient mice (75% reduction in substantia nigra T content as compared with control) were much more susceptible to MPTP toxicity than control mice, in terms of lethality and dopamine metabolite depletion in the substantia nigra (Odunze et al., 1990). However seemingly in conflict with these findings, the majority of investigators using this experimental PD model have found that vitamin E treatment is not protective. For example, Martinovits et al. (1986) reported that α-tocopherol (100 mg/kg body wt., i.p.) given daily, 2 days before, 1 day with and 4 days after MPTP administration, did not prevent the marked striatal dopamine depletions produced by MPTP (examined 10 days post-treatment). Likewise, subsequent studies have reported that MPTP-induced depletion of striatal dopamine could not be attenuated by pre-treatment of mice with a daily oral gavage of α-tocopherol (48 mg/kg body wt.) for 4, 8 or 12 weeks (Gong et al., 1991; Chi et al., 1992). In contrast to these negative findings, Perry et al. (1985), using the same PD model, reported that mice pretreated with a daily s.c. injection of very high levels of α-tocopherol (2350 mg/kg body wt.) for 48 h before and 72 h after MPTP administration resulted in partial protection against the loss of striatal dopamine content and dopaminergic neurons in the substantia nigra (observed 1 month following MPTP treatment). The reason for the conflicting findings on vitamin E protection in vivo is unknown but may be related to the high acute dose of T administered (in Perry’s study) and the ability of MPTP treatment to compromise the blood–brain barrier, enabling a pronounced delivery of T from the plasma to the brain during the toxic insult (Adams and Wang, 1994). This conclusion is also supported by the recent findings of Barc et al. (2002), who showed that again very high levels of TA (1000 mg/kg body wt., i.m.) administered 3 days before and 7 days after toxic MPTP treatment, partially prevented the observed inhibition of striatal dopamine uptake. The only other report of in vivo protection with vitamin E comes from the recent study of Roghani and Behzadi (2001) demonstrating that a low dose of d-α-tocopheryl succinate (TS) (20 mg TS/kg body wt., i.m.) administered three times a week for 1 month (following intrastratal 6-hydroxydopamine injection), protected rats against experimental PD (prevented substantia nigra pars compacta cell loss). The mechanism for this protection is unknown but these findings support our contention (outlined in the sections below) that TS has unique properties (as compared with other vitamin E derivatives) that result in the rapid enrichment of mito with cytoprotective levels of T.
A review of studies using in vitro vitamin E treatments in experimental cell models of PD again find conflicting results which, like the in vivo studies, are probably due to differences in the ability of various vitamin E treatments to rapidly enrich a critical cellular site with protective levels of T (previous studies indicate this site is mitochondrial) in dopaminergic cells of the substantia nigra. For example, in studies using dopaminergic cell cultures (rat PC 12 cells or human SH-SY5Y cells), vitamin E treatments (vitamin E form not always reported) were only protective against dopamine-induced cell death and ROS production when vitamin E was placed in the culture medium 24 h prior to the toxic dopamine insult and at a concentration of 100 μM or greater (Wei et al., 1996; Storch et al., 2000). In studies concluding that vitamin E is not cytoprotective, cells were incubated with up to 25 mM vitamin E but this antioxidant was administered at the same time as the toxic dopamine insult (Offen et al., 1996; Lai and Yu, 1997).

4.2. Clinical evidence for vitamin E-mediated protection in PD

As with the experimental PD studies, the effectiveness of dietary vitamin E supplementation in the prevention and treatment of clinical PD have been disappointing, again with several conflicting reports. Epidemiological investigations on the prevention of clinical PD with vitamin E intake (estimated from consumed food items) are inconclusive. In 1988, Golbe et al. reported an inverse relationship between the intake of food items high in vitamin E (e.g. nuts, plums, salad oil) and clinical PD in 81 New Jersey patients diagnosed with idiopathic PD and their same-sex sibling of similar age (control). These findings were later confirmed by de Rijk et al. (1997), and the inverse association of PD with the daily intake of vitamin E in foods appeared to be dose dependent (higher vitamin E intake associated with a lower incidence of PD) in these subjects from Rotterdam, the Netherlands. However, other investigators found no epidemiological evidence of an association between dietary vitamin E consumption in foods and clinical PD. These investigators questioned numerous subjects with a wide variety of ethnic backgrounds about past dietary habits such as over 100 PD patients and 280 age-matched controls without PD or neurological disease from New York City (Logroscino et al., 1996); 84 incident PD case subjects and 336 age-matched control subjects from Honolulu, Hawaii (Morens et al., 1996) and 342 PD patients and age-matched controls from the same German neighborhood or region (Hellenbrand et al., 1996).

Two clinical studies have also investigated the use of dietary vitamin E supplementation (given in addition to food) as a treatment for clinical PD (Fahn, 1992; The Parkinson Study Group, 1993). The largest study, DATATOP trial, was a double-blinded, placebo-controlled study involving the oral administration of 2000 IU dl-α-tocopherol/day to 400 PD patients recruited by 28 different academic medical centers in the US and Canada (The Parkinson Study Group, 1989). The subjects had diagnosed, untreated PD and the endpoint of the study was the time required for each patient to reach a stage when anti-PD medications had to be started (The Parkinson Study Group, 1993; Vatajelk et al., 1999). The results of this 2-year study showed that dietary vitamin E supplementation did not delay the onset of disability (and time to anti-PD therapy) associated with PD as compared with placebo controls. In contrast, findings from another study conducted in New York City, support the use of vitamin E in the treatment of clinical PD. Fahn (1992) investigated in 21 diagnosed PD patients receiving concomitant amantadine and anticholinergics, the effect of up to 12 years of daily consumption of 3200 IU α-tocopherol (stereoisomer form not reported) and 3000 mg of ascorbic acid. It is interesting to note that few adverse effects were reported in PD patients consuming 3200 IU α-tocopherol daily for up to 12 years. The results from this study showed that the vitamin E/C treatment was effective in postponing the need for the use of levodopa by an average of 2.5 years as compared with similar PD patients not consuming these vitamins. The author concluded from this study that the progression of PD might be slowed by the administration of high doses of T and ascorbate. Though there is no clear explanation for these conflicting reports, several
possibilities include: (1) the consumption of vitamin C enhanced the effect of vitamin E in the Fahn trial, (2) vitamin E protection against PD is dose dependent and the Fahn trial used a 60% higher dose (2000 vs. 3200 IU). Furthermore, in the DATATOP trial (and most likely in the Fahn trial, but not reported), the \( \alpha \)-tocopherol consumed was the dl form, which has recently been shown to be approximately 50% as active as the d form (Blatt et al., 2001; Leonard et al., 2002). Thus the amount of effective T consumed in the DATATOP study was approximately 1000 IU/day, and in the Fahn study, 1600 IU/day and these doses may have influenced the efficacy observed especially in light of the brain’s preference for T (Blatt et al., 2001); (3) the time period from PD diagnosis to administration of high doses of vitamin E in subjects appears to have been shorter in the Fahn study (Fahn, 1992) than the DATATOP trial (The Parkinson Study Group, 1989) and (4) the Fahn trial only reported data on 21 treated PD patients, was not a blinded or controlled study and the PD patients were permitted to take amantadine and anticholinergics.

We suggest that the DATATOP study and similar clinical trials for vitamin E as a treatment for PD are an inappropriate test of the therapeutic potential of this antioxidant. First, the patients participating in these trials often have late stage disease, in which the majority of the substantia nigra dopamine neurons have been eliminated or are irreversibly injured (Golbe et al., 1988; Vatassery et al., 1999). Certainly, demonstrating a significant effect on halting the progression of a chronic, late-stage disease will be extremely difficult, even with an effective antioxidant treatment. Second, an effective treatment (assuming the substantia nigra dopamine neurons are viable) will require a fast acting CNS antioxidant that rapidly halts oxidative stress-induced progression of this chronic disease. Based on our current knowledge of vitamin E kinetics, it seems unlikely that dietary supplementation with vitamin E will provide such a rapid delivery system for protective T to the CNS.

Thus we suggest that such clinical trials using short-term (several years) dietary vitamin E supplementation are not a proper test of the protective potential of this antioxidant. Instead, we propose that the critical target that requires protection in PD is the mitochondria located in the striatum or more specifically substantia nigra. We contend that protection against PD requires a vitamin E treatment that is capable of enriching striatal mito with protective levels of T and unfortunately it seems that short term vitamin E dietary supplementation often does not meet these criteria (at the doses previously given and due to its slow CNS accumulation). Accordingly we offer several new approaches for the use of vitamin E therapy in the prevention or treatment of PD. To prevent PD, we suggest that chronic administration of dietary vitamin E is required to slowly and continuously increase the T content of the brain and substantia nigra to a level that will result in mito enrichment and protection against PD, before substantial cell death occurs. Since PD is a chronic disease and patients typically do not exhibit signs of PD and thus extensive cell death until the sixth decade of life, long term, high dose vitamin E dietary supplementation (beginning by the third decade of life) is a potential clinical therapy for protection against this devastating disease. To effectively treat PD, we propose that an accelerated means (as compared with our current use of high doses of dietary vitamin E) must be found to enrich striatum mito with protective levels of T and obviously, this intervention is required as early as possible during this chronic disease process.

4.3. Enrichment of mitochondria with T is critical for cytoprotection against toxic oxidative stress

As previously mentioned, clinical and experimental evidence clearly support a critical role for mito-derived oxidative damage in PD. In fact during a cellular oxidative insult, it is known that important mito constituents, whose activities are required for optimal mito function, are preferentially oxidized and damaged as compared with other subcellular regions. For example, Vatassery (1993) reported that T in rat brain mito is more susceptible to oxidation than T in other subcellular fractions, during a severe oxidative challenge. Van Houten and co-workers (Mandavilli et al., 2000) reported that MPTP administration to mice led to
the substantia nigra specific-induction of mtDNA damage that was greater than nuclear DNA damage. In addition, researchers have shown that hyperoxia resulted in the inhibition of cell growth and selective inactivation of the mitochondrial-specific enzyme KGDH, which was not observed in oxygen-resistant cells (Gibson et al., 2000). Finally, cardiolipin, a phospholipid only found in abundance in the IMM, appears to be preferentially oxidized during an oxidative challenge and cardiolipin oxidation is clearly linked to mitochondrial electron transport chain dysfunction and detachment of cytochrome c from the IMM followed by the activation of caspases and induction of apoptosis (Fry and Green, 1981; Nomura et al., 2000; Lesnieski et al., 2001; Umansky et al., 2001).

Recently we demonstrated that the enrichment of hepatic mito with T is critical for cytoprotection against mito derived oxidative stress-mediated hepatocyte death (see Figs. 1 and 2; Fariss et al., 2001; Zhang et al., 2001a,b). This hypothesis originated from previous studies showing that TS treatment (25 μM) protected rat hepatocytes from a variety of toxic insults and thus may intervene in a common final pathway to cell death (Fariss et al., 1985). We and other investigators observed that TS is far more effective than T or TA in protecting isolated hepatocytes against many different acute toxic oxidative challenges including mito toxicants (Pascoe and Reed, 1987; Fariss et al., 1989; Carini et al., 1990; Fariss, 1990, 1997; Tirmenstein et al., 2000; Fariss et al., 2001; Zhang et al., 2001a,b). It is clear that TS cytoprotection is not selective for hepatocytes since similar protection was observed with many other cell types (Scott et al., 1987; Gogu et al., 1991; Trizna et al., 1992; Wey et al., 1993; Fariss et al., 1994), including dopaminergic PC12 cells exposed to toxic levels of glutamate and amyloid-beta-peptides (Pereira et al., 1999; Pereira and Oliveira, 2000). However, the investigators of these studies were unaware of the unique cytoprotective properties of TS. According to our studies in hepatocytes, TS cytoprotection is characterized by a dependence on an anionic charged TS molecule (Fariss, 1997), on the cellular uptake of TS, followed by hydrolysis and the release of the antioxidant T (Fariss, 1997; Ray and Fariss, 1994), on the

![Fig. 2. Time course of rotenone (ROT, 100 μM)/diethyl maleate (DEM, 1 mM)-induced cell death and Fe²⁺ (dose response)-induced cell death after 1 h incubation in hepatocytes isolated from control rats or rats treated with T or TS for 18 h (as described in Fig. 1). Hepatocyte suspension preparation and LDH leakage (cell death) measurements were conducted according to Fariss et al. (2001). Data are the mean ± S.E. and *P < 0.05, as compared with T treated.](image-url)
inhibition of cellular and mitochondrial lipid peroxidation (Fariss et al., 1989; Fariss, 1990; Selley, 1998; Zhang et al., 2001a,b) and on the maintenance of mitochondrial ultrastructure (Ray and Fariss, 1994). Thus, we hypothesize that TS protection is related to its unique ability to rapidly accumulate in hepatocytes and mito, followed by the release of T in sufficient quantities to prevent mitochondrial oxidative damage and cell death. In support of this hypothesis we have recently demonstrated that dietary vitamin E supplementation can also enrich rat hepatic mito and IMM with protective levels of T, thereby providing mito and cells protection against toxic oxidative stress (see Fig. 3). In the sections below, we describe experimental evidence and rationale for our proposal that parenteral vitamin E (e.g. TS) administration or dietary vitamin E supplementation may serve as a successful therapeutic strategy for the treatment or prevention of PD (by enriching substantia nigra mito with protective T).

4.4. Use of parenteral vitamin E succinate administration to enrich striatal mito with T and treat PD

Since mitochondrial oxidative stress appears to be a critical toxic event in the initiation and progression of PD, we propose that a successful antioxidant treatment will require the rapid protection of substantia nigra mito from oxidative damage. Unfortunately, such a rapid enrichment seems difficult to accomplish as we have shown that the acute exposure of hepatocytes and the liver to T or TA does not result in the mito accumulation of protective levels of T (evidence presented below, see Figs. 1 and 2). Instead, a more effective and rapid mito T delivery system must be developed. Accordingly, we suggest that TS is a prototype vitamin E derivative for such a delivery system. To briefly summarize our findings with TS, we have shown that hepatocytes incubated with TS (25 µM) for 60 min (as well as mito and microsomes isolated from these cells) are completely protected from Fe²⁺/C₂₇-induced lipid peroxidation (as determined with our assay of susceptibility to lipid peroxidation, Tirmenstein et al., 1998), while no protection was observed following 3 h T or TA treatments (Fariss et al., 2001). These results are in agreement with the cytoprotection data mentioned for TS in the previous section and clearly demonstrate the unique ability of in vitro TS treatment to

Fig. 3. Effect of dietary TA supplementation (35, 185, 1000 mg TA/kg diet) on T levels and susceptibility to lipid peroxidation in liver homogenates (Homog) and inner mitochondrial membranes (IMM) or on Fe²⁺-induced cell death (2 h incubation). Male S/D rats were given the diets for 7 days and then liver homogenate and IMM or hepatocytes were isolated and examined by methods described in Fig. 1. Vitamin E supplemented diets were prepared by Dyets Inc. (Bethlehem, PA). Data are the mean ± S.E. of three separate animals per treatment. *P < 0.05, as compared with 35 mg/kg group.
rapidly enrich cellular membranes, including mito, with protective levels of T. The importance of mito lipid peroxidation and T enrichment of mito in acute necrotic cell death and TS cytoprotection, respectively, are illustrated in our recent published studies using the mito complex I inhibitor, rotenone (ROT), in combination with a non-toxic concentration of the glutathione depletor, diethyl maleate (DEM), as a toxic oxidative challenge (Zhang et al., 2001a). In these studies, TS pretreatment resulted in complete protection against cell death induced by ROT/DEM (data not shown). These studies also clearly demonstrated that TS cytoprotection is dependent on the inhibition of mito lipid peroxidation (which precedes cell death and cellular lipid peroxidation), even when cellular and mito stores of GSH and ATP were depleted (Zhang et al., 2001a). Thus the critical toxic event for mito complex I inhibitors (Tirmenstein et al., 2000; Zhang et al., 2001a,b) such as ROT appears to be the induction of oxidative stress leading to mito lipid peroxidation.

Importantly, our recent in vivo studies also confirm that the enrichment of hepatic mito with T is a critical event for cytoprotection against toxic oxidative stress. Figs. 1 and 2 clearly show that acute TS administration results in a rapid enrichment of hepatic mito with TS and T, not observed with T or TA administration (Fig. 1), which protected against Fe$^{2+}$-induced mito swelling (data not shown) and ROT/DEM- and Fe$^{2+}$-induced necrotic hepatocyte death (Fig. 2), respectively. As shown in Fig. 1A, a single i.p. injection of T or TS resulted in a significant increase (3–5-fold) in liver homogenate T levels (18 h after administration), providing these membranes with complete protection against Fe$^{2+}$-induced lipid peroxidation (Fig. 1C). However, only TS administration resulted in a significant enrichment of hepatic mito membranes (including IMM, data not shown) with T and T ester (TS). Again this 3-fold enrichment of mito T following TS administration completely prevented Fe$^{2+}$-induced lipid peroxidation (Fig. 1C) and mito swelling (data not shown). Isolated hepatic mitochondria were not protected following isolation from T- or TA-treated rats (Fig. 1C). These in vivo results confirm that TS acts as a unique delivery system for T, rapidly accumulating in cellular and mito membranes and gradually releasing active T to prevent mito membrane oxidative damage, mito permeability transition (swelling), and oxidative cell death.

Based on these studies, we propose that vitamin E succinate administration (leading to enrichment of striatal mito T) may also prove beneficial in the treatment PD, where dopaminergic cell death is characterized by oxidative stress, lipid peroxidation, cellular GSH and ATP depletion, and elevated cellular Fe (toxic events that are remarkably similar to our hepatocyte studies). Unfortunately, the bioavailability of TS and T in striatal mito following TS administration is unknown. The CNS accumulation of TS and T are suggested, however, in the recent study of Roghani and Behzadi (2001) demonstrating that parenteral TS administration protects rats against experimental PD. We have recently found that s.c. injection of TS (100 mg/kg body wt.) given to rats daily for 7 days does indeed result in a significant accumulation of TS and T in the brain (increase of ca. 5–7 nmol/g brain as compared with controls, see Table 1). However, this level was only 15% of the TS measured in the other tissues (serum, liver, kidney, heart) obtained from the same animals. Clearly, the blood–brain barrier is impeding TS and T accumulation in the CNS following acute parenteral TS administration but this accumulation still appears to be four times faster than that observed for T enrichment of the CNS following equimolar

Table 1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Treatment</th>
<th>T</th>
<th>TS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>Vehicle</td>
<td>12.4</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>TS</td>
<td>32.5±2.0</td>
<td>34.9±4.0</td>
</tr>
<tr>
<td>Brain</td>
<td>Vehicle</td>
<td>20.4</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>TS</td>
<td>27.6±3.9</td>
<td>4.6±0.6</td>
</tr>
</tbody>
</table>

Note: Male S/D rats were given a s.c. injection of 100 mg/kg TS or saline (vehicle) daily for 7 days. Twenty-four hours after the final injection, serum and brain T and TS levels were measured according to the method of Fariss et al. (1989). Data points are the mean±S.E. of three separate animals (TS) or from a single animal (vehicle). ND is not detected.
1 g vitamin E/kg diet supplementation (see Table 2; Machlin and Gabriel, 1982). Therefore, as we have clearly shown that TS has the unique ability to rapidly accumulate in hepatic mito releasing protective T, we speculate that the CNS accumulation of TS will likewise provide enhanced mito protection to neuronal cells as compared with that observed for an equimolar concentration of T. Since TS has not been investigated as a potential treatment for PD, we still lack a basic understanding of the dosing conditions required to enrich substantia nigra mito with this vitamin E analog and whether such mito enrichment will protect against experimental PD. With future investigations devoted to these topics, we are optimistic that vitamin E derivatives using TS as the prototype will be developed and once administered will rapidly enrich striatal mito with adequate T to slow the progression of this devastating neurological disease.

4.5. Use of chronic dietary vitamin E supplementation to enrich striatal mitochondria with T and prevent PD

To prevent clinical late-onset PD, we propose that a therapeutic strategy to prevent mito oxidative stress and damage that leads to substantia nigra cell death is required. One potential strategy would be to inhibit the production of pro-oxidants during the initiation of this chronic disease. However, this option seems unrealistic as the environmental agents and endogenous factors (e.g. dopamine metabolism) responsible for pro-oxidant formation are unknown or not easily controlled. Instead, we propose that a chemoprevention strategy for PD should enhance the antioxidant capacity of mito membranes, especially IMM, with protective levels of T thereby neutralizing mito ROS-mediated damage and substantia nigra cell death. Unfortunately, we have a poor understanding of the factors that regulate T enrichment of mito in all tissues including the brain and the striatum. Our present knowledge of mito T enrichment is based on our rat liver studies with the parenteral administration of TS, T, and TA (as described above). To determine if dietary T supplementation can also lead to the enrichment of hepatic mito with protective levels of T, we fed rats a typical rodent diet (Purina 5001) that contained either 35 (control), 185 or 1000 mg TA/kg diet. As measured by food consumption (200 g rat consumes ca. 15 g diet/day; Yang and Desai, 1977), these supplements correspond to 0.5, 2.8, and 15 mg of TA/day per adult rat. These dietary vitamin E levels are clinically relevant and safe as they are equivalent to a 70 kg person taking daily, a 28 mg (amount in human diet + a multivitamin), 155 mg (amount in 200 IU T capsule), or 830 mg (amount in a 1200 IU T capsule) vitamin E supplement. These calculations are based on body surface area for a rat, 325 and 18 000 cm² for a human (Eaton and Klaassen, 2001). After 7 days on these diets, animals were sacrificed and mito isolated from 1 g TA diet-treated rats showed a dramatic increase in T levels in both the liver homogenate and IMM (as compared with 35 and 185 mg treated rats) and complete protection against lipid peroxidation (Fig. 3a and b). In contrast, liver homogenates and IMM isolated from 35 mg-treated rats were highly susceptible to lipid peroxidation. Interestingly, the 185 mg TA diet resulted in a 2-fold T enrichment of liver homogenate that resulted in complete resistance to lipid peroxidation but only marginal IMM T enrichment or protection (Fig. 3). Furthermore, only mito and hepatocytes isolated from rats fed a diet containing 1 g TA were completely protected (identical to that observed for parenteral TS treatment) against toxic oxidative insults (Fig. 3b and c). These results clearly demonstrate that dietary T supplementation can enrich hepatic mito with protective levels of T and this effect is indeed dose dependent. These data (including the parenteral TS data) suggest that a 3–5-fold increase in hepatic T levels are required for the enrichment of mito and IMM with protective levels of T. Thus we predict that dietary vitamin E treatments that enrich brain and striatum with T by 3–5-fold, will also lead to protective mito T enrichment in the same tissue. We recently examined the rate of T accumulation in the brains of rats fed diets containing 1 or 2 g TS/kg diet (similar results are observed with TS or TA in diet, as both ester forms are completely hydrolyzed in the gut) over a 7-week period (using both young, 1
month old, and aged, 19 months old, animals). The results from these studies are shown in Table 2 and clearly show that the accumulation of T in the brain is also dose dependent with the 2 g TS diet showing a 2-fold faster rate of accumulation at 0.44 nmol T/g brain per day. Based on this rate of accumulation and the observation that CNS accumulation of T is slow and constant (from our studies, and Machlin and Gabriel, 1982) and that T accumulation in the brain is uniform and will enrich striatum (Vatassery et al., 1988), we calculate that a 5-fold increase in brain T levels would be observed after approximately 6 months of feeding a 2 g TS/kg diet to rats. Consequently, we are optimistic that chronic vitamin E dietary supplementation (2 g TS/kg diet) for greater than 6 months (25% of a rodents life) will enrich brain and striatal mito with T and protect against experimental PD. This hypothesis awaits testing and confirmation. As the accumulation of T in the human brain, following consumption of high doses of vitamin E, appears to be slow and constant as observed for the rat (Vatassery et al., 1998), we speculate that patients consuming 1600–2000 IU d-\(\alpha\)-tocopheryl succinate beginning in the third decade of life may also benefit from enrichment of striatal mito with protective T and protection against mito oxidative stress-mediated neurodegenerative diseases such as PD. The adverse effects of such long term, high dose vitamin E consumption has not been adequately studied, however, previous clinical studies using similar doses for up to 12 years reported few adverse effects (Fahn, 1992).

5. Recommendation for vitamin E therapy in the prevention and treatment of Parkinson’s disease

Experimental and clinical evidence to date has identified mito oxidative stress and damage in the substantia nigra as critical events for the development of PD. Thus, we believe that successful therapeutic strategies for PD must target substantia nigra mitochondria. Clearly, we lack a basic understanding of the environmental events that are responsible for mito oxygen stress in this disease and thus reducing our exposure to such unknown agents is difficult. Instead, we recommend that developing therapeutic strategies to enrich these mito membranes with protective levels of antioxidants such as T may provide the means to prevent and treat PD. Therefore, to prevent PD, we propose the chronic administration of dietary vitamin E to slowly and continuously increase the T content of the brain and substantia nigra to a level that will result in mito enrichment and protection against PD. Since PD is a chronic disease and patients typically do not exhibit signs of PD until the sixth decade of life, we believe that long term, high dose vitamin E dietary supplementation (beginning by the third decade of life) may prove successful in preventing this devastating disease and such therapy requires investigation. In contrast, to effectively treat PD we propose that an accelerated means (as compared with our current use of high doses of dietary vitamin E) to enrich striatum mito with protective levels of T and an early intervention are required to halt the progression of this chronic disease. We

### Table 2

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Experiment 1 (1 g TS/kg diet per 7 weeks)</th>
<th>Experiment 2 (2 g TS/kg diet per 7 weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control diet</td>
<td>+TS diet</td>
</tr>
<tr>
<td>Serum</td>
<td>22.1±2.1</td>
<td>45.1±1.7*</td>
</tr>
<tr>
<td>Brain</td>
<td>19.2±2.9</td>
<td>32.4±5.0*</td>
</tr>
</tbody>
</table>

Note: 1-month (Exp. 1) and 19-month-old (Exp. 2) female S/D rats were fed a 1 g TS/kg diet or 2 g TS/kg diet, respectively, or 35 mg TA/kg diet (control), for 7 weeks, followed by the measurement of tissue T levels as described in Table 1. Data points are the mean ± S.E. of six separate animals per treatment. Accumulation rate was calculated by subtracting control diet T levels from TS diet T levels, divided by the number of days on the diet (49). *P < 0.05, as compared with control.
have identified a vitamin E derivative, TS, that possesses the unique ability to rapidly enrich mito with protective levels of T. Additional studies are required to demonstrate its effectiveness in neurodegenerative diseases such as PD.

Acknowledgements

This work was supported by NIH/NIEHS grant # ES05452 and the Gasper and Irene Lazzara Foundation.

References


