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Mitochondrial Dysfunction and Oxidative Damage in Alzheimer's and Parkinson's Diseases and Coenzyme Q_{10} as a Potential Treatment

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There is substantial evidence that mitochondrial dysfunction and oxidative damage may play a key role in the pathogenesis of neurodegenerative disease. Evidence supporting this in both Alzheimer's and Parkinson's diseases is continuing to accumulate. This review discusses the increasing evidence for a role of both mitochondrial dysfunction and oxidative damage in contributing to β-amyloid deposition in Alzheimer's disease. I also discuss the increasing evidence that Parkinson's disease is associated with abnormalities in the electron transport gene as well as oxidative damage. Lastly, I reviewed the potential efficacy of coenzyme Q as well as a number of other antioxidants in the treatment of both Parkinson's and Alzheimer's diseases.

KEY WORDS: Mitochondria; oxidative damage; free radicals; Alzheimer's; Parkinson's; coenzyme Q_{10}.

ALZHEIMER'S DISEASE TRANSGENIC MODELS: β-AMYLOID, ENERGY METABOLISM AND TRANSGENIC MODELS

There is a large body of evidence implicating impaired energy metabolism and oxidative damage in the pathogenesis of AD. α-Ketoglutarate dehydrogenase complex activity is severely decreased in postmortem AD brain (Gibson *et al*., 1998). This is unlikely simply to be secondary to cell loss, because the defect is also found in skin fibroblasts (Sheu *et al*., 1994). It however could be related vulnerability of the enzyme to oxidative damage. Genetic polymorphisms in one of the key components of α-ketoglutarate dehydrogenase dihydrolipoamide dehydrogenase is associated with AD (Kanamori *et al*., 2003). A truncated gene product was recently found, which is localized to the intermembrane space of mitochondria. If expression of the truncated gene product is reduced, there is a marked decrease in amounts of subunits of complexes I and IV of the mitochondrial electron transport chain and a decline of activity (Kanamori *et al*., 2003). There is a link between mitochondrial abnormalities and oxidative stress in AD postmortem tissue (Hirai *et al*., 2001), and oxidative damage occurs early in the pathogenesis of AD (Nunomura *et al*., 2001). Oxidative damage to lipids precedes β-amyloid deposition in a transgenic mouse model of AD (Pratico *et al*., 2001).

There is also a large body of evidence implicating β-amyloid in the pathogenesis of AD. All genes thus far identified as causing AD are involved with the processing of β-amyloid. Trisomy 21 inevitably results in AD pathology (Olson and Shaw, 1969), and the amyloid precursor protein (APP) gene is located on chromosome 21 (Kang *et al*., 1987). Mutations in the APP gene result in early onset autosomal dominant AD (Chartier-Harlin *et al*., 1991; Mullan *et al*., 1992). Mutations in presenilins, which also cause early onset autosomal dominant AD (Wisniewski *et al*., 1997), increase levels of the particularly fibrillogenic species $A\beta_{42}$ (Borchelt *et al*., 1996; Duff *et al*., 1996), through an effect on the γ-secretase (Strooper *et al*., 1998; Wolfe *et al*., 1999). Finally, the ε4 allele of apolipoprotein E (apoE) increases the risk of late onset AD (Strittmatter *et al*., 1993), and apoE4 binds directly to Aβ and promotes its fibrillogenesis (Castano *et al*., 1995).

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There are strong links between the mitochondrial and amyloid hypotheses. On one hand, mitochondrial dysfunction and oxidative stress may alter APP processing, leading to increased intracellular Aβ accumulation. Inhibition of cytochrome oxidase results in accumulation of potentially amyloidogenic C-terminal fragments (Gabuzda *et al*., 1994). Free radical stress increases cellular Aβ_{42} levels (Ohyagi *et al*., 2000). Uncoupling mitochondria with FCCP in normal astrocytes recapitulates the altered APP processing and intracellular accumulation of Aβ_{42} seen in astrocytes and neuronal cultures from fetal Down's syndrome brain (Busciglio *et al*., 2002). There is also evidence that oxidative stress increases the activity of β-secretase, the enzyme responsible for N-terminal cleavage of β-amyloid from the amyloid precursors protein (Drake *et al*., 2003; Tamagno *et al*., 2002). In Down's syndrome, there is evidence that oxidative damage precedes β-amyloid deposition (Nunomura *et al*., 2000).

On the other hand, β-amyloid may cause mitochondrial dysfunction and oxidative stress. β-Amyloid suppress mitochondrial succinate dehydrogenase and inhibits of PC12 cell redox activity (Kaneko *et al*., 1995; Sherman *et al*., 1994). Exposure of isolated rat brain mitochondria to β-amyloid caused a significant reduction in state 3 and state 4 respiration (Casley *et al*., 2002). β-Amyloid protein induces oxidative damage to mitochondrial DNA in PC12 cells (Bozner *et al*., 1997), and there is increased generation of reactive oxygen species in neurons cultured from fetal Down's syndrome (Busciglio and Yankner, 1995). Recently, a direct link between the mitochondrial and amyloid hypotheses was demonstrated, by showing that APP is physically targeted to mitochondria and impairs mitochondrial function in neuronal cells (Anandatheerthavarada *et al*., 2003).

There are now several transgenic animal models which show increased β-amyloid deposition. These include transgenic mice overexpressing APP with the V717F mutation, and the Swedish double mutation at positions 670/671 (Tg2576) (Hsiao *et al*., 1996; Masliah *et al*., 1996; Sturchler-Pierrat *et al*., 1997). These mice are analogous to recently described mice. These TgCRND8 mice have a double mutant form of the amyloid precursor protein 695 (KM 670/671 NL and V717F), under control of the PrP gene promoter (Chishti *et al*., 2001). The mice show thioflavin S-positive β-amyloid deposits at 3 months of age, and dense-cored plaques and neuritic pathology from 5 months of age. In the Tg2576 mice, β-amyloid deposits are associated with evidence of oxidative stress as assessed by immunostaining (Pappolla *et al*., 1998; Smith *et al*., 1998), and oxidative damage to lipids appears to precede β-amyloid deposition in AD transgenic mice (Pratico *et al*., 2001).

A recent paper showed that intracellular accumulated β-amyloid precedes both neurofibrillary tangles and synaptic dysfunction in a transgenic mouse expressing β-amyloid, presenilin, and tau mutations (Oddo *et al*., 2003). We examined the effects of crossing mice with a partial deficiency of manganese superoxide dismutase with Tg1995 mice (William *et al*., 1998). This markedly exacerbated β-amyloid deposition, providing direct evidence of a link between β-amyloid deposition and oxidative damage.

MITOCHONDRIAL DYSFUNCTION IN PD

The possible role of oxidative damage and mitochondrial dysfunction in PD has been strengthened by the finding that chronic infusions of the complex I inhibitor rotenone produce an animal model of PD in rats (Betarbet *et al*., 2000). The infusions produced a selective loss of substantia nigra dopaminergic neurons as well as cytoplasmic α-synuclein immunoreative inclusions closely resembling Lewy bodies. The mechanisms of neurotoxicity appears to involve oxidative damage (Scherer *et al*., 2002).

Evidence for mitochondrial dysfunction of idiopathic PD comes from a 30–40% decrease in complex I activity in the substantia nigra (Bindoff *et al*., 1989; Janetzky *et al*., 1994; Mann *et al*., 1992; Schapira *et al*., 1990). Reduced staining for complex I subunits in PD substantia nigra, but preserved staining for subunits of the other electron transport complexes, has been demonstrated immunohistochemically (Hattori *et al*., 1991). Strong support for a mitochondrial DNA encoded defect comes from studies which showed that complex I defects from PD platelets are transferable into mitochondrial deficient cell lines (Gu *et al*., 1998; Swerdlow *et al*., 1996). These defects are associated with increased free radical production, increased susceptibility to MPP^-, and impaired mitochondrial calcium buffering (Sheehan *et al*., 1997). Direct sequencing of mitochondrial complex I and tRNA genes failed to show homoplasmic mutations (Simon *et al*., 2000).

A number of other recent studies, however, provide genetic evidence that mitochondrial DNA abnormalities may contribute to PD pathogenesis. An out-of-frame cytochrome *b* gene deletion occurred in a patient with parkinsonism was associated with increased free radical production (Rana *et al*., 2000). A novel mitochondrial 12 SrRNA point mutation was found in a pedigree with parkinsonism, deafness, and neuropathy (Thyagarajan *et al*., 2000). We found parkinsonism occurred in association with the Leber's optic atrophy mitochondrial mutation G11778A (Simon *et al*., 1999). An increase in mitochondrial DNA deletions/rearrangements and novel complex I mutations were found in the substantia nigra of PD patients

(Gu *et al*., 2000; Richter *et al*., 2002). Lastly mitochondrial haplotypes in Caucasian patients (classified as haplotype J) markedly reduce the risk of developing PD (Van der walt *et al*., 2003). The mRNA for the NDI subunit of mitochondrial complex I is reduced by 25% in the substantia nigra melanized neurons in PD (Kingsbury *et al*., 2001).

OXIDATIVE DAMAGE IN PD

A great deal of interest has focused on the possibility that oxidative damage may play a role in the pathogenesis of PD. There are studies showing increased levels of malondialdehyde and cholesterol lipid hydroperoxides, markers for lipid peroxidation, in PD substantia nigra (Dexter *et al*., 1989, 1994). There are widespread increases in protein carbonyls in PD postmortem brain tissue (Alam *et al*., 1997). Concentrations of 8-hydroxy-2-deoxyguanosine, a marker of oxidative damage to DNA, are significantly increased in PD substantia nigra and stritum (Alam *et al*., 1997; Sanchez-Ramos *et al*., 1994; Zhang *et al*., 1999). There is evidence for nitrosyl radicals in PD substantia nigra (Shergill *et al*., 1996). Another means of looking for oxidative stress is to measure concentrations of reduced glutathione. Reduced glutathione is decreased in PD substantia nigra by approximately 50% (Perry *et al*., 1982; Perry and Yong, 1986; Riederer *et al*., 1989; Soficm *et al*., 1992). Individuals with incidental Lewy body disease may have presymptomatic PD, and they have a 35% reduction in reduce glutathione as compared with age-matched controls (Dexter *et al*., 1994).

Other studies showed an increase in oxidative damage to cytoplasmic DNA and RNA in substantia nigra in PD as detected using immunocytochemistry (Zhang *et al*., 1999). An increase in oxidative damage to DNA was also reported in leukocytes, serum, and CSF of PD patients (Kikuchi *et al*., 2002; Migliore *et al*., 2002). An increase in 3-nitrotyrosine immunoreactivity was reported in Lewy bodies in PD (Good *et al*., 1998). This finding was confirmed with antibodies specific for nitrated α-synuclein (Giasson *et al*., 2000). This finding provides a link between oxidative damage and protein aggregates, which are characteristic features of PD. Strengthening this is the observation that intracellular production of peroxynitrite induces α-synuclein aggregation (Paxinou *et al*., 2001). Other evidence shows that oxidative damage impairs ubiquitination and degradation of proteins by the proteasome (Jenner, 2003).

COENZYME Q_{10} AND NEUROPROTECTION

There is increasing interest in the potential usefulness of coenzyme Q_{10} (CoQ_{10}) to treat neurodengenerative diseases. CoQ_{10} serves as an important cofactor of the electron transport chain, where it accepts electrons from complexes I and II (Bayer, 1992; Dallner and Sindelar, 2000). CoQ_{10}, which is also known as ubiquinone, serves as an important antioxidant in both mitochondria and lipid membranes. It mediates some of its antioxidant effects through interactions with α-tocopherol (Bayer, 1992; Noack *et al*., 1994). Coenzyme Q_{10} blocks apoptosis by inhibiting activation of the mitochondrial permeability transition independently of its free radical scavenging activity (Papucci *et al*., 2003). Another potential neuroprotective mechanism of coenzyme Q_{10} is as a cofactor of mitochondrial uncoupling proteins (Echtay *et al*., 2000, 2002). Coenzyme Q_{10} is also an obligatory cofactor for mitochondrial uncoupling proteins (Echtay *et al*., 2000, 2002). Activation of these proteins reduces mitochondrial-free radical generation. Coenzyme Q induces mitochondrial uncoupling in the substantia nigra of primates, and this is associated with marked neuroprotection against MPTP toxicity (Horvath *et al*., 2003). Increased expression of mitochondrial uncoupling proteins protects against brain damage associated with both experimental stroke and epilepsy (Mattiasson *et al*., 2003; Sullivan *et al*., 2003).

CoQ_{10} diminished ischemia-induced neuronal injury in the hippocampus (Ostrowski, 2000). CoQ_{10} protects cultured cerebellar neurons against excitotoxin-induced degeneration (Favit *et al*., 1992). We studied the effects of administration of CoQ_{10} on lesions produced by mitochondrial toxins. Oral administrtion of CoQ_{10} produced dose-dependent neuroprotective effects against malonate-induced striatal lesions as well as depletions of ATP and increases in lactate concentrations (Beal *et al*., 1994). Administration of CoQ_{10} produced significant protection against dopamine depletions induced by MPTP administration (Beal and Matthews *et al*., 1997). Oral administration of CoQ_{10} for 1 week prior to coadministration of 3-nitropropionic acid resulted in a significant 90% neuroprotection against 3-nitropropionic acid induced striatal lesions (Matthews *et al*., 1998). We found that oral administration of CoQ_{10} starting at 50 days of age significantly increased life span of ALS transgenic mice (Matthews *et al*., 1998), and increased survival in HD transgenic mice by 14.5% (Ferrante *et al*., 2002). Administration of CoQ_{10} significantly delayed the development of motor deficits, weight loss, cerebral atrophy, and neuronal inclusions.

We administered CoQ_{10} at a dose of 360 mg per day to HD patients for 1–2 months (Korozhetz *et al*., 1997) CoQ_{10} therapy led to a significant 37% reduction in occipital cortex lactate concentrations, which reversed following discontinuation of therapy, indicating a therapeutic effect of CoQ_{10}. A tolerability study of CoQ_{10} in HD patients showed that there were minimal adverse effects at

doses of 600–1200 mg daily (Feigin *et al*., 1996). In the CARE-HD trial 360 patients were treated for 30 months (The Huntington Study Group, 2000). They were randomized to CoQ_{10} at 600 mg per day, remacemide at 600 mg per day or the combination in a 2×2 factorial design. The primary outcome variable was change in the Unified Huntington's Disease Rating Scale. In this trial, CoQ_{10} slowed decline on the total functional capacity measure scale by 14% over 30 months.

The CoQ analogue idebenone reduces cardiac hypertrophy in patients with Friedriech's ataxia (Hausse *et al*., 2002; Rustin *et al*., 1999). A study of the effects of CoQ_{10} in patients with Friedriech's ataxia showed improvement of cardiac and skeletal muscle bioenergetics (Lodi *et al*., 2001). Coenzyme Q_{10} was administered at 400 mg daily and after 3 months of treatment the cardiac phosphocreatine to ATP ratios showed a mean relative increase to 178% of initial values.

A phase II clinical trial in patients with Parkinson's disease enrolled 80 patients who were randomly assigned to placebo or CoQ_{10} at doses of 300, 600, or 1200 mg per day (Shults *et al*., 2002). The primary outcome measure was the Unified Parkinson's Disease Rating Scale, which was administered at screening, baseline, and 1, 4, 8, 12, and 16 months. The subjects were patients with early PD who did not require treatment (levodopa) for their disability. They were followed up for 16 months or until disability requiring treatment with levodopa had developed. The difference between the 1200-mg and placebo groups was significant with a $p = 0.04$, with an overall slowing of disability of 44% at 16 months.

ANTIOXIDANTS AND AD

A prior study showed that vitamin E has efficacy in slowing the progression of AD (Sano *et al*., 1997). Ginkgo biloba also may exert beneficial effects (Le Bars *et al*., 1997; Oken *et al*., 1998). The antioxidants curcurmin and melatonin exert beneficial effects on amyloid deposition in transgenic mouse models of AD (Lim *et al*., 2001; Matsubara *et al*., 2003). It is, therefore, possible that CoQ_{10} might similarly be beneficial in AD.

CONCLUSIONS

There is a large body of evidence implicating both mitochondrial dysfunction and oxidative damage in the pathogenesis of AD and PD. CoQ_{10} administration can increase brain and brain mitochondrial concentrations in brain in mature and older animals. There is substantial evidence that CoQ_{10} can act in concert with α-tocopherol as an antioxidant within mitochondria. CoQ_{10} administration is neuroprotective against ischemia and lesions produced by mitochondrial toxins including malonate, 3-nitropropionic acid, and MPTP. CoQ_{10} extends survival in a transgenic mouse models of ALS and HD. Initial clinical trials in Friedreich's ataxia, HD, and PD have shown beneficial effects. Several other antioxidants have the potential of ameliorating the progressive neurodegeneration which occurs in AD and PD. Lastly, it is possible that antioxidants may have additive or synergistic effects with agents targeting other modalities of cell death, such as apoptosis.

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