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Review

Oxidative stress induced-neurodegenerative diseases: the need for antioxidants that penetrate the blood brain barrier

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Abstract

Oxidative stress (OS) has been implicated in the pathophysiology of many neurological, particularly neurodegenerative diseases. OS can cause cellular damage and subsequent cell death because the reactive oxygen species (ROS) oxidize vital cellular components such as lipids, proteins, and DNA. Moreover, the brain is exposed throughout life to excitatory amino acids (such as glutamate), whose metabolism produces ROS, thereby promoting excitotoxicity. Antioxidant defense mechanisms include removal of O_2, scavenging of reactive oxygen/nitrogen species or their precursors, inhibition of ROS formation, binding of metal ions needed for the catalysis of ROS generation and up-regulation of endogenous antioxidant defenses. However, since our endogenous antioxidant defenses are not always completely effective, and since exposure to damaging environmental factors is increasing, it seems reasonable to propose that exogenous antioxidants could be very effective in diminishing the cumulative effects of oxidative damage. Antioxidants of widely varying chemical structures have been investigated as potential therapeutic agents. However, the therapeutic use of most of these compounds is limited since they do not cross the blood brain barrier (BBB). Although a few of them have shown limited efficiency in animal models or in small clinical studies, none of the currently available antioxidants have proven efficacious in a large-scale controlled study. Therefore, any novel antioxidant molecules designed as potential neuroprotective treatment in acute or chronic neurological disorders should have the mandatory prerequisite that they can cross the BBB after systemic administration.

Keywords: Neurodegenerative diseases; Alzheimer's disease; Parkinson's disease; Antioxidants; Free radicals; Blood brain barrier

Contents

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1. Biosynthesis and effects of free radicals

A free radical is any chemical species that contains one or more unpaired electrons. Unpaired electrons alter the chemical reactivity of an atom or molecule, usually making it more reactive than the corresponding non-radical, because they act as electron acceptors and essentially "steal" electrons from other molecules. This loss of electrons is called oxidation, and free radicals are referred to as oxidizing agents because they tend to cause other molecules to donate their electrons (Halliwell and Gutteridge, 1989). We are constantly exposed to free radicals created by electromagnetic radiation from the environment, both natural (e.g., radon, cosmic radiation) and man-made, and by internal cellular metabolism. The most common cellular free radicals are hydroxyl radical ($OH^{\bullet}$), superoxide radical ($O_2^{-\bullet}$), and nitric oxide ($NO^{\bullet}$) (Jenner and Olnaw, 1996; Simonian and Coyle, 1996).

Other molecules, such as hydrogen peroxide (H_2O_2) and peroxynitrate (ONOO), are not free radicals, but can lead to their generation through various chemical reactions. Free radicals and related molecules are often classified together as reactive oxygen species (ROS) to signify their ability to promote oxidative changes within the cell (Simonian and Coyle, 1996). Cells normally employ a number of defense mechanisms against damage induced by free radicals (Evans, 1993; Simonian and Coyle, 1996). Problems occur when production of ROS exceeds their elimination by the natural antioxidant defense system, or when the latter is damaged. This imbalance between cellular production of ROS and the ability of cells to efficiently defend against them, is called oxidative stress (OS) (Ebadi et al., 1996; Jenner and Olnaw, 1996; Simonian and Coyle, 1996). OS can cause cellular damage and subsequent cell death mainly by apoptosis in neurodegeneration because the ROS oxidize vital cellular components such as lipids, proteins, and DNA (Simonian and Coyle, 1996; Gorman, 1996).

Main generation of $\bullet O_2^-$ and H_2O_2

$$\text{Substrate} + O_2 - \xrightarrow{\textit{Mixed function oxidase}} \text{Product} + H_2O_2$$

$$\text{Xanthine} + O_2 - \xrightarrow{\textit{Xanthine oxidase}} \text{Uric acid} + \bullet O_2^- \text{ and } H_2O_2$$

Generation of •OH

$$H_2O_2 + Fe^{2+} \rightarrow OH^- + \bullet OH + Fe^{3+}$$

$$NO + \bullet O_2^- + H^+ \rightarrow ONOOH \rightarrow \bullet OH + NO_2$$

2. Antioxidants

Antioxidant defense mechanisms include: removal of O_2, scavenging reactive oxygen/nitrogen species or their precursors, inhibition of ROS formation, binding of metal ions needed for the catalysis of ROS generation and up-regulation of endogenous antioxidant defenses. The protective efficacy of antioxidants depends on the type of ROS that is generated, the place of generation (body barriers such as the blood brain barrier reduce the permeability of most antioxidants) and the severity of the damage (Halliwell, 1997; Halliwell et al., 1994). The antioxidant system can be classified into two major groups: enzymes and low molecular weight antioxidants (LMWA) as described in Table 1. The enzymes include a limited number of proteins: superoxide dismutase (SOD), catalase and peroxidase, as well as some supporting enzymes. The LMWA group of molecules can be further classified into indirect-acting antioxidants (e.g., chelating agents) and direct-acting antioxidants (e.g., scavengers and chain breaking antioxidants). The latter are extremely important in combating against OS. This subgroup contains several hundred compounds from a number of sources (both endogenous and exogenous). However, only a minority of these molecules, such as glutathione and NADPH, are synthesized by the cell itself. The majority, including ascorbic acid, lipoic acid, polyphenols, and carotenoids, are derived from dietary sources (Shohami et al., 1997).

3. Oxidative stress

3.1. Oxidative stress and excitatory amino acids

Although multiple factors can precipitate OS in cells, the neurotransmitter glutamate is the major effector of this process in the brain, primarily through activation of its ionotropic receptors. Glutamate and related excitatory amino acids account for most of the excitatory synaptic activity in the mammalian CNS and are released by an estimated 40% of all synapses. The ionotropic receptors can be distinguished by their pharmacological and electrophysiological properties: the N-methyl-D-aspartate (NMDA), the α-amino-3-hydroxy-5-methyl-4-isoxasole-proprionic acid (AMPA) and kainic acid (KA) receptors. Several studies provide evidence that the two phenomena are interrelated. The calcium-mediated effects of glutamate receptor activation, leading to neuronal degeneration, may involve a number of different pathways causing OS. Free radical-induced damage can occur by the stimulation of phospholipase A_2 (PLA_2) and the subsequent release of arachidonic acid and its derivatives (Dumuis et al., 1988). These substances and ROS enhance the release of glutamate, thereby promoting a vicious circle (Williams et al., 1989).

3.2. Oxidative stress and antioxidants in CNS

The excitatory amino acids and neurotransmitters whose metabolism produces ROS, are unique in the

Table 1
Antioxidants and ROS scavengers groups in neurodegenerative disorders

- ◆Endogenous enzymes, e.g., superoxide dismutase (SOD), catalase, glutathione peroxidase
- ◆Low molecular weight antioxidants (LMWA), e.g., Glutathione, tocopheroles (vitamine E), ascorbic acid (vitamin C), retinoic acid (vitamin A), melatonin, uric acid, lipoic acid
- ◆Endogenous antioxidant cofactors, e.g., coenzyme Q_{10}
- ◆Precursors and derivatives of endogenous antioxidants compounds and enzymes, e.g., acetylcysteine, carotenoids
- ◆Naturally occurring plant substances, e.g., flavonoids
- ◆Synthetic free radical compounds, e.g., Euk-8

brain as sources of OS. Other sources are generated by the high and constant use of oxygen in the mitochondria to supply the energy needs of these tissues. Free radicals are also produced by cytochrome P450 electron transport and the monoamine oxidase activity of the outer mitochondrial membrane.

3.3. Oxidative stress and neurodegenerative diseases

The brain is exposed throughout life to OS, and certain diseases of the brain and nervous system are thought to involve free radical processes and oxidative damage, either as a primary cause or as a consequence of disease progression.

3.3.1. Alzheimer's disease

Alzheimer's disease (AD) is a progressive neuropsychiatric disorder of unknown etiology. It is characterized by neuronal degeneration and cognitive deterioration, especially in the elderly (Flynn and Runho, 1999). OS has been implicated in the pathogenesis of AD (Markesbery, 1997) by the finding of several characteristics, such as enhanced lipid peroxidation, in specific areas of the brain in postmortem studies (Lovell et al., 1995). Several investigators detected an increase in the activity of catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase in the hippocampus and amygdala (Zemlan et al., 1989; Pappella et al., 1992). The suggestion that OS causes oxygen radical formation with resultant neurodegeneration and possibly plaque formation in the central nervous system, was supported by the study of Frautschy et al. (1991). Moreover Pappolla et al. (1998) provided evidence for the hypothesis that β-amyloid protein, the major constituent of the senile plaque, is neurotoxic and that such toxicity is mediated by free radicals in vitro and in a transgenic mouse model of AD.

3.3.2. Cognitive dysfunction in the elderly

Cognitive impairment is a common problem in the over-65-year age group, progressing to its most devastating form of clinical dementia, usually Alzheimer's dementia, in about 5% of this population (Hoffman et al., 1991). Goodwin et al. (1983) noted a correlation between memory function and vitamin C in the blood of healthy volunteers aged 60 or over. Accordingly, Perry et al. (1997) found a positive association of memory performance with β-carotene and vitamin C levels in plasma measured twice: 22 years and immediately before the tests. Another study with a larger sample group (n=335) reported that all the subjects with white matter lesions had lower plasma vitamin E levels (Breteler et al., 1994).

3.3.3. Parkinson's disease

Data from postmortem studies of brains from patients with Parkinson's disease (PD) suggest that OS plays an important role in neural degeneration of the pigmented dopaminergic neurons in the substantia nigra pars compacta (SNpc) (Fahn and Cohen, 1992). Apparently, there is a specific chemical fingerprint indicative of the damaging oxidative events: higher levels of cholesterol hydroperoxide, malondialdehyde, and protein adducts of 4-hydroxy-2-noneal (HNE) and of 8-hydroxy-2-deoxyguanosine, which point to the presence of ROS-induced DNA nicks (Jenner and Olnaw, 1996; Yoitaka et al., 1996). One of the suggested causes of OS in the SNpc is the production of ROS during the normal metabolism of dopamine. In the human SNpc, the oxidation products of dopamine may polymerize to form neuromelanin, which may also be toxic (Offen et al., 1999). Several studies have shown that dopamine is toxic to various cell cultures, causing programmed cell death (e.g., Ziv et al., 1994; Offen et al., 1995). N-methyl-4-phenyl-1, 2,3,6-tetrahydropyridine (MPTP) is a neurotoxin that produces biochemical and neuropharmacological changes in humans, lower primates and mice, which closely resemble those found in PD and also involve free radical formation (Akaneya et al., 1995). According to postmortem studies, the SNpc of PD patients shows a significant (>60%) reduction in GSH and a moderate (29%) increase in oxidized glutathione (GSSG) levels (Sian et al., 1994; Damier et al., 1993). This could be a critical primary event, leading to a weakening or deficiency of the natural antioxidative cellular defense mechanisms and thereby triggering degeneration of the nigral neurons, causing PD.

3.3.4. Huntington's disease

Huntington's disease is an autosomal neuronal disorder characterized as a movement disorder and caused by repetition of a CAG trinucleotide sequences encoding for a polyglutamine tract at the N terminus of the gene encoding a protein named huntingtin. There is a progressive, massive loss of neurons, particularly in the striatum (Bartzokis et al., 1999). Several postmortem studies showed increased iron levels in the striatum of patients with Huntington's disease, (Dexter et al., 1992; Chen et al., 1993). The striatum is characterized by an increase in iron concentrations, from very low levels at birth to high levels in older persons, therefore making the disease onset age-dependent (Bartzokis, 1997). Most hypotheses for the pathogenesis of Huntington's disease include a role for oxidative damage (Beal, 1996). Animals, as well as human postmortem studies, support the theory of metabolic dysfunction with concomitant OS (Gu et al., 1996; Loeffler et al., 1996; Browne et al., 1997). Excessive glutamate activation of excitatory receptors may also be involved (Olney and Gubareff, 1978) and may lead to ROS production. However, a

direct association between OS and Huntington's disease has not been reported.

3.3.5. Amyotrophic lateral sclerosis (ALS)

ALS is characterized by a selective and progressive degeneration of the lower motor neurons in the spinal cord and the upper motor neurons in the cerebral cortex, usually beginning in midlife. It can be sporadic or familial. Rosen and co-workers demonstrated 11 missense mutations in the gene encoding copper-zinc-superoxide dismutase (CuZn-SOD) in families with an autosomal dominant form of ALS (FALS). Postmortem studies of the frontal cortex and blood cells of FALS patients with missense mutations revealed a 40% reduction in SOD activity (Robberecht et al., 1994), but this was not found in sporadic ALS or FALS patients without SOD missense mutations. However, protein carbonyl content, a measure of protein oxidation, was found to be elevated by 85% in patients with sporadic ALS compared to controls, suggesting that OS may be involved in all types of ALS (Coyle and Puttfarcken, 1993). Levels of vitamin E and malondialdehyde (MDA) as a measure of lipid oxidation, increased over time in mutant CuZnSOD mice, as compared to controls (Hall et al., 1998). In patients with sporadic ALS there was a marked elevation over control levels in plasma 2-thiobarbituric reactive substances, which are products of lipid peroxidation. However, the plasma concentrations of antioxidants (α-tocopherol, β-carotene, ubiquinol-10 and glutathione) and the SOD activity in red blood cells were not significantly different between groups (Oteiza et al., 1997).

3.3.6. Schizophrenia and tardive dyskinesia

The presence of excess levels of ROS has been described for both schizophrenia and neuroleptic induced tardive dyskinesia (Lohr et al., 1990). Schizophrenia is a common psychiatric disorder affecting almost 1% of the population. The contribution of oxidative injury to the pathophysiology of schizophrenia is indicated by the increase in lipid peroxidation products in the plasma and CSF, and the altered levels of both enzymatic and non-enzymatic antioxidants in chronic naive first-episode patients (Reynolds, 1992; Mahadik and Scheffer, 1996). Furthermore, male schizophrenic patients were found to have lower levels of uric acid than control subjects, and the plasma levels of uric acid in the patient groups were significantly and inversely correlated with psychosis. Tardive dyskinesia is a movement disorder affecting 20–40% of patients treated chronically with neuroleptic drugs. Tsai et al. (1998) hypothesized that neuroleptics such as haloperidol, enhance striatal glutaminergic neurotransmission by blocking presynaptic dopamine receptors, thus promoting neuronal damage caused by OS.

3.3.7. Chemically-induced neurological disorders

Several neurotoxic chemicals have been shown to elevate the cerebral rate of ROS production in experimental animals. These include methyl mercuric chloride, cadmium, toluene, and other organic solvents (Lebel et al., 1990; Mattia et al., 1993). All of these agents are also capable of increasing intracellular levels of calcium ions (Bondy and Komulainen, 1988).

3.3.8. Brain aging

Aging in mammalian species appears to be the result of normal developmental and metabolic processes responsible for graying of the hair, decreases in the rate of wound healing and increases in susceptibility to disease and death. The most reliable risk factor for neurodegenerative diseases is normal aging. Studies have found evidence of oxidative damage to macromolecules (DNA, lipids, and proteins) especially in brains from elderly subjects, supporting the hypothesis that oxidative injury might directly cause the aging process. Additional links between OS and aging focus on mitochondria. Direct biochemical measurements of mitochondrial function demonstrate age-dependent increases in mitochondrial deletions, point mutations, and oxidative damage to the DNA. The mitochondrial DNA in the elderly population is particularly susceptible to OS probably due to its close proximity to the respiratory chain, limited repair mechanisms, few non-coding sequences and absence of histones (Cutler, 1991; Harman, 1992; Beal, 1995).

4. The blood brain barrier (BBB)

The brain needs a barrier that separates it from the blood, to permit the rigorous control of the brain microenvironment that is necessary for complex neural signaling. The blood brain barrier (BBB) is an endothelial barrier present in the capillaries that course through the brain (Reese and Karnovsky, 1967). According to ultrastructural studies, endothelial cells in brain differ fundamentally from those in most peripheral tissues, in two ways. First, they have very few endocytotic vesicles, thereby limiting the amount of transcellular flux. In addition, they are coupled by tight junctions or zipper-like structures that seal the cleft and restrict paracellular flux. Astrocyte processes that contact and influence endothelial cells do not form a true barrier in vertebrates, although they do so in invertebrates. In a few small brain regions such as the area postrema in the medula, subfornical organ and neurohypophysis, the classical BBB is physiologically absent.

4.1. Development of the BBB

The first known marker of brain endothelial cells in mice appears in the embryo, at day 10.5, even before

astrocytes are present (Qin and Sato, 1995). However, the time of actual barrier formation remains controversial (Saunders et al., 1991), partly because of the difficulty in assessing BBB function.

4.2. BBB permeability and drug delivery

Normally the tight junctions of the blood brain barrier permit the diffusion of only very small amounts of water-soluble compounds (paracellular aqueous pathway), while the large surface area of the lipid membranes of the endothelium offers an effective diffusive route for lipid-soluble agents (transcellular lipophilic pathway). Therefore, a potential route through which a therapeutic substance may cross the endothelium is by the lipid pathway. There is a good correlation between BBB penetration in vivo and the lipid solubility of a drug. Therefore, addition of hydrophobic groups to a molecule may help it to penetrate the brain. Prodrugs can also be made by linking the active compound to a lipophilic transport vector, such as pseudoglyceride or a nicotinoyl residue. However, although increasing the lipophilicity of a drug may increase its entry into the brain, it may also cause a reduction in biological activity by affecting drug interaction with receptor and/or plasma proteins. There are also sets of small and large hydrophilic molecules that can enter the brain by active transport (Rowland et al., 1992). For essential nutrients, such as glucose, purine bases, nucleotides, choline and certain large neutral amino acids (LNAA) (or related molecules, including L-DOPA), specific membrane-transporting proteins are present at relatively high concentrations in brain endothelial cells. These proteins can be used as another route for drug delivery. However, only molecules closely resembling the original substrate, will be transported through the BBB. Glucose derivatives, for example, can penetrate the BBB via glucose carrier while L-system amino acid carriers for LNAA are less efficient. There also seem to be systems that are capable of shuttling macromolecules into the brain, such as receptor-mediated (e.g., transferrin and insulin receptors) and adsorptive endocytosis (e.g., albumin and other plasma proteins).

One of the most important transporters is P-glycoprotein, which is highly expressed in the apical membrane of the endothelial cells and actively excludes certain undesired substances from the CNS. There are various methods which can be used to determine the rate of uptake of a drug into the brain parenchyma including: indicator dilution, brain uptake index, microdialysis, external registration, positron emission tomography (PET) scanning, in situ perfusion, and compartmental modeling (Bonate, 1995). In adult animals, barrier function is readily assessed by the introduction of dyes (e.g., macromolecular tracers: Evans blue which is serum albumin tracer, and micromolecular tracers: sodium fluorescein and [^{14}C] sucrose) or enzymatic tracers (e.g. horseradish peroxidase), into the circulation. In embryonic or neonatal animals, however, the addition of such tracers may actually disrupt the barrier by significantly increasing the volume/osmotic pressure of the blood (Rubin and Staddon, 1999).

4.3. Physical regulation of BBB permeability

The problem of drug entry into the brain has prompted researchers to develop methods to induce a transient opening of the tight junctions of the brain endothelial cells. There is a growing list of endogenous chemicals (including neurotransmitters and hormones but also inflammatory mediators) that can do so (Greenwood, 1992). In inflammatory states, and probably also in normal physiology, the brief opening of the BBB induced by these naturally occurring mediators may serve a useful function and be well tolerated by the brain.

4.3.1. Osmotic opening

Most of the clinical experience with physical regulation of the BBB opening, has come from "osmotic opening", in which an intracarotid artery injection of an inert hypertonic solution (generally mannitol or arabinose) is used to cause endothelial shrinkage and opening of tight junctions for a few hours (Gumerlock and Neuwelt, 1992). The method has been shown to increase the delivery of chemotherapeutic agents to patients with brain tumors.

4.3.2. Chemical opening

Chemicals offer the possibility of a more controlled and selective process, since it should be possible to devise a drug to open the barrier and one to close it within a precise time-window. Clinical trials have shown that bradykinin analog RMP7 and leukotriene LTC4 cause transient opening of the BBB (Black, 1990).

4.4. Pathological permeability of BBB

Obvious disruption of the BBB can be a relatively major part of the pathology following head trauma or other pathological states (e.g., cerebral ischemia). Moreover, it is now agreed that some lesions of the BBB, visible by gadolinium-enhanced magnetic resonance imaging (MRI), are associated with the progression of multiple sclerosis (Harris, 1991). Recent evidence indicates that capillary permeability is also influenced by stress (Friedman et al., 1996).

5. Antioxidants in the prevention and treatment of neurodegenerative disorders

The distribution of protective antioxidants in the brain has some interesting features. For instance, there is a relatively high concentration of the water-soluble antioxidant vitamin C in the brain. However, vitamin E concentrations are not remarkably different from those in other organs. The concentration of antioxidants varies within the different regions of the brain. For instance, the lowest concentration of vitamin E is found in the cerebellum (Vatassery, 1992). It has also been shown that enzymatic antioxidants, such as catalase, are found in lower concentrations in the brain, as compared to other tissues. These facts may also contribute to the potential OS in the brain Table 2.

5.1. Vitamin E (α-tocopherol)

Vitamin E is the most potent antioxidant that can break the propagation of the free radical chain reaction in the lipid part of the biological membrane. Among the antioxidants, it has shown some promise in the treatment of AD (Vatassery, 1992). Vitamin E, along with dietary fats, is absorbed from the intestine and secreted into the circulation in chylomicrons. It is transported in the circulation in plasma lipoprotein (McCormick et al., 1960). The liver controls vitamin E plasma concentrations through the incorporation of plasma very low-density lipoproteins (VLDL), by the α-tocopherol transfer protein (Traber and Sies, 1996). Genetic defects in this protein cause vitamin E deficiency in humans (Traber and Packer, 1995) which in turn lead to peripheral neuropathy due to the resultant impaired lipoprotein delivery of vitamin E to the nervous system. Alpha-tocopherol concentrations in the peripheral nerves are especially sensitive to variations in plasma vitamin E (Pillai et al., 1993). In rats, it was shown that in the long term, low levels of antioxidants, such as vitamin E, ascorbic acid and GSH in all tissues could lead to tissue peroxidisability. Vitamin E deficiency also influences the activities of SOD, catalase and glutathione peroxidase (De Kumar and Rukmini, 1988).

5.1.1. Vitamin E and AD

The hypothesis that OS is implicated in the pathogenesis of AD prompted a large, two year, double blind, placebo-controlled, randomized multi-center clinical trial with 2000 IU/day of vitamin E in 341 AD patients. The treatment was found to delay functional deterioration in moderately impaired AD patients (Sano et al., 1997). Studies with cocktails that included vitamin E are described below.

5.1.2. Vitamin E and PD

There has been much interest in the use of supplemental vitamin E as an antioxidant in preventing or slowing the progression of PD. Both animal models and clinical studies suggest that vitamin E deficiency contributes to nigral neurodegeneration and to the onset or progress of PD (Dexter et al., 1994).

5.1.2.1. Animal models 6-hydroxydopamine (6-OHDA) is a neurotoxin which is known to induce unilateral nigrostriatal lesions and is used as a model of unilateral Parkinsonism in animals, especially rats. Cadet et al. (1989) showed that pretreatment of 6-OHDA (in the striatum) lesioned rats with different types of vitamin E for a period of 1 month, caused a significant reduction in the apomorphine induced rotational behavior. Vitamin E also attenuated the toxic effects of the neurotoxin and its metabolites on striatal DA. Taken together, these data suggest that the decrease in turning behavior is probably related to the preventative action of the vitamin on 6-OHDA-induced DA depletion. In another study, pretreatment of rats with Vitamin E, caused significant

Table 2
Efficacy of antioxidants in the treatment of human neurodegenerative diseases

Disease	Antioxidant	Efficacy	References
Parkinson's disease	Vitamins E, C, A	−	Logroscino et al., 1996
	Vitamins E	+	De Rijk et al., 1997
	beta carotene + vitamin C+ flavonoids	−	
	Vitamin E	−	DATATOP 1989, 1993
	Vitamins E+C	+	Fahn, 1991
	GSH	+	Sechi et al., 1996
Alzheimer's disease	Vitamin E	+	Sano et al., 1997
	Vitamins E+C	+	Morris et al., 1998
Cognitive function	Vitamins E+C	+	Masaki et al., 1994
		−	Kalmijn et al., 1997
	Beta carotene	+	Jama et al., 1996
	Vitamin E	+	Schmidt et al., 1998
ALS	Cocktail	−	Vyth et al., 1996
Tardive dyskinesia	Vitamin E	±	Lohr and Caligiuri, 1996

Table 3
Efficacy of antioxidants in the treatment of neurodegenerative diseases in animal models

Experimental model	Antioxidant	Animal	Efficacy	References
Parkinson's disease	Vitamin E	Rat	+	Cadet et al., 1989; Perumal, 1992
	Vitamin E/C	Mice	−	Martinovits et al., 1986
	Vitamin E/Beta carotene		Partial	Perry et al., 1987; Yong et al., 1986
	Alpha-lipoate		+	Gotz et al., 1994
		Rat	Partial	Packer et al., 1997
			−	Seaton et al., 1996
	Coenzyme Q_{10}	Mice	+	Beal and Russell, 1997
	Euk-8		+	Susan, 1997
			Partial	Susan, 1997
	Melatonin	Rat	+	Jin et al., 1998; Chen, 1999
Alzheimer's disease	Alpha-lipoate	Mice	+	Stoll et al. 1993, 1994
Cognitive function	PBN	Rat	+	Sack et al., 1996
Methyl mercuric poisoning	GSH-glycosid	Mice	+	Choi et al., 1996
	Vitamin E/beta carotene		−	Anderson and Anderson, 1993
NMDA toxin	Alpha-lipoate	Rat	+	Greenmayere et al., 1994
Cadmium poisoning			+	Sumathi et al., 1994
Malonate toxicity	Coenzyme Q_{10}		+	Beal et al., 1994
3-np			+	Matthews et al., 1998

attenuation of the toxic effects of 6-OHDA on GSH and SOD levels in most brain regions. These results show that vitamin E can spare the antioxidant scavenging system from the injurious effects of 6-OHDA (Perumal, 1992) Table 3.

5.1.2.2. Clinical studies Several studies have attempted to slow the progression of PD by inhibiting nigral cell death. In one study, investigators found that oral intake of high doses of vitamin E (400–4000 IU/day for 5 months) failed to increase CSF vitamin E levels in patients (Pappert et al., 1996). However, these subjects were already showing clinical symptoms of the disease when the vitamin E was administered. Therefore, over 80% of the critically important neurons in the SNpc were already lost, prior to the initiation of treatment. Consumption of foods rich in vitamin E early in life may decrease the risk or delay the onset of PD (Tanner, 1992; Golbe et al. 1988, 1990Moarten, 1997). However, this issue remains unsolvedTable 4.

5.1.3. Vitamin E and Huntington's disease

Consistent with a possible role for iron as a risk factor in oxidative neurotoxicity, one clinical study has suggested that α-tocopherol treatment given early in the course of Huntington's disease, may slow the rate of motor dysfunction (Peyser et al., 1995).

5.1.4. Vitamin E and tardive dyskinesia

Of the 12 studies performed before 1996 on the use vitamin E for the treatment of tardive dyskinesia, nine showed some improvement. Interestingly, subjects who improved had milder symptoms of tardive dyskinesia at the onset. The remaining studies reported no effect of vitamin E (Lohr and Caligiuri, 1996). Further investigations are needed to shed more light on this issue.

In conclusion, vitamin E was shown to be effective clinically in only one report dealing with AD. However, other clinical trials and animal models provide conflicting data about the efficacy of vitamin E in the treatment of other neurological diseases. We could not find any

Table 4
Brain penetration of antioxidants in neurodegenerative disease/models of human/rodents

Antioxidant	Human/rat	Disease/model	Brain penetration	References
Vitamin E	Human	Parkinson's disease	−	Pappert et al., 1996
Alpha-lipoate	Rat	Ischemia	+	Panigrahi, 1996
Coenzyme Q_{10}		3 Nitro propionic acid	+	Matthews et al., 1998
			−	Beal and Russell, 1997; Zhang et al., 1995
Oxidized form of vitamin C			+	Agus et al., 1997
Vitamin D and A derivatives			Restricted	Pardridge et al., 1985
GSH			+	Kannan et al., 1990

study that checked the entry of vitamin E into the brain. Therefore, its efficacy in neurodegenerative diseases is still questionable.

5.2. Vitamin C (ascorbic acid)

Humans and other primates cannot synthesize this vitamin, whereas most mammals (e.g., rat and mouse) produce it endogenously in the liver (Chatterjee et al., 1975). Ascorbic acid is oxidized to dehydroascorbate, which can undergo irreversible hydrolysis to 2,3-diketo-L-gulonic acid, with decarboxylation to CO_2 and components of the pentose phosphate cycle or to oxalic acid plus threonic acid. Vitamin C has a variety of roles, one of which is the regeneration of vitamin E (Chan, 1993). Vitamin C is found at higher than plasma levels in a variety of tissues, including the brain (there is a greater than 10-fold gradient between the concentration of ascorbic acid in brain and serum) (Frei and England, 1989; Schriber and Trojan, 1991; Rose and Bote, 1993). It is believed to be a critical cofactor of dopamine β-hydroxylase, and to be involved in catecholamine biosynthesis. It also inhibits peroxidation of membrane phospholipids, and acts as a scavenger of free radicals (Path, 1990).

5.2.1. Brain penetration

Agus et al. (1997) found that vitamin C can cross the BBB in its oxidized form. It readily enters the brain and is retained in brain tissue in the form of ascorbic acid. This transport is probably implemented via the glucose transporter in the BBB, the GLUT1 receptor. The author concluded, therefore, that increasing blood concentrations of dehydroascorbic acid could increase vitamin C concentrations in the brain. There is no report showing vitamin C efficacy in clinical studies when it was given alone. However, it was shown to have some benefit when it was given in combination with other vitamins (see below).

5.3. Carotenoids

Like vitamin E, the carotenoids are natural lipid-soluble antioxidants (Machlin and Bendick, 1987). β-Carotene is the best known carotenoid because of its importance as a vitamin A (retinol) precursor. It is known to possess antioxidant activity somewhat analogous to that of vitamin E.

5.3.1. Vitamins A and analogs

Pardridge et al. (1985) measured the transport of retinol and retinoic acid, through the rat BBB after their injection into the common carotid artery. They showed that only 5% or less of vitamin A derivatives entered the brain in the presence of albumin and specific high-affinity binding proteins in plasma. Another study showed that peroxidation in rat brain mitochondria was inhibited by the fat-soluble vitamins especially retinol and retinol acetate, but also by retinoic acid, retinol palmitate, and retinal at concentrations of 0.1 to 100 mmol/l (Patmanatha, 1989). The brain is not generally recognized as an organ that requires vitamin A. However, Macdonald et al. (1990) found that brain tissue does contain cellular vitamin A-binding proteins and a nuclear receptor protein for retinoic acid in special structures of the BBB. This suggests that a significant movement of retinol across the BBB may occur. In conclusion, none of the studies checked the clinical value of vitamin A alone. However, one study showed low permeability in the brain.

5.4. Cocktails of vitamins

5.4.1. Alzheimer disease (AD)

To examine the possible correlation between the intake of vitamins E and C and the incidence of AD, a stratified random sample of 91 persons over the age of 65 years was selected from a disease-free population. After an average follow-up period of 4.3 years, none of the 27 vitamin E users had AD, compared with the 2.5 predicted on the basis of age, sex and years of education (p=0.03). None of the 23 vitamin C users had AD compared with 3.2 predicted (p=0.04). These data suggest that the intake of high-dose of vitamins E and C supplements may reduce the risk of AD (Morris et al., 1998).

5.4.2. Cognitive function in the elderly

Masaki et al. (1994) found that after adjustment for age, education and presence of stroke, cognitive function was not significantly related to supplement intake 20 years prior. However, cognitive function showed a significant correlation with the intake of both vitamin C and E supplements for a 4-year duration and with the intake of the vitamins in supplements concurrent to the measurement of function. A later study revealed no association of antioxidants with either cognitive impairment or decline (Kalmijn et al., 1997). Jama et al. (1996) reported a cross-sectional inverse relationship of β-carotene with cognitive impairment, but Schmidt et al. (1998) found that only low levels of α-tocopherol were significantly associated with cognitive functioning.

5.4.3. Parkinson's disease (PD)

5.4.3.1. Animal models for PD MPTP is a neurotoxin that damages nigrostriatal dopamine neurons in several species. To determine if antioxidant administration can prevent MPTP toxicity, Martinovits et al. (1986) divided C57 black mice into three trial groups. Group 1 was injected s.c. with MPTP (30 mg/kg) once, daily for two days, alone or with ascorbic acid (1 mg/kg) and vitamin

E (100 mg/kg) i.p.; Group 2 was injected once with MPTP (30 mg/kg), alone or with ascorbic acid (200 mg/kg) two days before, on the same day as MPTP and again 4 days after; Group 3 was injected once with MPTP (15 mg/kg) alone or with ascorbic acid (500 mg/kg) and vitamin E (100 mg/kg), given 90 min before and again 90 min after. Mice were decapitated 7, 10 or 30 days, respectively, after receiving MPTP. Results showed that MPTP caused marked striatal depletions (40–70% greater than controls), which were unchanged by co-treatment with the various antioxidants. In another study, investigators found that pretreatment of mice receiving one injection of 40 mg/kg MPTP with large doses of vitamin E (1000 mg/kg) or β-carotene (200 mg/kg), prevented loss of GSH and partially protected the dopaminergic nigrostriatal neurons from MPTP-induced damage (Perry et al., 1987; Yong et al., 1986). However, in a study on marmosets, the same team (Perry et al., 1987) failed to confirm these protective effects.

5.4.3.2. Clinical studies In one population-based, case-control study of the possible association of food or supplement dietary intake of vitamins with antioxidant activity: E, and C and A (carotenoids and retinol) and PD, no significant differences were observed between patients (n=110) and control subjects (n=287) (Logroscino et al., 1996). Another study examined whether a high dietary intake of vitamin E (10 mg/d), beta-carotene (1 mg/d), vitamin C (100 mg/d), and flavonoids (10 mg/d), could decrease the risk of PD, and found that individuals with higher vitamin E intake had PD significantly less often than those with lower vitamin E intake. Intake of beta-carotene was also inversely related to PD, but not significantly. Intake of vitamin C and flavonoids was not associated with PD. The author concluded that a high intake of dietary supplements may protect against the occurrence of PD (De Rijk et al., 1997, The Rotterdam Study). The Parkinson study group (deprenyl and tocopherol antioxidant therapy of parkinsonism (DATATOP 1989, 1993)) in a blind-labeled trial that started in 1989, sought to reduce ROS-induced damage in 800 patients with vitamin E (2000 IU/day) and/or deprenyl (10 mg/d). However, results showed no neuroprotection and clinical benefit. However, in another study by Fahn (1991), a high dose of vitamin E (3200 mg/d) combined with vitamin C (3000 mg/d) was administered to 15 patients with early PD before levodopa treatment, as a preliminary open-labeled trial. Levodopa became necessary 2.5 years later in the group taking antioxidants compared to controls. The differences between these two trials may be due to the elevation of the vitamin E dose and the addition of vitamin C in the latter.

5.4.4. Amytrophic lateral sclerosis

N-acetylcysteine (NAC), vitamins C and E, N-acetylmethionine (NAM), dithiothreitol (DTT) (200 mg/kg) or its isomer dithioerythritol (DTE) were administered (s.c. injection or orally) to 36 patients with ALS. Those with a history of heavy exposure to metal were also given meso-2,3-dimercaptosuccinic acid (DMSA, 250 mg/kg). The antioxidants did not cause harm to the patients, but neither did they prolong their survival (Vyth et al., 1996).

5.4.5. TPA-induced brain lipid peroxidation

Bagchi et al. (1998) showed that treatment with grape seed proanthocyanidin extract (GSPE) (25–100 mg/kg), succinates (VES) (100 mg/kg) and beta-carotene (50 mg/kg), protected mice against 12-O-tetradecanoylphorbol-13-acetate (TPA) induced lipid peroxidation in brain and hepatic tissue, and reduced DNA fragmentation by approximately 50%, 14%, 31% and 11%, respectively. The inhibition of GSPE was dose-dependent.

5.4.6. Methyl mercuric chloride toxicity

The administration of 40 mg/l of methyl mercuric chloride (CH_3HgCl) to mice via their drinking water stimulated lipid peroxidation in liver, kidney and brain; semisynthetic dietary supplements of vitamin E (10/100/1000 mg/kg) or β-carotene ($10^3/10^4/10^5$ IU/kg) had no protective effect. Indeed, excess beta-carotene further enhanced lipid peroxidation (Anderson and Anderson, 1993). In summary, the reported studies show contradictory data on the effectiveness of treatment of neurodegenerative diseases with vitamin combinations. Whether the vitamins can cross the BBB is still not known.

5.5. Melatonin

Melatonin (N-acetyl-5-methoxytryptamine) is an indoleamide secreted by the pineal gland (which is located in the dorsal surface of the hypothalamus) and has structural similarities to serotonin. It is so called because it has the ability in certain fish, reptiles and amphibians to temporarily turn the skin a dark color, by stimulating production of the pigment melanin. Today, melatonin is known as a stringent biological modulator of mood, sleep, retinal physiology, sexual behavior, seasonal-reproductive physiology and behavior, circadian rhythms, and immunological functions. Melatonin is highly lipophilic and, when administered exogenously, can readily cross the BBB to access neurons and glial cells. Moreover, there is experimental evidence that melatonin influences aging and age-related processes and disease states. These roles are apparently related to its potency as a free radical scavenger (Beyer et al., 1998). Several studies have assessed the neuroprotective effects of melatonin as an antioxidant. (Jin et al., 1998) performed a unilateral lesion of SNpc in rats with the neurotoxin 1-methyl-4-phenylpyridinium (MPP^+). He found increased lipid peroxidation (by 117% compared to

controls), and decreased tyrosine hydroxylase activity (60% of controls) in the SNpc 4 hours later. Treatment with melatonin, however, led to almost complete recovery of both the lipid peroxidation reduction (99% of control) and tyrosine hydroxylase (TH) levels (96% of control). One week after MPP^+ infusion, the untreated rats had a further reduction of tyrosine hydroxylase activity (52% of control). In contrast rats given continuous melatonin treatment (twice a day for 5 days), showed a partial, but not statistically significant, recovery of tyrosine hydroxylase activity (71% of control). Another study showed the potent protective effect of melatonin against oxidative damage induced by kainic acid (KA), a glutamate receptor agonist in rat brain. Kainic acid was directly injected into the unilateral striatum of adult SD rats. The group was administered melatonin (10 mg/kg) i.p. 1 h before and 1, 3 and 5 after. Three days later, the control group showed significant apoptotic cortical neuronal death, whereas apoptosis was significantly attenuated in the melatonin treated group. Biochemical studies have indicated that kainic acid can induce OS, as manifested by a decrease in total GSH and GSSG and an increase in the GSSG/GSH ratio in the striatum and cortex compared with the contralateral brain regions. In the kainic acid injected striatum, melatonin did not reduce OS, but in adjacent areas, OS was significantly reduced by melatonin (Chen, 1999). In conclusion, although melatonin can pass the BBB, as shown in animal studies, its use in the treatment of neurodegenerative diseases is limited since the possible effects on other systems have not been fully characterized.

5.6. Alpha-lipoic acid in neurological disorders

The metabolic antioxidant α-lipoate (thioctic acid, 1,2-dithiolane-3-pentanoic acid; 1,2-dithiolane-3 valeric acid; and 6,8-dithiooctanoic acid) is absorbed from the diet and crosses the BBB. It is taken up and reduced in cells and tissues to dihydrolipoate, which is exported to the extracellular medium. Hence, protection is afforded to both intracellular and extracellular environments. Both α-lipoate and especially dihydrolipoate are potent antioxidants. For example α-lipoate was shown to scavenge hydroxyl radicals, singlet oxygen, and nitric oxide. In addition, α-lipoate chelates a number of transition metals, regenerates through redox cycling of other antioxidants (such as vitamin C and vitamin E), and raises intracellular levels of glutathione (Packer et al., 1997).

5.6.1. Aging and memory loss

Alpha lipoate has shown potential for the treatment of the age-related behavioral decline that is associated with AD (Stoll et al. 1993, 1994). Aged mice (20–23 months) that received oral alpha-lipoate (100 mg/kg for 15 d) exhibited improved performance in an open field memory test and 24 h after the first test, treated animals exhibited better results than untreated young animals (Stoll et al. 1993, 1994). Alpha-lipoate treatment had no effect on memory in young animals. Another study demonstrated that i.p. administration of alpha-lipoate or dihydrolipoate (10 mg/kg, for 10 d), decreased rat striatum lesions induced by excitotoxins, which affect NMDA receptors and which may lead to calcium influx, as well as the generation of free radicals. In animals that received NMDA, striatal lesion size was reduced by 49% with alpha-lipoate treatment and by 41% with dihydrolipoate treatment. In animals receiving malonic acid, lesion size was reduced by 45% with alpha-lipoate treatment and by 68% with dihydrolipoate (Greenmayere et al., 1994).

5.6.2. PD

Gotz et al. (1994) showed that pretreatment with alpha-lipoate, in mice given MPTP and diethylthiocarbamate (which potentiates the effects of MPTP) did not restore dopamine levels in the striatum, but did maintain the normal ratio of reduced oxidized ubiquinone. In addition, alpha-lipoate treatment increased ^{14}C-deoxyglucose uptake in the SNpc/pr (Jenner et al., in Press). The alpha-lipoate stimulated the alternation of glucose utilization combined with normalization of reduced and oxidized ubiquinone, suggesting that alpha-lipoic acid may potentially correct metabolic abnormalities in PD. In rats in which a 6-OHDA lesion was induced in the SN, pretreatment with alpha-lipoic acid for 5 days partially prevented the neurotoxic effect of 6-OHDA, as shown by the partial protection of dopamine, homovanillic acid, and dihydroxyphenylacetic acid levels (Youdim, personal communication in Packer et al., 1997). However, when the destructive effect of 6-OHDA was potentiated by glutathione depletion with buthionine sulfoximine (BSO) administration, lipoate treatment did not reverse either the glutathione depletion or the 6-OHDA toxicity (Seaton et al., 1996).

5.6.3. Cadmium poisoning

The brain is the major target in acute cadmium poisoning, causing metabolic alterations, even in small doses (Shah and Panet, 1991), and production of free radicals (Manca et al., 1991). This may lead to oxidative damage, which in turn enhances peroxidation of membrane lipids (Axelsson et al., 1968). In rats injected with cadmium chloride, 30 min post lipoate treatment (30 mg/kg i.p) completely abolished the cadmium-induced lipid peroxidation in brain, and normalized ATPase activity, catalase activity and glutathione levels (from 37% of control in rats treated with cadmium alone, to 104%, Sumathi et al., 1994).

5.6.4. Wilson's disease

Wilson's disease is a genetic disorder of copper metabolism in which copper accumulation in the liver is

associated with neurologic and psychiatric abnormalities. Studies have shown that alpha-lipoate, a copper chelator (On et al., 1995; Sigel et al., 1978) is a promising candidate agent for the treatment of this disorder, increasing copper excretion in the urine, normalizing liver function, and reducing the severity of symptoms.

In conclusion, alpha-lipoate is a small lipid molecule that might pass the BBB and be effective. More controlled clinical studies should be performed in order to evaluate its safety and efficacy.

5.7. Coenzyme Q_{10}

Coenzyme Q_{10} (ubiquinone) is a mobile and lipid-soluble compound in the hydrophobic core of the phospholipid bilayer of the inner membrane of the mitochondria. It is an essential cofactor in the electron transport chain, where it accepts electrons from complex 1 and 2 (Beyer, 1992; Ernster and Dallner, 1995; Do et al., 1996) and transfers them, one at a time, to complex 3. It is composed of a redox active quinoid moiety and a hydrophobic tail and serves as an important antioxidant in both mitochondria and lipid membranes (Noac et al., 1994; Forsmark et al., 1997). The predominant form of coenzyme Q in humans contains 10 isoprenoid units in the tail, whereas the predominant form in rodents contains 9 isoprenoid units in the tail (coenzyme Q_9). Coenzyme Q_{10} levels are known to decrease with aging in both human and rat tissues (Beyer et al., 1985; Kalen et al., 1989). This decrease may be caused by reduced synthesis or age-dependent increases in lipid peroxidation that can reduce coenzyme Q_{10} levels (Forsmark et al., 1997).

Based on findings that energy metabolism and oxidative damage in the mitochondria play a role in the pathogenesis of neurodegenerative diseases (Beal 1992, 1995), several studies have suggested that coenzyme Q_{10} could exert a beneficial therapeutic effect. Some studies in 1–2 month-old animals found no increase in brain concentrations after oral administration of coenzyme Q_{10} (Zhang et al., 1995; Beal and Russell, 1997). Others noted a significant attenuation of lesions produced by intrastriatal administration of malonate, as well as malonate-induced depletions of ATP and increases in lactate concentrations (Beal et al., 1994). In another study, when 12 and 24-month old rats were fed with 200 mg/kg coenzyme Q_{10}, a significant increase in cerebral cortex mitochondrial concentrations of the factor was noted. Striatal lesions (closely resembling those found in Huntington's disease) induced by systemic administration of 3-nitropropionic acid (an irreversible inhibitor of succinate dehydrogenase) were markedly attenuated, and the life span significantly increased in a transgenic mouse model of ALS (Matthews et al., 1998). It was also found that coenzyme Q_{10} treatment attenuated dopamine depletions produced by MPTP in older mice (Beal and Russell, 1997).

In conclusion, most of the animal model studies showed that coenzyme Q_{10} can be beneficial in treatment against variety of toxins, although measurement of the coenzyme Q_{10} could not be found in the treated animal brain. Clinical trials should be preformed in order to evaluate its efficacy in patients.

5.8. Reduced glutathione (GSH)

Reduced glutathione (GSH)-gamma-glutamylcysteinylglycine is a ubiquitous tri-peptide, formed from the amino acids glutamate, glycine, and cysteine by two ATP-dependent enzymatic reactions (Richman and Meister, 1975). The availability of cysteine is critical for the synthesis of GSH in most cells (Meister and Anderson, 1983). GSH is a major intracellular antioxidant and its antioxidant activity depends upon the thiol group within the molecules. Intracellular GSH is maintained in its thiol form by glutathione disulfide (GSSG) reductase, which requires NADPH. GSH plays a critical role in detoxification of peroxides and electrophilic toxins as a substrate for GSH peroxidase and GSH-S-transferase (Larsson et al., 1983; Meister and Anderson, 1983). Nearly all the plasma GSH derived from GSH synthesized in the cytosol of hepatocytes and released by carrier-mediated transport (Ookhtens et al., 1985). Plasma GSH is cleared by the kidney and other organs (e.g., intestine and lungs) by carrier-mediated transport and breakdown by gamma-glutamyltranspeptidase (GGT) and dipeptidase (Meister, 1982). Deficiencies of GSH (by buthionine sulfoximine, which inhibits γ-glutamyl transpeptidase, the producing enzyme of GSH), demonstrate the need for cellular protection from endogenous ROS.

5.8.1. GSH and BBB penetration

Information on the origin of brain glutathione and its possible transport from blood to brain is limited. Kannan et al. (1990) found a substantial uptake of ^{35}S-labeled glutathione by rat brain using the carotid artery injection technique. The brain uptake index was similar with or without the use of the irreversible gamma-glutamyl transpeptidase inhibitor, acivicin. The radioactivity taken up was predominantly (>83%) in the form of GSH, suggesting BBB crossing by a saturable and specific mechanism. However, the overall penetration is very limited (<1%) and therefore GSH is not used to CNS diseases.

5.8.2. PD

In an open-label clinical study, GSH (600 mg×2/d for 30 d, i.v.) was administered to 9 patients with early, untreated PD. All patients improved significantly, with a 42% decline in disability. The therapeutic effect lasted for 2–4 months after GSH was stopped (Sechi et al.,

1996). Although these results show the efficacy of GSH the study sample was small and uncontrolled. further studies are needed on this topic.

5.8.3. Methyl mercury poisoning

To facilitate GSH transport into cells, a new compound made from a glycoside of GSH, (GSH-glyc) was synthesized, and administered i.p. or orally (40 mg/kg per day for 3/7 days) to C57BL/6J mice. GSH concentrations in brain and liver rose to a significantly higher level than normal. Methyl mercury (MeHg) poisoning of untreated mice with multiple doses of methylmercuric chloride (MMC) induced severe toxic effects associated with marked depletion of brain and liver GSH, leading to death. However, animals primed with GSH-glyc and given MMC and GSH-glyc concurrently were devoid of toxic signs (Choi et al., 1996). Thus, although the permeability of GSH or GSH-conjugated substances is very limited, modifications of the GSH derivatives for improved penetration may have clinical potential.

5.9. SOD and catalase activity molecules

Superoxide dismutase (SOD) is the most widely studied large protein molecule. SOD converts superoxide to hydrogen peroxide (H_2O_2) as follows: $2O_2^{\bullet -}+2H^+ \rightarrow H_2O_2+O_2$ (Farberreaction). Different forms have been described. One form, containing copper and zinc at its active site, is found in the cytoplasm of cells, and an isoform of this molecule is present in extracellular fluids such as plasma. A third isoform containing manganese at its active site is located in the mitochondria. Trace metals such as copper, zinc and manganese are essential for maintaining the antioxidant activity of SOD. A novel study, performed by Melov et al. (2000) (Fridovich et al., 1989), showed that augmentation of the natural antioxidant system of wild type worms (*Caenorhabditis elegants)* with Euk 8 and its analog, Euk 134, increased their mean life span by 44%. It was also effective in the treatment of prematurely aging worms, and resulted in the normalization of their life span (a 67% increase). The author concluded that the results supported the theory that ROS is a major determinant of life span and that it can be counteracted by pharmacological intervention.

Catalase is a member of the peroxidases that contains heme at its active site. Catalases are found in peroxisomes in most tissues and they are believed to cross membranes easily (Halliwell and Gutteridge, 1989). They reduce hydrogen peroxide ($H_2O_2 \rightarrow H_2O+O_2$), which is directly produced by some enzymes (e.g., monoamine, xanthine oxidase), to water and oxygen. The activity of this enzyme is lower in the brain than the liver. Euk-8, a salen-manganese complex, may be regarded as a prototype molecule of a new class of synthetic catalytic scavengers with combined SOD and catalase activity. Baudry et al. (1993) confirmed SOD activity of Euk-8 and other salen-manganese complexes. Catalase activity was demonstrated via its ability to generate oxygen in the presence of hydrogen peroxide. Euk-8 was shown to be effective in the treatment of neurodegenerative disease in two in vivo models. In the first, mice received intraventricular injections of 6-OHDA and i.p. injections of Euk-8. Extensive damage, measured by binding of [^{3}H] mazindol, a ligand for the dopamine uptake protein, was observed in the brain hemisphere ipsilateral to the site of injection of 6-OHDA at a relatively high dose. Protection by Euk-8 was significant, but only partial. However, in the same mice, a diminished degree of damage was also detected in the contralateral hemisphere. On this side, full protection by Euk-8 was achieved (Doctrow et al., 1997). Euk-8 has also been found to protect mice against the neurodegenerative effects of MPTP. Mice injected with MPTP, exhibited a substantial loss of dopaminergic neurons, also assessed by [^{3}H] mazindol binding. Oral treatment with Euk-8 (via the drinking water) completely protected mice from MPTP-induced neurotoxicity. These models indicate that, when administered peripherally, Euk-8 can be beneficial in the treatment of 6-OHDA and MPTP toxicity. However, its permeability through the BBB was not measured.

6. Conclusions

Due to increased exposure to environmental damage, our endogenous antioxidant defense system is not completely effective. It seems reasonable to propose that antioxidants are very important in diminishing the cumulative effects of oxidative damage. Since OS has been implicated in the pathogenesis of many neurological, particularly neurodegenerative, diseases, antioxidants of widely varying chemical structures have been investigated for use as therapeutic agents. Most of the papers hereby reviewed checked the efficacy of antioxidants in the treatment of neurodegenerative diseases. Although some showed a degree of efficiency when used in animal models or in small clinical studies, none of the antioxidants were examined in a large-scale controlled study and the data is conflicting. The rationale for antioxidant treatment in the CNS is based on established observations and experiments in vitro. However, in practice, the drugs used failed to provide real neuroprotection. Potential reasons for these mixed results include inappropriate use of specific antioxidant/s for a given disease or, stage of disease progression or the use of suboptimal doses. Another limitation is the insufficient knowledge of BBB penetration of different antioxidants when used systemically. Therefore, CNS drug design that enables BBB transport will depend on new knowledge of the molecular and cellular biology of brain capillary endo-

thelial transport processes. In addition, methods of targeting drugs to specific sites within the brain are necessary to produce efficient drugs with minimal side effects. It is also important to determine whether antioxidants can be used as prophylactics, in order to slow down the progression of neurodegenerative diseases such as AD and PD in populations that are at high risk, such as the elderly.

References

Agus, D.B., Sanjiv, S.G., Pardridge, W.M., Spielholz, C., Baselga, J., Carlos Vera, J., Golde, D.W., 1997. Vitamin C crosses the blood brain barrier in the oxidized form through the glucose transporters. Journal of Clinical Investigation 100 (11), 2842–2848.

Akaneya, Y., Takahasi, M., Hatanaka, H., 1995. Involvement of free radicals in MPP+ neurotoxicity against rat dopaminergic neurons in culture. Neuroscience Letters 193, 53–56.

Anderson, R.H., Anderson, O., 1993. Effects of dietary α-tocopherol and β-carotene on lipid peroxidation induced by methyl mercurin chloride in mice. Pharmacology and Toxicology 73, 192–201.

Axelsson, B., Dahlyren, S.E., Piscator, M., 1968. Renal lesion in the rabbit after long term exposure to cadmium. Archives of Environmental Health 17, 245–248.

Bagchi, D., Garg, A., Krohn, R.L., Bagchi, M., Bacgchi, D.J., Balmoori, J., Stohs, S.J., 1998. Protective effects of grape seed proanthocyanids and selected antioxidants against TPA-induced hepatic and brain lipid peroxidation and DNA fragmentation, and peritoneal macrophage activation in mice. General Pharmacology 30 (5), 771–776.

Bartzokis, G., 1997. Magnetic resonance imaging of brain iron. In: Connor, J.R. (Ed.), Metals and Oxidative Damage in Neurological Disorders. Plenum Press, New York, pp. 41–56.

Bartzokis, G., Cummings, J., Perlman, S., Hance, D.B., Mintz, J., 1999. Increased basal ganglia iron levels in Huntington's disease. Archives of Neurology 56, 569–574.

Baudry, M., Etienne, S., Bruce, A., Puluchi, M., Jacobsen, E., Malfroy, B., 1993. Salen manganese complexes are superoxide dismutase mimics. Biochemical and Biophysical Research Commununications 192 (2), 964–968.

Beal, M.F., 1992. Does impairment of energy metabolism result in excitoxic neuronal death in neurodegenerative illnesses? Annals of Neurology 31, 119–130.

Beal, M.F., Henshaw, R., Jenkins, B.G., Rosen, B.R., Schultz, J.B., 1994. Coenzyme Q_{10} and nicothinamide block striatal lesions produced by the mitochondrial toxin malonate. Annals of Neurology 36, 882–888.

Beal, M.F., 1995. Aging, energy and OS in neurodegenerative diseases. Annals of Neurology 38, 357–366.

Beal, M.F., 1996. Mitochondria, free radicals and neurodegeneration. Current Opinion in Neurobiology 6, 661–666.

Beal, M.F., Russell, T.M., 1997. Coenzyme Q_{10} in the central nervous system and its potential usefulness in the treatment of neurodegenerative diseases. Molecular Aspects of Medicine 18, S169–S179.

Beyer, R.E., Burnett, B.A., Cartwright, K.J., Edington, D.E., Falzon, M.J., Krietman, K.R., Kuhn, T.W., Ramy, B.J., Rhee, S.Y.S., Rosenwasserm, J. et al., 1985. Tissue coenzyme Q (ubiquinone) and protein concentrations over the life span of the laboratory rat. Mechanisms of Aging and Development 32, 267–281.

Beyer, R.E., 1992. An analysis of the role of coenzyme Q in free radical generation and as antioxidant. Biochemistry and Cellular Biology 70, 390–403.

Beyer, C.E., Steketee, J.D., Saphier, D., 1998. Antioxidant properties of melatonin — an emerging mystery. Biochemical Pharmacology 56, 1265–1272.

Black, K.L., King, W.A., Ikezaki, K., 1990. Selective opening of the blood-tumor barrier by intracarotid infusion of leukotriene. Journal of Neurosurgery 72, 912–916.

Bonate, O.L., 1995. Animal models for studying transport across the blood-brain barrier. Journal of Neuroscience Methods 56, 1–15.

Bondy, S.C., Komulainen, H., 1988. Intracellular calcium as an index of neurotoxic damage. Toxicology 49, 35–41.

Breteler, M.M.B., Amerongen, N.M., Swieten, J.C., Claus, J.J., Grobbee, D.E., Van Gijn, J., Hofman, A., van Harshamp, A., 1994. Cognitive correlates of ventricular enlargement and cerebral white matter lesions on magnetic resonance. Imaging the Rotterdam Study. Stroke 25, 1109–1115.

Browne, S.E., Bowling, A.C., MacGarrey, U. et al., 1997. Oxidative damage and metabolic dysfunction in Huntington's disease: selective vulnerability of the basal ganglia. Annals of Neurology 41, 646–653.

Cadet, J.L., Katz, M., Jackson-Lewis, V., Fahn, S., 1989. Vitamin E attenuates the toxic effects of intrastriated injection of 6-hydroxydopamine (6-OHDA) in rats: behavioral and biochemical evidence. Brain Research 476, 10–15.

Chan, A.C., 1993. Partners in defense. Vitamin E and vitamin C. Canadian Journal of Physiology and Pharmacology 71 (9), 725–731.

Chatterjee, I.B., Majunder, A.L., Nandi, B.K., Subramanian, N., 1975. Synthesis and some major functions of vitamin C in animals. Annals of the New York Academy of Sciences 258, 24–47.

Chen, J.C., Hurdy, D.A., Hucharczyk, W. et al., 1993. MRI of human postmortem brain tissues correlative study between T2 and assays of iron and ferritin in Parkinson and Huntington's disease. American Journal of Neurological Research 14, 275–281.

Chen, S.T., 1999. The antioxidant melatonin reduces cortical neuronal death after intrastriatal injection of haingate in the rat. Experimental Brain Research 124, 241–247.

Choi, B.N., Yee, S., Robles, M., 1996. The effects of glutathione glycoside in methyl mercury poisoning. Toxiciology and Applied Pharmacology 141, 357–364.

Coyle, J.T., Puttfarcken, P., 1993. Oxidative stress, glutamate and neurodegenerative disorders. Science 262, 689–695.

Cutler, R.G., 1991. Human longevity and aging: possible role of reactive oxygen species. Annals of the New York Academy of Sciences 621, 1–28.

Damier, P., Hirsch, E.C., Zhang, P., Agid, Y., Javoy-Agid, F., 1993. Glutathione peroxidase, glial cells and Parkinson's disease. Neuroscience 52, 1–7.

DATATOP, 1989. Parkinson study group: a multicenter controlled clinical trial in early Parkinson's disease. Archives of Neurology 46, 1052–1060.

DATATOP, 1993. Parkinson study group. Effects of tocopherol and deprenyl on the progression of disability in early Parkinson's disease. New England Journal of Medicine 328, 176–183.

DATATOP, 1996. Parkinson study group. Impact of deprenyl and tocopherol treatment on Parkinson's disease in DATATOP patients requiring levodopa. Annals of Neurology 39, 37–45.

De Kumar, A., Rukmini, D., 1988. Physiologcial antioxidants and antioxidative enzymes in vitamin E-deficient rats. Toxicology Letters 44, 47–54.

De Rijk, M.C., Breteler, M.M., den Breeijen, J.H., Lauren, L.J., Grobbee, D.E., Van der Meche, F.G., Hoffman, A., 1997. Dietary antioxidants and Parkinson's disease. The Rotterdam Study. Archives of Neurology 54 (6), 1762–1765.

Dexter, D.T., Jenner, P., Schapira, A.H., Marsden, C.D., 1992. Alternations in levels of iron, ferritin and other trace metals in neurodegenerative disease affecting the basal ganglia. Annals of Neurology 32 (Suppl.), S94–S100.

Dexter, D.T., Nanayakkaral, A., Goss-Sampson, M.A. et al., 1994.

Nigral dopaminergic loss in vitamin E deficient rats. Neuroreport 5, 1773–1776.

Dumuis, M., Sebben, H., Haynes, J.P., Pin, J., Bockaert, A., 1988. NMDA receptors activate the arachidonic acid cascade system in striatal neurons. Nature 336, 68.

Do, T.Q., Schultz, J.R., Clarke, C.F., 1996. Enhanced sensitivity of ubiquinone-deficient mutants of *Saccharomyces cerevisae* to products of autooxidized polyunsaturated fatty acid. Proceedings of the National Academy of Sciences of the USA 93, 7534–7549.

Doctrow, S.R., Huffman, K., Marcus, C.B., Musleh, W., Bruce, A., Baudry, M., Malfroy, B., 1997. Salen-manganese complexes: combined superoxide dismutase/catalase mimics with broad pharmacological efficacy. Advances in Pharmacology 38, 247–269.

Ebadi, M., Srinivasan, S.K., Baxi, M.D., 1996. Oxidative stress and antioxidant theory in Parkinson's disease. Progression in Neurobiology 48, 1–19.

Ernster, L., Dallner, G., 1995. Biochemical, physiological and medical aspects of ubiquinon function. Biochimica et Biophysica Acta 127, 195–204.

Evans, P.H., 1993. Free radicals in brain metabolism and pathology. British Medical Bulletin 49, 577–587.

Fahn, S., 1991. An open trial of high dosage antioxidants in early Parkinson's disease. American Journal of Clinical Nutrition 53, 3805–3825.

Fahn, S., Cohen, G., 1992. The oxidant stress hypothesis in Parkinson's disease. Evidence supporting it. Annals of Neurology 32, 804–812.

Flynn, B.L., Runho, A., 1999. Pharmacological management of Alzheimer's disease part II: antioxidants, antihypertensives and Ergoloid derivatives. The Annals of Pharmacotherapy 33, 188–197.

Forsmark, A.P., Lee, C.P., Dallner, G., Ernster, L., 1997. Lipid peroxidation and changes in the ubiquinone content and the respiratory chain enzymes of submitochodrial particles. Free Radical Biology and Medicine 22, 391–400.

Frautschy, S.A., Baired, A., Cole, G.M., 1991. Effects of injected Alzheimer β-amyloid cores in rat brain. Proceedings of the National Academy of Sciences of the USA 88, 8362–8366.

Frei, B., England, L. et al., 1989. Ascorbate is an outstanding antioxidant in human blood plasma. Proceedings of the National Academy of Sciences of the USA 86, 6377–6381.

Fridovich, I., 1989. Superoxide dismutases: An adaptation to a paramagnetics gas. Journal of Biological Chemistry 264, 7761–7764.

Friedman, A., Kaufer, D., Stener, J., Hendler, I., Sorey, H., Tur-Kaspa, I., 1996. Pyridostigmin brain penetration under stress enhances neuronal excitability and induced early immediate transcriptional response. Nature Medicine 2, 1382–1385.

Golbe, L.I., Farrel, T.L.M., Davis, P.H., 1988. Case control study of early life dietary factors in Parkinson's disease. Archives of Neurology 45, 1350–1353.

Golbe, L.I., Farrell, T.L.M., Davis, P.H., 1990. Follow up study of early-life protective and risk factor in Parkinson's disease. Movement Disorders 5, 66–70.

Goodwin, J.S., Goodwin, J.M., Garry, P.J., 1983. Association between nutritional status and cognitive functioning in a healthy elderly population. JAMA 249, 2917–2921.

Gorman, A.M., McGowan, A., O'Neil, C., Cotter, T., 1996. Oxidative stress and apoptosis in neurodegeneration population. Journal of Neurological Science 139 suppl, 45–52.

Gotz, M.E., Dirr, A., Burger, R., Wanetzky, B., Weinmuller, N., Chan, W.W., Chen, S.C., Reichmann, H., Rausch, W.D., Rieterer, P., 1994. Effects of lipoic acid or redox state of coenzyme in mice treated with 1-methyl-4-phenyl 1,2,3,6-tetrahydropyridine and diethyldithiocarbonate. European Journal of Pharmacology 266, 291–300.

Greenwood, J., 1992. Experimental manipulation of the blood brain and blood retinol barriers in the physiology and pharmacology of the blood brain barrier. In: Bradbury, M.W.B. (Ed.). Springer-Verlag, pp. 459–486.

Greenmayere, J.T., Garcia-Osuna, N., Greene, J.G., 1994. The endogenous cofactors, thioctic acid lesions and dihydrolipoic acid, are neuroprotective against NMDA and malonic acid lesions of striatum. Neuroscience Letters 171, 17–20.

Gu, M., Gash, M.T., Mann, V.M., Jany-Agid, F., Cooper, J.M., Schapira, A.H., 1996. Mitochondrial defect in Huntington's disease caudate nucleus. Annals of Neurology 39, 385–389.

Gumerlock, M.K., Neuwelt, E.A., 1992. Therapeutic opening of the blood brain barrier in man. In: Bradbury, M.W.B. (Ed.). Springer-Verlag, pp. 525–542.

Hall, E.D., Andrus, P.K., Oostreen, J.A., Fleck, T.J., Gurney, M.E., 1998. Relationship of oxygen radial-induced lipid peroxidative damage to disease onset and progression in a transgenic model of familial ALS. Journal of Neuroscience Research 53 (1), 66–77.

Halliwell, B., Gutteridge, J.M.C., 1989. Free Radicals in Biology and Medicine, 2nd ed. Clarendon Press, Oxford.

Halliwell, B., 1994. Free radicals, antioxidants, and human disease: curiosity, cause or consequence? The Lancet 344, 721–724.

Halliwell, B., 1997. Antioxidants: the basics — what they are and how to evaluate them. Advances in Pharmacology 38, 3–20.

Harman, D., 1992. Role of free radicals in aging and disease. Annals of New York Academy of Sciences 673, 126–134.

Harris, J.O., Frank, J.A., Patronas, N., Mcfarlin, D.E., Mcfarland, H.F., 1991. Serial gadolinium-enhanced magnetic resonance imaging scans in patients with early, relapsing-remitting multiple sclerosis: implications for clinical trials and natural history. Annals Neurology 29, 548–555.

Hoffman, A., Grobbee, D.E., De Jong, P.T.V.M., Van den Ouweland, A., 1991. Determinants of disease and disability in the elderly. The Rotterdam Elderly Study. European Journal of Epidemiology 7, 403–412.

Jama, W.J., Launer, L.J., Witteman, J.C.M., den-Breeijn, H., Breteler, M.M.B., Hofman, A., 1996. Dietary antioxidants and cognitive function in a population-based sample of older persons: the Rotterdam study. American Journal of Epidemiology 144, 275–280.

Jenner, P., Olnaw, C.W., 1996. Oxidative stress and the pathogenesis of Parkinson's disease. Neurology 47 (Suppl.), S161–S176.

Jenner, P., Seaton, T.A., Mardsden, C.D., Altered ^{14}C-deoxy-glucose incorporation in rat brain following treatment with α-lipoic acid. Diab. Stoffw. in Press.

Jin, B.K., Shin, D.Y., Jeong, M.Y., Gwang, M.R., Baik, H.W., Yoon, K.S., Cho, Y.H., Joo, W.S., Kim, Y.S., Baik, H.H., 1998. Melatonin protects nigral dopaminergic neurons from 1-methyl-4-phenylpyridinium (MPP+) neurotoxicity in rats. Neuroscience Letters 245, 61–64.

Kalen, A., Appelkvist, E.L., Daliner, G., 1989. Age related changes in the lipid composition of rat and human tissues. Lipids 24, 579–584.

Kalmijn, S., Feshens, E.J.M., Launer, L.J., Kromhout, D., 1997. Polyunsaturated fatty acids. antioxidants and cognitive function in very old men. American Journal of Epidemiology 145, 33–41.

Kannan, R., Uuhlenhamp, J.F., Jaendidier, E., Trinh, H., Oouhbtens, M., Laplowitz, N., 1990. Evidence for carrier-mediated transport of glutathione across the blood brain barrier in the rat. Journal of Clinical Investigation 85, 2009–2013.

Larsson, A., Orrenius, S., Holmgren, A., Mannervik, B., 1983. Functions of glutathione. Biochemical physiological toxicological and clinical aspects. Raven Press, New York.

Lebel, C.P., Ali, S.F., McKee, M., Bondy, S.C., 1990. Organometal-induced increases in oxygen reactive species: the potential of 2′7′-dichlorofluorescin diacetate as an index of neurotoxic damage. Toxicology and Applied Pharmacology 104, 17–24.

Loeffler, D.A., Lewitt, P.A., Juneau, P.L. et al., 1996. Increased regional brain concentration of ceruloplasmin in neurodegenerative disorders. Brain Research 738, 265–274.

Logroscino, G., Marker, K., Cote, L., Tang, M.X., Shea, S., Mayeux,

R., 1996. Dietary lipids and antioxidants in Parkinson's disease: a population-base case control study. Annals of Neurology 39, 89–94.

Lohr, J.B., Kuczenski, R., Bracha, H.S., Moir, M., Joste, D.V., 1990. Increased indices of free radical activity in the cerebrospinal fluid of patients with tardive dyskinesia. Biological Psychiatry 28, 533–539.

Lohr, J.B., Caligiuri, M.P., 1996. A double-blind placebo-controlled study of vitamin E treatment of tardive dyskinesia. Journal of Clinical Psychiatry 57, 167–173.

Lovell, M.A., Ehmann, W.D., Butler, S.M., Markesberg, W.R., 1995. Elevated thiobarbituric acid reactive substances and antioxidant enzyme activity in the brain in Alzheimer's disease. Neurology 45, 1594–1601.

Macdonald, P.N., Boh, D., Ong, D.E., 1990. Localization of cellular retinol-binding protein and retinol-binding protein in cells comprising the blood brain barrier of rat and human. Proceedings of the National Academy of Sciences of the USA 87, 4265–4269.

Machlin, L.J., Bendick, A., 1987. Free radical tissue damage: protective role of antioxidant nutrients. FASEB Journal 1, 1441–1445.

Mahadik, S.P., Scheffer, R.E., 1996. Oxidative injury and potential use of antioxidants in schizophrenia. Prostaglandins, Leukocytes and Essential Fatty Acids 55, 45–54.

Manca, D., Ricard, A.C., Trottier, B., Chevalier, G., 1991. Studies on lipid peroxidation in rat tissues following administration of low and moderate doses of cadmium chloride. Toxicology 67, 303–323.

Markesbery, W.R., 1997. Oxidative stress hypothesis in Alzheimer's disease. Free Radical Biology and Medicine 23, 134–147.

Martinovits, G., Melamed, E., Cohen, O., Rosenthal, J., Uzzan, A., 1986. Systemic administration of antioxidants does not protect mice against the dopaminergic neurotoxicity of 1-methyl-4-phenyl 1,2,5,6-tetrahydropyridine (MPTP). Neuroscience Letters 69, 192–197.

Masaki, K.H., White, L.R., Petrovitch, H., Ross, G.W., Curb, J.D., Ardo, E., Grove, J., 1994. The influence of prior and concurrent use of aspirin and vitamins on cognitive function scores in elderly Japanese American men. Neurobiology of Aging, (Suppl. 1) 574.

Matthews, R.T., Yang, L., Browne, S., Baik, M., Beal, M.F., 1998. Coenzyme Q10 Administration increases brain mitochondrial concentrations and exerts neuroprotective effects. Proceedings of the National Academy of Sciences of the USA 95 (15), 8892–8897.

Mattia, C.J., Adams, J.D., Bondy, S.C., 1993. Free radical induction in the brain and liver by products of toluene catabolism. Biochemistry and Pharmacology 46, 103–110.

Melov, S., Ravenscroft, J., Malik, S. et al., 2000. Extension of life span with Superoxide Dismutase/Catalase Mimetics. Science 289, 1567–1569.

McCormick, E.C., Cornwell, D.G., Brown, J.B., 1960. Studies on the distribution of tocopheral in human serum lipoproteins. Journal of Lipid Research 1, 221–228.

Meister, A., 1982. Amino acids and glutathione. In: Snell, E.E. (Ed.), Amino Acids, Fermentations and Nucleic Acid: A Symposium. Annual Review Inc, Palo Alto, CA, pp. 5–27.

Meister, A., Anderson, M.E., 1983. Glutathione. Annual Review of Biochemistry 52, 711–760.

Morris, M.C., Bechett, L.A., Scherr, P.A., Herbert, L.E., Bennett, D.A., Field, T.S., Evans, D.A., 1998. Vitamin E and vitamin C supplement use and risk of incident Alzheimer's disease. Alzheimer's Disease and Associated Disorders 12 (3), 121–126.

Noac, K.H., Kube, U., Augustin, W., 1994. Relations between tocopheral depletion and coenzyme Q during lipid peroxidation in rat liver mitochondria. Free Radical Research 20, 375–386.

Offen, D., Ziv, I., Gorodin, S., Barzilai, A., Malik, A., Melamed, E., 1995. Dopamine induced programmed cell death in mouse thymocytes. Biochimica et Biophysica Acta 1268, 171–177.

Offen, D., Gorodin, S., Melamed, E., Hanania, J., Malik, Z., 1999. Dopamine melanin is actively phagocytized by PC12 cells and cerebellar granular cells: possible implications for the etiology of Parkinson's disease. Neuroscience Letters 260, 101–104.

Olney, J.W., Gubareff, T., 1978. Glutamate neurotoxicity and Huntington's chorea. Nature 271, 557–559.

On, P., Tritschler, H., Wolff, S.P., 1995. Thioctic (lipoic) acid. A therapeutic metal chelating antioxidant? Biochemical Pharmacology 50, 123–126.

Ookhtens, M., Hobdy, K., Corrasee, M.C., Ty, A.W., Kaplowitz, N., 1985. Sinusoidal efflux of glutathione in the perfused rat liver: evidence for a carrier mediated process. Journal of Clinical Investigation 75, 258–265.

Oteiza, P.I., Uchitel, O.D., Carrasquedo, F., Duborovski, A.L., Roma, J.C., Fraga, C.G., 1997. Evaluation of antioxidants, protein, and lipid oxidation products in blood from sporadic amyotrophic lateral sclerosis patients. Neurochemical Research 22 (4), 535–539.

Packer, L., Tritschler, H., Wessel, K., 1997. Neuroprotection by the metabolic antioxidant and α-lipoic acid. Free Radicals in Biology and Medicine 22, 359–378.

Panigrahi, M., Sadguna, Y., Shivakumar, B.R., Kolluri, S.V., Roy, S., Packer, L., Ravindranath, V., 1996. Alpha-lipoic acid protects against reperfusion injury following cerebral ischemia in rats. Brain Research 717, 184–188.

Pappella, M.A., Omar, R.A., Kim, K.S., Rubakis, U.K., 1992. Immunohistochemical evidence of antioxiodant stress in Alzheimer's disease. American Journal of Pathology 140, 621–628.

Pappert, E.J., Tangney, C.C., Goetz, C.G., Ling, Z.D., Lipton, J.W., Stebbins, G.T., Carney, P.M., 1996. Alpha-tocopherol in the ventricular cerebrospinal fluid of Parkinson's disease patients: dose response study and correlations with plasma levels. Neurology 47, 1037–1042.

Pappolla, M.A., Chyan, Y.J., Omar R.A., et al. 1998. Evidence of oxidative stress and in vivo neurotoxicity of beta-amyloid in a transgenic mouse model of Alzheimer's disease: a chronic oxidative paradigm for testing antioxidant therapies in vivo.

Pardridge, W.M., Sakiyama, R., Coty, W.A., 1985. Restricted transport of vitamin D and derivatives through the rat blood brain barrier. Journal of Neurochemistry 44, 1138–1141.

Path, J., 1990. Cellular functions of ascorbic acid. Biochemistry and Cell Biology 68, 1166–1173.

Patmanatha, N.D., 1989. Effects of vitamin A and its analogs on nonenzymatic lipid peroxidation in rat brain mitochondria. Journal of Neurochemistry 52, 585–588.

Perry, T.L., Yong, V.W., Hansen, S., Jones, K., Bergeron, C., Foulks, J.G., Wright, J.M., 1987. α-tocopherol and β-carotene do not protect marmosets against the dopaminergic neurotoxicity of N-methyl-4-phenyl 1,2,3,6-tetrahydroxypyridine. Journal of the Neurological Sciences 81, 321–331.

Perry, W.J., Perry, P., Stahelin, H.B., 1997. The relation between antioxidants and memory performance in the old and very old. Journal of the American Geriatric Society 45, 718–724.

Perumal, A.S., Gopal, V.B., Tordzro, W.K., Cooper, T.B., Cadet, J.L., 1992. Vitamin E attenuates the toxic effects of 6-hydoxydopamine on free radical scavenging system in rat brain. Brain Research Bulletin 29, 699–701.

Peyser, C.E., Folstein, M., Chase, G.A. et al., 1995. Trial of α-tocopherol in Huntington's disease. American Journal of Psychiatry 152, 1771–1775.

Pillai, S.R., Traber, M.G., Steiss, J.E., Kayden, H.J., 1993. Depletion of adipose tissue and peripheral nerve α-tocopheral in adult dogs. Lipids 28, 1095–1099.

Qin, Y., Sato, T.N., 1995. Mouse multidrug resistance 1a/3 gene is the earliest known endothelial cell differentiation marker during blood brain barrier development. Development Dynnamics 202, 172–180.

Reese, T.S., Karnovsky, M.J., 1967. Fine structural localization of a blood brain barrier to exogenesis peroxidase. Journal of Cell Biology 34, 207–217.

Reynolds, G.P., 1992. Developments in the drug treatment of schizophrenia. TIPS 13, 116–121.

Richman, P.G., Meister, A., 1975. Regulation of gamma-glutamylcystein, synthetase by non-allosteric feedback inhibition by glutathione. Journal of Biological Chemistry 250 (4), 1422–1426.

Robberecht, W., Sapp, P., Viaene, M.U., Rosen, D., McLenna, Y.D., Hames, J., Horovitz, R., Theys, P., Brown, R., 1994. Cu/Zn superoxide dismutase activity in familial and soporadic amytrophic lateral sclerosis. Journal of Neurochemistry 62 (1), 1384–1387.

Rose, R.C., Bote, A.M., 1993. Biology of free radical scavengers: an evaluation of ascorbate. FASEB Journal 7 (12), 1135–1142.

Rowland, L.P., Fink, M.E., Rubin, L.L., 1992. Cerebrospinal fluid: blood brain barrier brain oedema and hydrocephalus. In: Kandel, E.R., Schwartz, J.H., Jessle, T. (Eds.), Principles of Neural Science. Elsevier, New York, pp. 1050–1060.

Rubin, L.L., Staddon, J.M., 1999. The cell biology of the blood brain barrier. Annual Review of Neuroscience 22, 11–28.

Sack, C.A., Socci, D.J., Crandall, B.M., Arentash, G.W., 1996. Antioxidant treatment with phenyl-alpha-tert butyl nitrone (PBN) improves the cognitive performance and survival of aging rats. Neuroscience Letters 205 (3), 181–184.

Sano, M., Erresto, C., Thomas, R.G., Melville, R.K., Schafer, K., Grundman, M. et al., 1997. A controlled trial of selegiline, alpha tocopherol or both as treatment for Alzheimer's disease. New England Journal of Medicine 336, 1216–1222.

Saunders, N.R., Dziegielewska, K.M., Mollgard, K., 1991. The importance of the blood brain barrier in fetuses and embryos. Trends in Neuroscience 14, 14–15.

Schmidt, R., Hayn, M., Reihart, B., Roob, G., Schmidt, H., Schumacher, M., Watzinger, N., Launer, L.J., 1998. Plasma antioxidants and cognitive performance in middle aged and older adults: results of the Austrian stroke prevention study. Journal of the American Geriatric Society 46, 1407–1410.

Schriber, M., Trojan, S., 1991. Ascorbic acid in the brain. Physiology Research 40, 413–418.

Seaton, T.A., Jenner, P., Marsden, C.D., 1996. Thiocitic acid does not restore glutathione levels of protection against the potentiation of 6-hydroxydopamine toxicity induced by glutathione depletion in rat brain. Neurological Transactions 103, 315–329.

Sechi, G., Deeden, M.G., Bua, G., Sutta, W., Deian, G.A., Pes, G., Rosati, G., 1996. Reduced intravenous glutathione in the treatment of early Parkinson's disease. Progress in Neuropsychopharmacology and Biological Psychiatry 20, 1159–1170.

Shah, J., Panet, H.C., 1991. Effect of cadmium on Ca^{2+} transport in brain microsomes. Brain Research 566, 127–130.

Shohami, E., Yannai, E.B., Horowitz, M., Kohen, R., 1997. Oxidative stress in closed head injury: brain antioxidant capacity as an indicator of functional outcome. Journal of Cerebral Blood Flow and Metabolism 17, 1007–1019.

Sian, J., Dexter, D.T., Less, A.J. et al., 1994. Alteration in glutathione levels in Parkinson's disease and other neurodegenerative disorders affective basal ganglia. Annals of Neurology 36, 348–355.

Sigel, H., Prijs, N.B., McCormick, D.B., Shih, J.C.H., 1978. Stability of binary and tertiary complex of α-lipoate and lipoate derivatives in Mn^{2+}, Ca^{2+} and Zn^{2+} in solution. Archives of Biochemical Biophysics 187, 208–214.

Simonian, N.A., Coyle, J.T., 1996. Oxidative stress in neurodegenerative diseases. Annual Review of Pharmacological Toxicology 36, 83–106.

Stoll, S., Hartmann, H., Cohen, S.D., Muller, W.E., 1993. The potent free radical scavenger α-lipoic acid improves memory in aged mice: putative relationship to NMDA receptor deficits. Pharmacology, Biochemistry and Behavior 46, 799–805.

Stoll, S., Rostoch, A., Bartsch, R., Lorn, E., Meichelboch, A., Muller, W.E., 1994. The potent free radical scavenger alpha-lipoic acid improves cognition in rodents. Annals of the New York Academy of Sciences 717, 122–128.

Sumathi, R., Devi, V.H., Varalashmi, P., 1994. DL α-lipoic acid protection against cadmium-induced tissue lipid peroxidation. Medicinmal Science Research 22, 23–25.

Tanner, C., 1992. Epidemiology of Parkinson's disease. Neurological Clinician 10, 317–327.

Traber, M.G., Packer, L., 1995. Vitamin E: beyond antioxidant function. American Journal of Clinical Nutrition 62 (suppl), 15015–15095.

Traber, M.G., Sies, H., 1996. Vitamin E in humans. Demand and delivery. Annual Review of Nutrition 16, 321–347.

Tsai, G., Goff, D.C., Chang, R.W., Flood, J., Baer, L., Coyle, J.T., 1998. Markers of glutomatergic neurotransmission and oxidative stress associated with tardive dyskinesia. American Journal of Psychiatry 155 (9), 1207–1213.

Vatassery, G.T., 1992. Vitamin E: Neurochemistry and implication for Parkinson's disease. Annals of the New York Academy of Sciences 669, 92–110.

Vyth, A., Timner, J.G., Bossyut, P.M.M., Louwerse, E.S., Vianney De Jong, J.M.B., 1996. Survival in patients with amyotrophic lateral sclerosis, treated with an array of antioxidants. Journal of the Neurological Sciences 139 (suppl), 199–203.

Williams, J.H., Errington, M.L., Lynch, M.A., Bliss, T.V., 1989. Arachidonic acid induces a long-term activity-dependent enhancement of synaptic transmission in the hippocampus. Nature 341, 739–742.

Yoitaka, A., Hyttori, N., Uchida, K., Tanaka, N., Stadtman, E.R., Mizuno, Y., 1996. Immunohistochemical detection of 4-hydroxynonenal protein adducts in Parkinson's disease. Proceedings of the National Academy of Sciences of the USA 93, 2696–2701.

Yong, V.W., Perry, T.L., Krishman, A.A., 1986. Depletion of glutathione in brain stem of mice caused by N-methyl-4-phenyl-1, 2,3,6-tetrahydropyridine is prevented by antioxicyte pre treatment. Neuroscience Letters 63 (1), 56–60.

Zemlan, F.P., Theinhaus, O.J., Bosmann, H.B., 1989. Superoxide dismutase activity in Alzheimer's disease: possible mechanism for paired helical formation. Brain Research 476, 160–162.

Zhang, Y., Aberg, F., Appelkvist, E.L., Dallner, G., Ernsteir, L., 1995. Uptake of dietary coenzyme Q supplement is limited in rats. Journal of Nutrition 125, 446–453.

Ziv, I., Melamed, E., Nardi, N., Luria, D., Achiron, A., Offen, D., Barzilai, A., 1994. Dopamine induces apoptosis like cell death in cultured sympathetic neurons: a possible novel pathogenic mechanism in Parkinson's disease. Neuroscience Letters 170, 136–140.