Oxidative stress and neurodegenerative disease

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ABSTRACT

Several disease conditions are believed to be related to oxygen free radical formation including a number of neurodegenerative disorders. Therapy using free radical scavengers (antioxidants) has been used to prevent, delay or modify the progress of many neurological disorders. The optimum antioxidant therapeutic options have to be tailored and modified individually. This is because the biochemistry of oxidative pathophysiology is still a complex matter. In this review the role of oxidative stress and the potential therapeutic effect of some antioxidants is discussed in a number of neurodegenerative disorders including Alzheimer disease, Parkinson's disease and multiple sclerosis.

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Oxidative stress is caused by exposure to reactive oxygen intermediates, such as superoxide anion (O_2^-) hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH), which can damage proteins, nucleic acids and cell membranes. A number of studies also suggest that the effects of this oxidant are integrally linked to the damage caused by hypochlorous acid (Ho) and reactive nitrogen intermediates as nitric oxide (NO), peroxynitrates (HOONO) and nitrosthiols (RSNO).[1] The cell continuously expresses enzymes that detoxify the reactive oxygen species or repairs the damage caused by them.[2]

Oxidative stress. Free radicals and other reactive oxygen species (ROS) are formed as by-products of oxygen metabolism. The main step is based on transfer of 4 electrons, taken from 2 molecules of reduced nicotinamide adenine dinucleotide (NADH), to one molecule of dioxygen (O_2), the result is production of water as follows:

$$2\ NADH + 2\ H^+ + O_2 \rightarrow 2\ NAD^+ + 2\ H_2O.$$

Five to ten percent of oxygen that enters cellular respiration receives less than 4 electrons necessary for the complete reduction and is converted into superoxide (O_2^-) or hydrogen peroxide (HO_2).[3] The presence of an efficient antioxidant system has been developed to detoxify these reactive species before they can cause damage to cellular structures, such as, the presence of superoxide dismutase which converts O_2^- into H_2O_2, and this is rapidly reduced by catalase and glutathione peroxides.[4,5] Under pathological conditions an increase in free radical formation or an exhaustion of antioxidant defense system takes place and the results are excessive cell damage. Rapid metabolism of free radicals is an important step since accumulation of free radicals can lead to the production of more reactive and more toxic radical species. For example, H_2O_2 can react with ferrous iron (Fe^{2+}) to undergo Fenton-type activation.[6] Nitric oxide (NO) on the other hand combines with O_2^- to form peroxynitrate ($ONOO^-$), this molecule is toxic by itself, but it can decompose in the presence of iron to form Fenton-like products.[7] Fenton-like products account for most of the structural damage to unsaturated phospholipids, proteins or deoxyribonucleic acid (DNA). An important piece of information is the correlation of DNA oxidative damage and cancer, which can be seen through the association of smoking and lung cancer as the best example. The mechanism involved is the iron catalyzed Fenton reaction, where Fe^{2+} reduces H_2O_2 to hydroxyl radical as follows:

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

This can immediately react with DNA; the result is breakage of DNA strands with the release of the base and consequently increased mutation, cancer and age-related pathologies.[8,9]

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Antioxidant. Antioxidants are endogenous or exogenous compounds that either reduce the formation of free radicals or react with and neutralize them and so protect the cell from oxidative injury. Two types of antioxidants are present; endogenous or exogenous antioxidants and both can be lipid soluble; for example, vitamin E or water soluble; for example, vitamin C. For a particular antioxidant compound to enter the brain, it must penetrate the blood-brain barrier (BBB) to reach a therapeutic concentration within the central nervous system (CNS). Thus, antioxidants which readily pass through the BBB are good candidates for use in neurological disorders. An example is pyrolopyrimidineses, a novel class of antioxidants, which inhibit lipid peroxidation, has proved to have an excellent BBB penetrance and are neuroprotective in animal models, of both focal and generalized cerebral ischemia.[10,11] Another example is coenzyme Q^{10} (ubiquinone) is a lipid-soluble mitochondrial antioxidant cofactor that readily crosses the BBB and has been shown to be neuroprotective in several animals models of neurodegenerative disease.[12] Different antioxidants have distinctive effects in protecting either nucleic acid, proteins or lipids from oxidative stress damage. Thus, a combination therapy of different free radical scavengers having distinctive effect is a good practice for probable synergistic effect. This strategy could be better than single agents. May et al,[13] and Liu et al[14] proved that the use of vitamin E and vitamin C together is superior to either agent alone. Another example is when Lethem and Orrell[15] concluded that the use of a catalase might augment the potential beneficial effects of exogenously administered superoxide dismutase. An important point to bear in mind is the fact that dietary intervention may alter free radicals mediated injury. The intake of tea, containing flavonoid compounds and tomatoes which are rich in non-vitamin A carotenoid lycopene 20 is examples of naturally occurring antioxidants.[16]

Oxidative stress, antioxidant and Parkinson's disease. The idea of involvement of oxidative stress in Parkinson's disease emerged from the fact that metabolism of dopamine monoamine oxidase B (dopamine MAOB) might produce excessive amounts of H_20_2. Further, more the ability of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyrodine (MPTP) a nigral toxin to inhibit complex I of the mitochondrial respiratory chain via its metabolite 1-methyl1-4-phenylpyridinium (MPP+) is another factor. In addition, there is a decrease in the level of complex I of the mitochondrial respiratory chain in Parkinson disease, which is restricted to substantia nigra and does not occur in other brain areas or in other basal ganglia degenerative disorders.[17] Postmortem brain tissue from patients dying with Parkinson's disease has revealed alteration in a range of biochemical indices indicative of oxidative stress, including alteration in iron handling, mitochondrial function and antioxidant defenses in substantia nigra.[18,19] The level of total iron in substantia nigra in Parkinson's disease is elevated by 40% and this is limited to Zona compacta of substantia nigra.[20,21] Melanin with its antioxidant properties, accumulates in subpopulation of neurons of substantia nigra, which are more susceptible to degeneration in Parkinson's decease.[22] Neuromelanin complexes with iron, which may increase the susceptibility of melanized neurons to oxidative damage,[23] thus, the metabolism of dopamine in the presence of high concentration of reactive iron may have a synergistic action in promoting oxidative stress. Another possible evidence of the role of oxidative stress mechanism in substantia nigra in Parkinson's disease is reduction in the level of reduced glutathione (GSH), without a corresponding increase in the level of oxidized glutathione (GSSG). Those changes are restricted to substantia nigra and do not occur in other brain areas, in addition, they do not occur in other related basal ganglia degenerative disorders.[24-26] Recent evidence suggests that GSH depletion may serve as a general trigger for dopaminergic cell death in Parkinson disease, as shown by DNA fragmentation and appearance of DNA loading on agarose gel in alcohol cell line of neuronal origin.[27]

Susceptibility to develop Parkinson disease has been linked to abnormalities of P450 enzyme function. At least 2 P450 enzymes are found in nigral dopaminergic neurons, CyP2E1 is one of them (a potent generator of free radicals), which may contribute to nigral pathology in Parkinson's disease. In addition, inducible nitric oxide synthetase (NOS) leads to massive release of NO, prostaglandin E2 (PGE2), and leukotrienes that produce a toxic effect in the CNS. Early onset Parkinsonism following flu encephalitis during World War I was due to induction of inducible NOS in cells of substantia nigra dopaminergic neurons leading their death.[28] Antioxidants such as melatonin, vitamin C and E probably play an important acute and chronic role in reducing or eliminating the oxidant damage produced by NO.[29]

In 1993, the Parkinson study group trial of 800 patients with early untreated Parkinson disease, concluded that treatment with vitamin E at 2000 IU/ day had no effect in delaying the need for L-dopa therapy in patients who were followed up over a mean period of 14 months.[30] In another study, treatment with 10 mg of Selegiline per day, a selective MAOB inhibitor, prevents MPTP-induced neurotoxicity by blocking its conversion to MPP. Selegiline delayed the need for L-dopa by a median time of approximately 9 months. Selegiline is thought to exert a therapeutic effect by selectively inhibiting the enzyme MAOB and thus increasing brain dopamine levels. In addition, Selegiline protects against MPTP toxicity by scavenging the highly toxic radical MPP.[31,32] Furthermore, a Scandinavian study confirmed that early treatment with Selegiline delays the need for L-dopa. In addition, the same study showed that there was no significant symptomatic deterioration during a 2 month washout period of

Selegiline withdrawal prior to initiation of L-dopa therapy, confirming the concept that Selegiline could be neuroprotective in Parkinson's disease.[33] The above trials indicate the lack of efficacy of vitamin E in Parkinson's disease, and thus, it is important to know the underlying disease mechanisms before choosing the proper antioxidant compounds for evaluation and then considering them for treatment. The evidence mentioned earlier, indicative of mitochondrial disorder, supports the use of antioxidants with good mitochondrial penetrance; for example, coenzyme Q^{10} which could be of benefit in this disease.

Oxidative stress, antioxidant and Alzheimer's disease. Alzheimer's disease is the most common of the neurodegenerative diseases and advancing age being the most important risk factor. Other risk factors include head injury. The occurrence of Alzheimer's disease follows a familial form as autosomal dominant inheritance, but it also occurs as sporadic illness. The involvement of neurofibrillary tangles, and the presence of senile plaques is the main pathology in Alzheimer's disease. However, there is also evidence suggestive that oxidative damage may also play a role in the pathological process. The involvement of the mitochondria defect in the electron-transport enzymes through a deficiency in cytochrome oxidase activity in platelets from Alzheimer's disease patient.[34] This was presented as a reduction in catalytic activity and normal amounts of cytochromes $a^{+}a3$, suggesting that reduced complex-IV activity is a consequence of abnormal catalytic activity rather than decreased enzyme levels.[35,36] Other studies showed an increase level of iron in the pre-frontal cortex and alteration in the level and distribution of ferritin.[37,38] Furthermore, copper, zinc-dependent superoxide dismutase is elevated in the temporal cortex. In addition, there are diminished levels of complex IV and α-Ketoglutarate dehydrogenase (α-KGDH).[39,40] Other evidence showed increased levels of 8-hydroxy-2-deoxyguanosine in the parietal cortex and increase in oxidative damage to mitochondrial DNA.[41] A consequence of mitochondrial dysfunction may be increased free-radicals production. Consistent with this fact, scientists found that mitochondrial DNA showed a threefold increase in concentrations of 8-hydroxy-2-deoxyguanosine (a marker for oxidative damage to DNA).[41] Other studies have shown increased tissue concentration of markers of lipid peroxidation as well as protein carbonyl groups.[42] Another important factor in the pathogenesis of Alzheimer's disease is the accumulation of 34-43 amino acid called amyloid beta peptide (Aβ). This can directly induce oxidative stress under cell culture condition and might, therefore, also initiate oxidative reaction in vivo. Amyloid beta peptide is processed from a much longer precursor molecule and can be found in senile plaques as protein deposits, which are neurotoxic to cortical cell culture.[43,44]

Under normal conditions, free radical scavenging systems such as superoxide dismutase, catalase and glutathione peroxide, protect neurons against oxidative damage. On the other hand, during aging the efficacy of such antioxidant defense systems decreases. Thus, an increasing oxidative burden triggered by Aβ leads ultimately to a loss of function and neuronal cell death. The accumulation of Aβ can directly induce lipid peroxidation.[45-47] Amyloid beta peptide also attracts and activates microglia which in turn generates NO. Nitric oxide reacts with superoxide radicals and produces peroxynitrate (an aggressive free radicals).

A British double-blind placebo-controlled randomized trial of 341 community patients with moderate Alzheimer's disease, showed that treatment with vitamin E at 2001 IU/d or Selegiline 10 mg/d was associated with an average 7.4 month delay to reach one of the following markers of disease progression. This includes death, institutionalization, and loss of ability to perform activities of daily living or progression to severe dementia during a 2-year treatment period. Combination therapy of both vitamin E and Selegiline did not prove additional benefit compared with either drug alone.[48] In vitro, vitamin E was used to prevent Aβ-induced neuronal disintegration. Mitochondrial activity assays (MTT test) and cell lysis assays (trypan blue exclusion, cell counting) showed that Aβ-toxicity was almost completely prevented by vitamin E at a concentration of 100 μg/ml.[49] Consequently, it might be concluded that several Alzheimer's diseases related neurotoxins effects (Aβ, glutamate) can be blocked by the protective activity of vitamin E. As the incidence of Alzheimer's diseases is rapidly increasing in post-menopausal women,[50] it has been implicated that estrogen plays a neuro-protective effect in the CNS.[51] Scientists found out that female sex hormone binds to intracellular receptors which can lead to changes in the genetic program of estrogen responsive neuron cells and increase out growth of dendrites and improve synaptogenesis in nerve cells.[52,53] In addition, Behl et al[54] in 1995 showed that estrogens could act as powerful antioxidants in vitro. Estradiol 17.β and 17.α, protect hippocampal cells in dispersed cell culture and in tissue slide culture against glutamate, Aβ and peroxide-induced oxidative cell death.[55,56] It is worth mentioning that the chemical structure of estrogen is very similar to the chemical structure of vitamin E, since both molecules consist of a long lipophilic tail and mesomeric ring system.

Consequently, it seems that estrogens can be used in clinical trials of Alzheimer's disease. On the other hand, one has to keep in mind that estrogen is a female sex hormone with a feminizing effect when used in male patients. Furthermore, estrogen appears to inhibit dopamine transporter function and such an effect could prevent neurotoxic agents from entering dopamine nerve terminals, thereby decreasing nigrostriatal neurodegeneration.[57]

Oxidative stress, antioxidant and multiple sclerosis. Myelin destruction is one of most classical feature of

central neuronal system pathology. In multiple sclerosis (MS) the mechanism of demyelination may involve the cooperation of the immune system and free radical generating system of oligodendrocytes. Since myelin is enriched with iron, thus, demyelination will expose iron and supply the necessary substrates for iron catalyzed hydroxyl radical attack, which leads to various lipid peroxidation products including isoprostanes.[57,58] Greco et al[59] in 1999 proved that lipid peroxidation occurs in MS brain in vivo. They measured CSF δ-epi-$PGF_2\alpha$ in subjects with definite MS, and found that the level of CSF δ-epi-$PGF_2\alpha$ was 3 times higher than in patients with other non-inflammatory neurological diseases, or in non-neurological patients undergoing subdural anesthesia.[59] It is worth mentioning that increased level of lipid peroxidation in MS patients occurs also at the erythrocyte level.[60] In addition glutathione peroxidase activity was found decreased in MS patients.[61,62] A more recent study showed a significant reduction in plasma and lymphocytes ubiquinone, plasma vitamin E, and erythrocyte glutathione peroxidase. In MS patients, blood antioxidant deficiency was associated with significantly higher levels of plasma polyunsaturated fatty acids. They concluded that the blood of patients with MS shows signs of significant oxidative stress. They also suggested, the possibility of counteracting it by antioxidant administration plus an appropriate diet.[63] But there is controversy regarding levels of glutathione peroxidase. A more recent study conducted on MS patients in the active phase by using antibody-based enzyme immuno-assay on serum samples, showed increased levels of glutathione peroxidase in the active phase compared to those in non active phase of MS and controls.[64] They suggested that these enzymes reflect the activity of the defense of MS. In addition, iron metabolism plays a role in the pathogenesis of MS, Zeman et al[65] demonstrated a lower level of transferrin among relapsing remitting, secondary progressive and primary progressive MS.[65] A recent study conducted in Boston, United States of America found no association between intake of fruits and vegetables and risk of MS. They concluded that use of vitamin C, vitamin E and multivitamin supplements were unrelated to risk of MS.[66]

In conclusion, it is quite clear that there is considerable evidence to support a role for oxidative stress as a pathological cause in a number of neurodegenerative disorders. Free radicals may be part of a cascade, which characterizes neuronal cell death and may provide a biochemical tool by which the pathological process can be inhibited. More studies on the action of ROS and their sources may lead to a better understanding of the basis of neurodegenerative diseases and to the development of appropriate therapeutic agents.

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