Role of Oxidative Stress in the pathogenesis of neurodegenerative disorders

Essay

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To partial fulfillment of
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2013

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Neurodegenerative diseases are characterized by progressive dysfunction and death of cells that frequently affect specific neural systems, implying some form of selective vulnerability. Morphologically, neuronal loss is associated with gliosis and, frequently, with misfolding and aggregation of proteins leading to the relentless accumulation of abnormal extracellular and intracellular filamentous deposits in specific cell types (Fig.1), mainly neurons and glia, representing the core features/hallmarks of many neurodegenerative disorders. (Mattson, 2006).

Fig. 1. Accumulation of filamentous proteins in different types of neurodegenerative diseases.
Investigations have revealed potential common pathogenic mechanisms underlying many diverse neurodegenerative disorders. They include:

1. abnormal protein dynamics with protein misfolding, defective protein degradation, and aggregation. (Fig.1)
2. oxidative stress (OS) and formation of free radicals/reactive oxygen species (ROS).
3. impaired bioenergetics and mitochondrial dysfunctions.
4. fragmentation of neuronal Golgi apparatus (GAs).
5. disruption of cellular/axonal transport.
6. actions and mutations of molecular chaperones.
7. dysfunction of neurotrophins.

All these mechanisms are interrelated in complex vicious circles finally leading to cell death, the basic molecular cascades of which are still to be elucidated. (Skovronsky, 2006).

➢ Protein aggregation

A causative link between the formation of protein aggregates and neurodegeneration has been established, which may occur as a result of the toxic action of substances produced during early phases, and soluble oligomers and protofibrillar derivatives of misfolded proteins may play a pathogenic role. The “toxic oligomer” hypothesis is supported by the finding that a single monoclonal antibody can recognize a common conformational epitope that is displayed by several disease-associated proteins, including β-amyloid (Aβ), α-synuclein, and Polyglutamine containing peptides. In general, the identity of the underlying protein determines which neurons are affected and, hence, the clinical manifestation of each disease.

(Fornai F, 2005), (Kayed R, 2003)
Abnormal interaction between normal highly soluble brain proteins alters their conformation, and/or misfolding gradually converts them into insoluble polymeres with characteristic cross-pleated β-sheet structures, and promotes the assembly of these pathological conformers into filaments that progressively accumulate in a disease- and protein-specific manner in the cytosol or nuclei of affected brain cells (neurons and/or glia) or in the extracellular space (Fig. 2) (Ross CA, 2004)

Fig. 2. Model of protein misfolding and fibrillation leading to the deposition of aggregated proteins in cells and extracellular space.

Progressive intracellular accumulation of disease proteins can result from one or more of the following pathological processes: (a) abnormal synthesis and folding of proteins, (b) abnormal interaction of diverse proteins with other proteins, (c) overproduction of protein constituents, (d) impaired degradation and turnover of proteins, (e) altered post-translational modifications of newly synthesized proteins, (f) abnormal proteolytic cleavage, (g) improper expression or altered gene splicing, (h) insufficient molecular chaperone activity, and (i) impaired intracellular transport of proteins, especially those targeted for axonal transport.
The first mechanisms have been the subject of intense investigations, but less attention has been focused on the role of the axonal transport in mechanisms underlying neurodegenerative disorders. (Roy S, 2005).

Despite the fact that many of the amyloidogenic proteins associated with neurodegenerative disorders are expressed systematically, the resulting proteinopathy is generally restricted to the central nervous system (CNS). In vivo, these changes develop insidiously over the lifetime of an individual, even though, in general, they usually do not manifest clinically until middle or late life. The causes of this prolonged process are poorly understood, but it almost certainly reflects the requirement of the progressive damage of specific vulnerable brain regions or neuronal networks before clinical manifestation occurs. Further factors may be unfavorable kinetics of protein misfolding, oligomerization, and fibrillation, that are, in turn, linked to many age-related metabolic impairments. A key unanswered question is whether these aggregates contribute to the onset and progression of neurodegeneration, are mere bystanders resulting from an alternative pathway, or even play a neuroprotective role. (Richard IH, 2002).

Alterations in cellular homeostasis that affect protein folding in the ER trigger a signaling pathway known as the unfolded protein response (UPR). The initially cytoprotective UPR will trigger an apoptotic cascade if the cellular insult is not corrected. ER stress is caused by disturbances in the structure and function of the ER with the accumulation of misfolded proteins and alterations in the calcium homeostasis. The ER response is characterized by changes in specific proteins, causing translational attenuation, induction of ER chaperones, and degradation of misfolded proteins. In the case of prolonged or aggravated ER stress, cellular signals leading to cell death are activated. ER stress has been suggested to be involved in some human neuronal diseases, such as Parkinson’s disease (PD), Alzheimer’s disease (AD) and prion diseases, as well as other disorders. (Hoozemans JJ, 2005).
The cellular response to these aggregates includes (1) the recruitment of chaperones or proteins involved in the folding of nascent translational products and in the resolubilization of aggregated polypeptides, and (2) the ubiquitination of aggregates, suggesting cellular attempts to degrade deposits of these mutant proteins via the ubiquitin–proteasomal pathway (UPP). *(Myung J, 2001).*

![Diagram of the ubiquitination process](image)

**Fig. 3.** Steps and components in the ubiquitination of substrate proteins in the ubiquitin–proteasomal pathway *(McNaught KS, 2001).*

The presence of ubiquitinated proteins within neuronal inclusions is one of the hallmarks of neurodegeneration. These inclusions contain various components of the Ubiquitin–proteasomal pathway (UPP) which operates as an intracellular protein-clearing system. The Ubiquitin–proteasomal system (UPS) consists of a multienzymatic pathway placed mainly in the ER which becomes activated during
OS and works to process misfolded protein-mediating reactions that link abnormal proteins with multiple Ubiquitin (Ub) molecules as a signal for degradation domains and may promote degradation of ubiquitinated proteins. \textit{(Hegde AN, 2004)}

The accumulation of misfolded proteins within the Endoplasmic reticulum (ER) results in a highly specific Ubiquitin–proteasomal pathway (UPP) which, when activated, leads to either a reduction in Endoplasmic reticulum (ER) stress or apoptotic cell death. \textit{(Mori K, 2000)}.

The presence of Ubiquitin (Ub) and other specific proteins of the UPS in cellular inclusions reinforced the notion that the Ubiquitin–proteasomal pathway (UPP) plays a pivotal role in their formation in Parkinson’s disease (PD), multiple system atrophy (MSA) and other synucleinopathies. Lewy bodies (LBs), the morphological hallmark of Parkinson’s disease (PD) and dementia with Lewy bodies (DLBs), have a distinct central parkin and Ub-positive domain, with α-synuclein in the periphery. \textit{(McNaught KS, 2003), (Wenning GK, 2005)}

The importance of the Ubiquitin–proteasomal pathway (UPP) has been observed also in other neurodegenerative disorders, e.g. in HD characterized by striatal degeneration with nuclear inclusions of the truncated ubiquitinated protein Huntingtin, chaperones, and proteasomes, in amyotrophic lateral sclerosis (ALS) showing skein-like inclusions in motor neurons rich in Ub proteasome and some chaperone proteins, in spinocerebellar ataxies (SCAs), with neuronal intranuclear inclusions containing ataxin-1 and several chaperones; in Alzheimer’s Disease (AD), Parkinson’s disease (PD), and neuronal intranuclear inclusion disease. \textit{(Johnson MD, 2005)}.

When the capacity of the proteasome system to degrade misfolded proteins is overwhelmed, aggregation occurs and proteins are
moved to Ub-rich structure termed the “aggresome” as a general response to discrepancies in protein turnover. *(Dinakopoulos AC, 2005)*

It forms part of the cellular response to aggregated proteins and appears as inclusions in a number of proteinopathies. Aggresomes have been reported for Superoxide dismutase (SOD), parkin, α-synuclein, and prion proteins. Disease-related prion proteins forming aggresomes in neuronal cells lead to caspase activation and apoptosis. *(Kristiansen M, 2005)*

It is not clear whether aggresome formation is causative or protective, although data suggested that they serve a cytoprotective function, facilitating the degradation of toxic proteins. Misregulation of degradation of misfolded proteins leads to their accumulation with inhibition of axonal transport, thus facilitating the accumulation of ubiquitinated proteins in the cell body and eventual cell dysfunction. *(Walsh DM, 2005)*

- **Oxidative stress (OS) and formation of free radicals**

Oxidative stress (OS) occurs when the production of free radicals or their products are in excess of antioxidant defense mechanisms. OS, resulting from increased formation of hydrogen peroxide and oxygen-derived free radicals, can damage biological molecules and initiate a cascade of events, including dysfunction of mitochondrial respiration, excitotoxicity, and a fatal rise in cytosolic calcium, and, thus, is a major factor of the cytopathology of many neurodegenerative disorders. The generation of Reactive oxygen species (ROS) during early-stage protein aggregation may be a common, fundamental molecular mechanism underlying the pathogenesis of oxidative damage, neurodegeneration and cell death in different neurodegenerative diseases. However, it remains unclear how mitochondrial oxidative stress may induce neuronal
death. In a variety of tissues, cumulative oxidative stress, disrupted mitochondrial respiration, and mitochondrial damage are associated with, and may indeed promote cell death and degeneration. 

*(Van Houten B, 2006)*

Among the various free radicals generated in the living organism, hydroxyl radical and peroxynitrite are the most potent and can damage cells via non-selective oxidation of proteins, lipids, fatty acids, and nucleic acid. They are formed via the Haber–Weiss and Fenton reaction between H2O2 and reduced transition metals (usually iron II or copper). *(Emerit J, 2004)*

Proteins are initial targets of Reactive oxygen species (ROS), and protein radicals generated by ROS can oxidize Glutathione (GSH), suggesting that radicals are important for oxidative stress. In Alzheimer’s disease (AD), aberrant metal homeostasis may contribute to the formation of ROS and toxic Aβ oligomers, thus, facilitating the formation of amyloid plaques. Alternatively, not superoxide itself but the protoneated form, the hydroxyl radical, can initiate lipid peroxidation reactions. Another mechanism of lipid peroxidation has been attributed to increased formation of peroxynitrite from nitric oxide (NO) and superoxide. Reduction of the resulting oxidized transition metal ions (Fe(II) or Cu(II)) by vitamin C or other reductants regenerates the “active” transition metal and leads to the process of redox cycling and the catalytic production of free radicals. Cellular reductants are often diminished in neurodegenerative disorders. *(Cash AD, 2004)*

Iron is a powerful promoter of free radical damage, able to catalyze generation of highly reactive hydroxyl, alkoxy, and peroxy radicals from H2O2 and lipid peroxides, respectively. Although most iron in the brain is stored in ferritin, “catalytic” iron is readily mobilized from injured brain tissue. As a result of a loss of iron homeostasis, the brain becomes vulnerable to iron-induced oxidative stress (OS). *(Honda K, 2004)*
There is increasing evidence that iron misregulation is involved in the mechanisms that underlie many neurodegenerative disorders. Conditions such as neuroferritinopathy and Friedreich ataxia (FRDA) are associated with mutations in genes that encode proteins involved in iron metabolism, and as the brain ages, iron accumulates in regions that are affected by Alzheimer’s disease (AD) and Parkinson’s disease (PD). (Zecca L, 2004)

High concentrations of reactive iron can increase OS-induced neuronal vulnerability, and iron accumulation might increase the toxicity of environmental or endogenous substances. Examination of distinct antibodies against neurofibrillary tangles (NFTs) that recognize unique epitopes of tau in Alzheimer’s disease (AD) after treatment with 4-hydroxy-2-nonenal (HNE) recognized tau only in the phosphorylated state. These findings not only support the idea that OS is involved in NFT formation, but also show that HNE modifications of tau promote and contribute to the generation of the major conformational properties defining NFTs. (Liu Q, 2005).

Increased levels of oxidative damage to DNA, lipids, and proteins have been detected in postmortem tissues from patients with Alzheimer’s disease (AD) and Parkinson’s disease (PD), amyotrophic lateral sclerosis (ALS), Progressive supranuclear palsy (PSP), and related disorders, and at least some of these changes may occur early in disease progression. Recent studies showed that lipid peroxidation is an early event in the brain in amnestic mild cognitive impairment (MCI) suggesting that oxidative damage occurs early in the pathogenesis of AD. (Markesbery WR, 2005)

Increased OS has also been described in amyotrophic lateral sclerosis (ALS), where disruption of Zn metabolism in motoneurons is important in both sporadic and familial ALS. Enhanced basal oxidodradical products, lipid peroxide, perturbed calcium
homeostasis, and increased nitrotyrosine in lower motoneurons of both transgenic mice and human ALS are present.  
(Kabashi E, 2006)

Table 1. Comparison of the cytotoxic action of iron-induced oxidative stress (OS) with findings in substantia nigra (SN) of Parkinson disease (PD) and in Alzheimer’s disease (AD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Iron-induced OS</th>
<th>PD</th>
<th>AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue iron</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Fe++/Fe+++ ratio</td>
<td>?</td>
<td>↑</td>
<td>?</td>
</tr>
<tr>
<td><strong>Antioxidants</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂O₂-Scavenging glutathione</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Superoxide dismutase (SOD)</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Glutathione peroxidase</td>
<td>↓</td>
<td>↓</td>
<td>/↓</td>
</tr>
<tr>
<td>Catalase</td>
<td>?</td>
<td>–↓</td>
<td>↓</td>
</tr>
<tr>
<td>8-HOG</td>
<td>?</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>H₂O₂ + OH</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td><strong>Lipid peroxidation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malondialdehyde</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Lipid (hydro) peroxidase</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>04-Hydroxynonenal protein</td>
<td>?</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td><strong>Protein peroxidation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbonyls</td>
<td>↑</td>
<td>?</td>
<td>↑</td>
</tr>
<tr>
<td>Nitrotyrosine (peroxynitrite)</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Complex I and II activities</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Ca++ homeostasis/uptake</td>
<td>↑</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>l-Ferritin</td>
<td>↑</td>
<td>↓/↑/–</td>
<td>↑</td>
</tr>
<tr>
<td>Transferrin-binding sites</td>
<td>?</td>
<td>↓</td>
<td>↓</td>
</tr>
</tbody>
</table>

– Unchanged; ↑ Increased; ↑↑ Strongly increased; ↓ Decreased; ? Unknown.
Impaired bioenergetics and mitochondrial dysfunction

Mitochondria provide energy from aerobic metabolism; oxidative phosphorylation is the principal source of high-energy compounds. Impaired bioenergetics and dysfunction of mitochondrial energy metabolism leads to reduced ATP production, impaired calcium puffering, and generation of reactive oxygen species (ROS). Mitochondria are both targets important sources of ROS. Generation of reactive oxidants, including ROS, is increased in damaged mitochondria and in cells with compromised mitochondrial function. Conversely, acute exposure to high levels of oxidants can induce the mitochondrial permeability transition (MPT), uncouple oxidative phosphorylation with catastrophic effects on mitochondrial energetics, and contribute to cytotoxicity via necrosis and/or apoptosis through release of cytochrome c, etc. Increasing evidence implicates both proteasomal dysfunction and impaired bioenergetics (mitochondrial dysfunction) in aging and neurodegenerative disease (Kwong JQ, 2006).

The Ubiquitin–proteasomal pathway (UPP) is known to require ATP at several steps. Therefore, impaired mitochondrial function may impair its activity. More importantly, increased production of reactive oxygen species (ROS) leads to damaged misfolded proteins requiring degradation by the UPP. Parkin prevents mitochondrial swelling and cytochrome c release. (Muftuoglu M, 2004)

On the other hand, proteasomal inhibition reduced mitochondrial complex I and II activities, increased mitochondrial ROS production, and increased the presence of damaged mitochondria in autophagosomes. (Sullivan PG, 2004)
There are several pathways by which both mitochondrial dysfunction and protein aggregation may interact (Fig. 4). Both aggregated SOD and Aβ in the mitochondrial matrix could contribute to cell death by triggering apoptosis. *(Vijayvergiya C, 2005)*

In Alzheimer’s (AD), there is a large body of evidence implicating impaired energy metabolism and oxidative damage. Reduced Cyclooxygenase (COX) activity will decrease more of the mtDNA-encoded subunit II than of IV suggested mitochondrial dysfunction. Several studies using cybrid analysis reported a COX decrease in AD brain tissue that could be transferred to mitochondrial-deficient cell lines, indicating that this defect may be caused by inherited mtDNA mutations, which, however, could not be replicated by others. *(Onyango IG, 2005)*

Alzheimer’s (AD) brains showed an average 50% reduction in mtRNA content that, together with other changes, is likely to reduce
oxidative phosphorylation. However, spectroscopic enzyme analysis of respiratory complexes in brain homogenates did not support the primary contribution of mitochondrial respiratory chain dysfunction in the pathogenesis of AD. Mitochondrial dysfunction in AD may also be caused by impaired axonal transport that is accompanied by proximal collection of mitochondria which could contribute to loss of distal synapses (Stokin GB, 2005).

In Parkinson’s disease (PD), the association between neurodegeneration and mitochondrial dysfunction or oxidative damage, or both, stems from studies in the mitochondrial permeability transition pore (MPTP) model showing inhibition of complex I, leading to reduction in mitochondrial ATP production and increased generation of reactive oxygen species (ROS). Both contribute to neuronal cell death via decreased protein pumping and reduced voltage differential across the inner mitochondrial membrane that would elicit opening of the mitochondrial permeability transition pore (PTP) and subsequent initiation of apoptosis. (Fiskum G, 2003)

- Fragmentation of neuronal Golgi apparatus

Mechanisms involved in Golgi fragmentation include: (a) the dysregulation by mutant Superoxide dismutase (SOD1) of the microtubule-destabilizing protein Stathmin, (b) the disruption by mutant Superoxide dismutase (SOD1) of the neuronal cytoplasmic dynein, (c) the coprecipitation of mutant SOD1 with Heat shock protein (Hsp25) and (Hsp27), (d) the reduction of detyrosinated microtubules by aggregated tau which resulted in non-apoptotic cell death, and (e) the disruption by mutant growth hormone of the trafficking from the rough endoplasmic reticulum (ER) to the Golgi apparatus (GA). These data indicate that neuronal Golgi fragmentation is an early and probably irreversible lesion in neurodegeneration, caused by a variety of mechanisms. Golgi
fragmentation is not secondary to apoptosis but it may “trigger” apoptosis. (GonatasNK, 2006)

➢ Disruption of cellular/axonal transport

The system of axonal transport is associated with three basic components: the cargo, the moving motor proteins, the rails on which the cargo moves, and various adaptative and regulatory proteins. Defects in any of these departments can lead to dramatic changes, and potential neurodegeneration. Three recent developments have highlighted the significance of disrupted cellular/axonal transport in human neurodegenerative diseases: (a) the discovery of human motor protein mutations in these disorders, (b) axonal transport defects in animal and in vitro cellular models harboring human mutations, and (c) newly discovered roles for pathogenic proteins like Amyloid precursor protein (APP), tau, presenilin, and synuclein, in the regulation of axonal transport, (Roy S, 2005)

➢ Action and mutations of molecular chaperones

Molecular chaperones have essential roles in many cellular processes, including protein folding, targeting, transport, degradation, and signal transduction. Conditions of stress are characterized by a robust increase in the synthesis of heat shock proteins (HSPs) that are crucial for recovery from stress-induced protein damage. Almost all HSPs, classified into six main families on the basis of their molecular mass, function as molecular chaperones, and the number of diseases that are known to be caused by their mutations are increasing. Under certain pathological conditions, the capacity of the protein quality control machinery (activation of molecular chaperones, Ubiquitin–proteasomal pathway (UPP), and lysosome-mediated autophagy) is exceeded
and misfolded proteins accumulate to dangerous levels. Accumulation of the aggregation prone proteins activates signal transduction pathways that control cell death, including JNK (C-jun N-terminal kinase) pathway that controls viability of a cell in various models of Parkinson’s disease (PD) and Huntington disease (HD). (Meriin AB, 2005)

➢ Dysfunction of neurotrophines

There is growing evidence that reduced neurotrophic support is a significant factor in the pathogenesis of neurodegenerative diseases. Neurotrophines regulate development and the maintenance of the vertebrate nervous system. They affect neuronal survival; influence synaptic function and plasticity, and are central to many aspects of the nervous system function. The neurotrophins are able to bind to different receptors, and bind to a common receptor p75wtr, and each of the neurotrophins also binds to one of the family of Trk receptors. By dimerization of the Trk receptors, and subsequent transphosphorylation of the intracellular kinase domain, signaling pathways are activated. Since (neurotrophic factors) NTFs in neurons are subject to retrograde and, in at least some cases, to anterograde transport from and to targeting neurons, their effects may be related to synthesis in local or remote sites or to changes in axonal transport. Observations in Alzheimer’s disease (AD) brain indicate increase in neurotrophic factor (NTF) and decrease in brain-derived neurotrophic factor (BDNF) in surviving neurons of hippocampus and certain neocortical regions, and decrease of TrkA in cortex and nucleus basalis. (Salehi A, 2004)

Decreased retrograde transport of nerve growth factor (NGF) in human brain and mouse Advances in Understanding of Neurodegeneration 35 models leads to loss of neuronal markers and shrinkage of neurons in the cholinergic basal forebrain, rather than due to decreased synthesis. Thus, nerve growth factor (NGF) and receptor TrkA may have a prominent role in both the etiology and
treatment of Alzheimer’s disease (AD). In Parkinson’s disease (PD) brain, decreases in the neuronal content of Neurofibrillary tangles (NFTs) and on their receptors have been observed, and starting clinical results have been found using intraparenchymal injection of related NGFs, glial-derived neurotrophic factor (GDNF), for the treatment of PD. In Huntington disease (HD), the mutant protein Huntington leads to a downregulation of BDNF in the basal ganglia, leading to neuronal loss, opening up the possibility of BDNF therapy. In amyotrophic lateral sclerosis (ALS), nerve growth factor (NGF) concentrations and BDNF were strongly upregulated in early stages of the disease, whereas the levels of other NGFs gradually increased during the course of the disorder. *(Mutoh T, 2000)*

➢ “Neuroinflammatory” processes

Chronic inflammatory reactions in the CNS have been implicated as contributory factors in the pathogenesis of neurodegenerative disorders. Components related to AD neuroinflammation include microglia and astrocytes, the classic and alternate pathways of the complement system, the pentraxins, acute-phase proteins, neuronal-type nicotinic acetylcholine receptors (AChRs), peroxisomal proliferation-activated receptors (PPARs), as well as “pro-inflammatory” cytokines and chemokine. In animal models and human brains, both the microglia and astrocytes have been shown to generate Aβ, one of the main components of senile plaques, which, itself may act as a pro-inflammatory agent inducing the activation of glia and many of the inflammatory components. *(McGeer PL, 2004)*.

All these substances may lead to increased formation of ROS and upregulation of genes that produce toxic agents such as reactive nitrogen species (RNS). Footprints of oxygen-free radicals and peroxynitrite attack have been detected in postmortem AD brain, which, at least in part, are produced by activated microglia, and may be an important progression factor in AD. *(Wong A, 2001)*
NEURONAL DEATH – THE FINAL PATHWAY

Based on distinct morphologic criteria and biochemical features, three major mechanisms of neuronal demise are: apoptosis, a specific form of gene-directed programmed cell death (PCD); (oncotic) necrosis, a passive killing of the cell; and autophagic degeneration. Morphologically, apoptotic cell death is characterized by chromatin condensation (pyknosis), nuclear fragmentation, cell shrinkage, and plasma membrane blabbing. Eventually, the cell breaks into small membrane-surrounded fragments (apoptotic bodies), which are cleared by phagocytosis in vivo without inciting an inflammatory response, phagocytotic activity being balanced by positive and negative signals. (Almeida CJ, 2005).

Oxidative stress, free radicals and Redox imbalance

Introduction to ROS (reactive oxygen species)

In the structure of atoms and molecules, electrons usually associate in pairs, each moving within a defined region of space around the nucleus. This space is referred to as the atomic or molecular orbital. One electron in each pair has a spin quantum number of $+\frac{1}{2}$, and the other, $\frac{1}{2}$. The process of removing electrons is called oxidation, and the substance receiving electrons becomes reduced. The reactions involved in electron transfer are called redox (reduction oxidation) reactions. A free radical is any species capable of independent existence (hence the term free) that contains one or more unpaired electrons. (Halliwell, 2007)
Free radicals are sometimes reactive, although the chemical reactivity of radicals varies over a wide spectrum. Consideration of this broad definition shows that there are many free radicals in chemistry and biology. The simplest is the atomic hydrogen with one proton and a single electron, which must therefore be unpaired. Hence, removal of a hydrogen atom from a biological molecule leaves behind an unpaired electron on the atom to which hydrogen was originally attached. The diatomic oxygen molecule O2 qualifies as a radical in as much as it possesses two unpaired electrons, each located in a different orbital but both having the same spin quantum number: that is the reason why O2 itself has a relatively low reactivity in contrast to other radicals, which can be highly reactive. Radicals can be formed by the loss of a single electron from a nonradical, or by the gain of a single electron by a nonradical. Radicals can react with other molecules in a number of ways. Thus, if two radicals meet, they can combine their unpaired electrons and join to form a covalent bond. A radical might donate its unpaired electron to another molecule. Or, it might catch an electron from another molecule to pair. There are reactive oxygen molecules such as H2O2 that do not fit the definition of free radicals, so all the reactive species, radicals or not, are called reactive oxygen species (ROS). (Sohal RS, 1997)

More than 90% of the oxygen that enters human cells is used for the production of energy. Mitochondria produce more than 80% of the adenosine triphosphate (ATP) needed by the mammalian cells. During this process, four electrons are added to each O2 molecule, resulting in the formation of two molecules of water. An estimated 1 5% of the O2 taken into cells, however, forms partially reduced O2 species, the reactive oxygen species (ROS). Some of them contain an unpaired electron and are therefore referred to as free radicals: The Superoxide Anion Radical, Hydrogen Peroxide, Hydroxyl Radical, Singlet Oxygen, Peroxyl Radical, and Nitric Oxide and Peroxynitrite Anion. (Halliwell, 2006)
Physiological Roles of ROS

In healthy aerobes, there is a balance between the production of reactive oxygen species (ROS) and antioxidant defenses. In health, the cell has become well equipped to cope with the normal production of ROS. Indeed, continuous low concentrations of ROS induce expression of antioxidant enzymes and related defense mechanisms. A large body of evidence has been accumulated that living organisms have not only adapted to a coexistence with free radicals but have developed various mechanisms for the advantageous use of free radicals in various physiological functions. (Kirkwood TB, 2005)

Infectious diseases were a powerful driver of natural selection in early human civilizations. Indeed, reactive oxygen species (ROS) participate directly in defense against infection and also are important coordinators of the inflammation. Resident macrophages of the brain in the normal situation, fight against infection by means of ROS. ROS are well recognized for playing both deleterious and beneficial roles, which in most cases depend on their concentration. At high reactive oxygen species (ROS) concentrations there are harmful effects, and in a low moderate concentration ROS are involved in physiological roles in cellular response to noxious stimuli. It was suggested that the main effects of ROS on cells are through their actions on signaling pathways rather than causing nonspecific damage to macromolecules. (Maher P, 2000)

Normally, reactive oxygen species (ROS) participate in many signal transduction pathways that are essential for the many functions of the brain such as memory and learning. With aging, when these pathways deteriorate, there is an accumulation of high concentrations of ROS, which cause age-associated neurodegenerative disorders. The CNS thus evolved specific signaling pathways. (Sugawara T, 2004)
Free radicals and neurodegenerative disorders

The various neurodegenerative diseases (diseases in which neurons degenerate and die) have a variety of different symptoms, affect different parts of the brain, and have different causes. They have in common impaired mitochondrial function, increased oxidative damage, defects in the ubiquitin-proteosome system, presence of abnormal aggregated proteins, changes in iron metabolism, and some involvement of excitotoxicity and of inflammation. It seems likely that all these events are involved in a vicious cycle and that any of them could initiate neuronal cell death, rapidly recruiting the others to its destructive purpose. Oxidized proteins are usually removed by the proteosome. Inhibition of the proteosome allows abnormal proteins to accumulate and produces OS, but how this is done is still unclear. Finally, ROS-producing agents could initiate neurodegeneration, because reactive oxygen species (ROS) damage mitochondria, cause rise in Ca2+ , and may inhibit proteosome function. The iron content of most brain areas increases with age, and iron and other metals promote the aggregation of several proteins. How do neurons die in these various diseases? Sometimes they die by necrosis, as in excitotoxicity, and sometimes, probably, by apoptosis. However, as more studies are done, the role of intermediate types of cell death, with features of both necrosis and apoptosis, is becoming more prominent. (Halliwell, 2007)

Free Radicals and oxidative stress

The central nervous system is particularly vulnerable to oxidative stress (OS) as it utilizes large amounts of dioxygen but harbors relatively poor concentrations of antioxidants and related enzymes. Moreover, it contains a very high amount of polyunsaturated lipids, the most vulnerable biomacromolecule to oxidation. (Sayre LM, 2005)

Free radicals in Parkinson’s disease (PD) comprise mainly oxygen radicals (reactive oxygen species (ROS) or oxides of nitrogen
(reactive nitrogen species (RNS). Moreover, transition metals like iron and copper contribute to the generation of oxidative stress (OS) as they have the ability to change oxidation numbers by one, allowing them to donate or accept single electrons. This ability makes them powerful catalysts of free-radical reactions. ROS are generated as a result of normal metabolism. However, the deleterious condition termed oxidative stress (OS) occurs when ROS or RNS due to an excessive production overwhelms the protective defense mechanisms of a cell resulting in functional disruption and ultimately in cell death. The most important oxygen species in humans are H2O2, superoxide radical (O•−2) and hydroxyl radical (HO•). Reactive nitrogen species include the radical nitric oxide (NO) and peroxynitrite (ONOO•−). Although it is the neuronal loss of the substantia nigra (SN) that leads to many of the clinical symptoms of PD it is obvious that 30–40% of the changes in parameters of OS found in homogenates of nigral tissue cannot be restricted to these cells that account for only 1–2% of the whole nigral cell population. (Jenner P, 2003)

Changes also occur in other cell types, predominantly in glial cells, implying a concept of general metabolic failure in the SN of Parkinson’s disease (PD) patients. The reason why it is the SN that is the target of the high degree of oxidative stress (OS) in PD may lay in its high energy metabolism and the high content of dopamine in its neuronal cells although dopaminergic cells are normally endowed with quite a number of protective mechanisms. Moreover, neuropathological studies have shown that primarily long fibers with scarce myelinization needing more energy degenerate. (Braak H, 2003)

Lack of antioxidant capacities of long fibers makes them especially vulnerable to oxidative stress (OS). It is not yet clear, whether OS is a primary cause of neurodegeneration or a consequence of other pathogenetic factors. Studies in patients with incidental Lewy body disease (ILBD), which is supposed to represent presymptomatic Parkinson’s disease (PD), implicated that with the exception of
reduced GSH levels there is no conclusive evidence of other markers of OS at an early stage of neurodegeneration. (Jenner P, 2003)

Studies demonstrating oxidative dimer formation as the critical rate-limiting step for fibrillogenesis of α-synuclein provide an explanation that overproduction of reactive oxygen species (ROS) and/or impairment of cellular antioxidative mechanisms are primary events both in the initiation and in the progression of Parkinson’s disease (PD). It is therefore highly possible that oxidative stress (OS) may be both an initiator of neurodegeneration and a component of the pathogenetic process accelerating neuronal loss. (Krishnan S, 2003)

Factors contributing to the generation of oxidative stress (OS)

Metabolism of dopamine produces reactive oxygen species (ROS) and might therefore account at least in part for the selective vulnerability of the SN pars compacta (SNC) in Parkinson’s disease (PD). Already during the process of dopamine synthesis, cytotoxic products like reactive dopamine quinone products may be formed. (Choi HJ, 2003)

After synthesis, dopamine is directly taken up into synaptic vesicles. Here, dopamine is protected from oxidation by a very low pH that stabilizes the catechol structure and confers a milieu where protons are very strongly bound to oxygen atoms. The other mechanism protecting neurons from autooxidation of dopamine involves dopamine metabolism by monoamine oxidase (MAO). (Graumann R, 2002)

Autooxidation of dopamine leads to the production of dopaquinone and O•–2. This reaction is catalyzed by metals, oxygen or enzymes like tyrosinase or xanthine oxidase. O•–2 is either metabolized into H2O2 or it reacts with nitric oxide, generating the strongly reactive peroxynitrite (ONOO•–). In the second step, dopaquinone is
Thesis

cyclized to aminochrome. This may then be polymerized leading

to the formation of neuromelanin (NM) or may be conjugated with

gSH and reduced by one- or two-electron transfer catalyzed by

quinone reductases. *(Graumann R, 2002)*

The conjugated aminochrome leukoaminochrome-GSH is very

stable in contrast to unconjugated aminochrome-reduced forms.

Also, two-electron oxidation of aminochrome, which is catalyzed

by DT-diaphorase is supposed to be neuroprotective, as the

autooxidation rate of the produced o-hydroxychinone

(leukoaminochrome) is very low. Therefore depletion of glutathione

(GSH) or changes in the function of DT-diaphorase constitutes

reduced cellular defense mechanisms leading to increased formation

of reactive oxygen species (ROS). It is generally assumed, that in

Parkinson’s disease (PD) autooxidation of dopamine may therefore

be the consequence of an overproduction of dopamine, an inhibition

or low expression of synaptic vesicle catecholamine transporters or

inhibition or low expression of monoamine oxidases (MAO).

*(Graumann R, 2002)*

Dopamine linked to genetic defects and mitochondrial dysfunction

Besides the direct contribution to the generation of reactive oxygen

species (ROS), dopamine has been shown to form covalent

oxidative adducts with α-synuclein leading to its retention in a

protofibrillar form, which is capable of permeabilizing synthetic

vesicles enhancing dopamine leakage. In cultured human

dopaminergic neurons, mutant α-synuclein has been shown to even

trigger an elevation of cytosolic dopamine, enhancing dopamine-

dependent toxicity. *(Volles MJ, 2001)*

The large amount of Neuromelanin (NM) in the Substantia nigra

compacta (SNc) is unique to humans. It is generally regarded to be

the result of the oxidation of dopamine and noradrenaline. This,

however, has been questioned due to the fact that not all

dopaminergic neurons of the SN contain NM and long-term l-

DOPA treatment does not seem to enhance NM concentration in
surviving neurons. As in Parkinson’s disease (PD) primarily NM-containing neurons degenerate, with the largest pigmented neurons being preferentially lost, a cytotoxic effect of NM contributing to oxidative stress (OS) has been proposed. Conversely, the less pigmented ventral tier of the substantia nigra (SN) is the first to degenerate in PD. (Gibb WR, 1992)

Neuromelanin (NM) is an excellent chelator of metal ions, especially iron. Iron bound to NM accounts for 10–20% of the total iron in the substantia nigra (SN) in normal subjects aged 70–90 years. It has been supposed that the amount of iron determines the role of NM: In the situation of normal iron levels, this redox-active metal is sequestered. In the presence of excess iron, however, NM promotes the formation of ROS and fosters the release of iron into the cytoplasm. Additionally, NM can bind a variety of potentially toxic substances like MPP+ (1-methyl-4-phenyl-2 3-dihydropyridinium ion), the neurotoxic metabolite of MPTP (1-methyl-4-phenyl-1 2 3 6-tetrahydropyridine) or pesticides suggesting a contribution to neurotoxin-mediated neurodegeneration. (Faucheux BA, 2003)

It can be hypothesized that not neuromelanin (NM) itself but rather its interaction with iron, catechols and neurotoxic metabolites may account for its contribution to oxidative stress (OS). A possible radical cross-linking between the polycatecholic framework of NM and the isoprenoid chain of dolichol, a lipid component of intact NM granules has been described. (Fedorow H, 2005)

The content of iron, which is essential for many biological processes including its role as a cofactor for the synthesis of dopamine, is, under physiologic conditions, higher in the basal ganglia and SN than in most other regions of the brain. (Rausch WD, 1988)

In Parkinson’s disease (PD), iron content of the Substantia nigra compacta (SNc) is additionally about 35% elevated. However, it is
not the increase in total iron that implicates OS as long as a concomitant increase in proteins keeps it stored in a redox inert form. In PD, an increase of the Fe (III): Fe (II) ratio from 2:1 to almost 1:2 has been found. *(Dexter DT, 1993)*

An important site of iron release is microglia. Here, superoxide and a number of oxidized catechols may lead to the release of iron from ferritin thereby contributing to free-radical-induced cell damage. Increased levels of iron and Fe (II) enhance the conversion of H2O2 to •OH via the Fenton reaction and favor a greater turnover in the Haber–Weiss cycle, which leads to an amplification of oxidative stress (OS). *(Riederer P, 1993)*

Oxidative stress (OS) may increase the levels of free iron. The mechanisms include the release of iron from ferritin by O•−2, from heme proteins like hemoglobin and cytochrome c by peroxides and from iron–sulfur proteins by ONOO•−. Besides the contribution to the formation of highly reactive oxygen species (ROS), iron has been shown to interact with α-synuclein enhancing the conversion of unfolded or α-helical conformation of α-synuclein to β-pleated sheet conformation, the primary form in Lewy bodies (LBs). Colocalization of proteins involved in brain iron metabolism and LBs is a further implication for the involvement of iron in the neurodegenerative process in Parkinson’s disease (PD). It is not entirely clear yet, at what time in the pathophysiological cascade of PD iron accumulation occurs. Iron accumulation induced by toxin-mediated neurodegeneration in animal models suggests it to be a secondary phenomenon. However, high iron diet, fed to weanling mice, has been shown to lead to marked reduction of SN glutathione levels, a finding known to occur very early in PD. *(Goetz ME, 2004), (Hochstrasser H, 2005)*

Data from recent transcranial ultrasound studies also imply iron accumulation to occur very early in the disease process constituting rather a primary cause of the disease in idiopathic Parkinson’s
disease (PD). In contrast, in patients with monogenetic PD the ultrasound finding indicates less iron accumulation. Therefore, it may be possible that iron contributes to different degrees at different stages to the pathophysiological cascade of PD. In idiopathic PD, a more causative role earlier in the disease process may be postulated, while in monogenetically caused PD other factors may have greater influences on disease development and progression. Interestingly in single cases of apparently “idiopathic” PD, an association of sequence variations in some genes encoding for iron-metabolizing proteins within the brain and PD has been established while such an association could be ruled out in others. (Borie C, 2002), (Felletschin B, 2003)

Copper is on the one hand, essential for the function of key metabolic enzymes but may enhance production of reactive oxygen species (ROS) when it reacts uncontrollably on the other hand. These reactions may even be aggravated under conditions of oxidative stress (OS) as exposure to ONOO• may lead to the release of copper from ceruloplasmin. Dopaminergic neurons are especially vulnerable as copper neurotoxicity seems to depend on dopamine-mediated copper uptake. (Deplazes J, 2004)

Exogeneously acquired increased copper levels may be deleterious as shown by a population-based case-control study which provided evidence that chronic occupational exposure to copper is associated with Parkinson’s disease (PD). Moreover, dietary and pharmacological manipulations of copper modify the course of the disease in mouse models of PD in ways that suggest a role for this metal in disease pathogenesis. It has been shown that copper may accelerate aggregation of α-synuclein to form fibrils and that copper-mediated stress is linked to mitochondrial dysfunction as a result of decreased activity of cytochrome c oxidase. (Mattson MP, 2004), (Rossi L, 2004)

Manganese is essential for a number of enzymes including SODs, arginase, hydrolase and carboxylase enzymes. However, chronic
exposure to even moderate amounts of manganese over longer periods of time may induce Parkinsonism similar to idiopathic Parkinson’s disease (PD). Similarly, combination of high intake of iron and manganese has been found to be related to PD. (Powers KM, 2003)

The main pathophysiological impact of manganese is supposed to be the promotion of rapid dopamine oxidation in the brain leading to severe destruction of brain tissue at the striatum and pallidum. Moreover, it may incur depletion of levels of peroxidase and catalase. (HaMai D, 2004)

In vitro and animal models suggest that manganese directly inhibits mitochondrial function preferentially by inhibiting mitochondrial complex III. Additionally, manganese has been shown to accelerate α-synuclein fibril formation. (Zhang J, 2003)

Not all metals enhance production of reactive oxygen species (ROS). Zinc, which on the one hand may contribute to the generation of oxidative stress (OS) by interference with the mitochondrial complex I, may act on the other hand as an antioxidant by displacing iron ions from their binding sites and inhibiting iron-dependent radical reactions. It exerts this influence by binding to thiol groups, inhibiting nitric oxide synthase and inducing Zn2+-containing, antioxidative proteins. (Mendez-Alvarez, 2002)

Patients with Parkinson’s disease (PD) showed a significantly decreased zinc status established by a zinc tally test and also the CSF levels of zinc were significantly decreased in PD patients as compared with controls. Similarly, deficiency of magnesium, a cofactor for multiple enzymes, may increase oxidative damage. Moreover, in vitro studies have shown that magnesium may inhibit the aggregation of α-synuclein induced either spontaneously or by incubation with iron. (Goltz N, 2002)
Mitochondrial dysfunction and OS

It has been suggested that mitochondrial complex I inhibition may be one central cause of sporadic Parkinson’s disease (PD). The site of mitochondrial ROS production most strongly implicated in PD is complex I, the first of five complexes of the mitochondrial respiratory chain. A minor contribution to the overall reactive oxygen species (ROS) generation is attributed to the ubiquinone site. A decline of about 30% in the activity of complex I, has been found in SNc of PD patients. The decrease in activity is matched by a decrease in protein content that appears to reflect an mtDNA defect and reduced production of complex I subunits. *(Dawson TM, 2003) (Beal MF, 2004)*

Because of the reduced activity, less NAD is formed resulting in the impairment of proton pumping and electron transport. Consequences are an increase of reactive oxygen species (ROS), proteasome inhibition, accumulation of oxidatively modified proteins, with consequent aberrant protein accumulation, promotion of expression of proapoptotic proteins and eventually cell death. *(Shamoto-Nagai M, 2003)*

Decline of complex I activity is followed by reduced ATP production resulting in decreased energy for the cell and DNA damage and may influence complex downstream signal transduction processes. *(Kotake Y, 2003)*

The first exotoxin described to contribute to the pathogenesis of Parkinson’s disease (PD) was MPP+ (1-methyl-4-phenyl-2 3-dihydropyridium ion), the highly toxic metabolite from MPTP(1-methyl-4-phenyl-1 2 3 6-tetrahydropyridine). Other substances known to cause or promote PD by interference with the respiratory chain are transition metals zinc, copper and manganese, and dieldrin or the insecticide rotenone, a specific inhibitor of complex I. *(Sherer TB, 2002)*
Animal models underscored the causative link between these substances and the development of Parkinson’s disease (PD). One example is that chronic infusion of the complex I inhibitor rotenone in rat brain leads to selective loss of dopaminergic neurons and the formation of cytoplasmic α-synuclein inclusions. As not all individuals exposed to the same environmental toxins develop PD a genetically determined susceptibility is probable. In the brain of PD patients, changes in the mitochondrial genome have been found to result in an increase in the number and variety of mtDNA deletions/rearrangements. Moreover, there have been several reports of mtDNA mutations in rare maternally inherited pedigrees of parkinsonism. However, because of the clinically and pathologically differing phenotype from idiopathic PD, there is little convincing evidence to support a causal role of mt DNA variations in PD. 

(Gu G, 2002), (Thyagarajan D, 2000)

Consequences of Redox imbalance

Oxidative stress (OS) damage compromises all biomacromolecules – polynucleotides, proteins, sugars and lipids, leading to a critical failure of biological functions and finally, cell death. Because of their high reactivity, free radicals cannot be measured directly. However, there are a number of indices for OS in the substantia nigra (SN) of Parkinson’s disease (PD) patients. Their localization and primary targets depend on the sites of their formation (Schipper HM, 2004):

• Lipid peroxidation of membranes that are crucial for cell viability, occurs as a consequence of direct reaction of fatty acids of polar lipids with oxygen or a reaction catalyzed either by metals like iron or by NADPH cytochrome P-450 reductase. It leads to the formation of free radical intermediates and semistable peroxide. Increased levels of secondary products like conjugated dienes,
hydrocarbon gases (e.g. ethane) and carbonyl compounds (e.g. malondialdehyde) and decreased levels of polyunsaturated fatty acid have been demonstrated. *(Schipper HM, 2004)*

- Oxidation of proteins may occur directly as protein side chains are oxidized leading to a loss of function of proteins and a deactivation of enzymes. Often, thiols of proteins involved in the regulation of enzyme activity are directly oxidized. Increase of malondialdehyde has been suggested to lead to intra- and inter-molecular cross-links of proteins. Conformational changes leading to an increase in hydrophobicity may result in aggregation or precipitation of proteins, which can no longer be subjected to the normal protein degradation pathway. Additionally, oxidative damage of proteins may occur by the adduction of secondary products like oxidation of sugar i.e. glycoxidation, or of polyunsaturated lipids, i.e. lipoxidation. *(Dalle-Donne I, 2005) (Sayre LM, 2001)*

- DNA and RNA damage are major consequences of oxidative stress (OS). Exposure of nucleic acids to reactive species may result in strand breakage, nucleic acid–protein cross-linking and nucleic base modification. Base modification, cross-linking of DNA–DNA and DNA–proteins, sister chromatid exchange and single- or double-strand breaking may lead to disruption of transcription, translation and DNA replication. Increased levels of 8-hydroxy-2_hydroxyguanine and thymidine glycol indicating DNA base damage have been demonstrated in the substantia nigra (SN) and striatum of Parkinson’s disease (PD) brain. Mitochondrial DNA (mtDNA), which is transiently attached to the inner mitochondrial membrane where a large amount of ROS is produced, is particularly vulnerable to oxidative damage. Moreover, DNA repair mechanisms in the mitochondria are less efficient than in the nucleus. Therefore, ROS-mediated mtDNA damages may contribute to mitochondrial dysfunction generated by endogenous reactive intermediates which act directly on mitochondrial proteins. RNA oxidation has also been observed in neurons of Parkinson’s disease (PD) patients.
Reactive oxygen species (ROS) interfere with signal transduction and gene expression affecting cell death. Within neurons, the intracellular pathways of signaling and gene expression affecting cell survival are especially vulnerable to redox changes. Complex interactions of various sites of signal transduction with radicals via modification of enzymes leading to altered signal transduction and eventually altered gene expression have been described. These include increased formation of Oxidized glutathione (GSSG) resulting in the inactivation of protein phosphatases, which again negatively regulate protein kinases leading to increases in apoptosis and expression of inflammatory genes. On the other hand, ROS can effectively delay activation of caspases and calpains which are important executors of apoptosis. Therefore, a balanced redox equilibrium is important to maintain the pathways important for cell survival in neurons.

Therapeutic outlook

All three factors leading to pathological cascades – redox imbalance, influences of specific genes and mitochondrial dysfunction – seem to be closely linked and interconnected in a way that disturbance in each one of the systems has a deleterious influence on the other, promoting its dysfunction with the final consequence of neuronal death. Therapeutic strategies should, therefore, focus on influencing these contributing factors in all diseases. Concerning redox imbalance, much effort has been taken to develop therapeutic strategies to prevent the deleterious effects of reactive oxygen species (ROS) either by directly scavenging or by triggering protective mechanisms inside the cell. Several agents exerting antioxidative influence by modulating cellular energy metabolism in animal models seem promising as neuroprotective agents. These include coenzyme Q10, creatine, Ginkgo biloba,
nicotinamide, acetyl-l-carnitine as well as non-narcotic analgesics, acetaminophen and aspirin. (Beal MF, 2003) (Maharaj DS, 2004)

Alpha-lipoate may serve as an antioxidant by reducing glutathione disulfide increasing intracellular glutathione levels. Also, the developed subtype-selective inhibitors of Inducible nitric oxide synthase (iNOS) and Neuronal nitric oxide synthase (nNOS) could exert a neuroprotective influence by diminishing oxidative stress (OS). Effort to find ways of decreasing the elevated iron levels in animal models of Parkinson’s disease (PD) has led to the discovery of attenuation of iron-mediated dopaminergic neurodegeneration by the iron chelator desferal, lisuride and neuroprotection by the brain permeable iron chelator VK-28 (5[4-(2hydroxyl)-piperazine-1methyl]-quinoline-8-ol). (Youdim MB, 2004)

Diet is becoming increasingly important as there is evidence that patients may influence disease development and progression by adapting specific dietary concepts. However, data concerning diet and nutritional supplements is controversial. The role of nutritionally administered iron has been supported by an epidemiological study revealing that iron taken in the highest quartile compared with those in the lowest quartile leads to an increased risk of Parkinson’s disease (PD). (Powers KM, 2003)

The vitamins, tocopherol (vitamin E) and vitamin C are potent free-radical scavengers. However, although an important role of vitamin E for neuroprotection following amyloid Aβ-peptide-mediated neuronal damage in vitro has been established, the DATATOP trial showed that it could not reduce the dosage of l-DOPA in Parkinson’s disease (PD), indicating that restriction of its site of action to specific compartments may not be sufficient for radical defense in PD or that the beginning of the therapeutic application might have been too late. (Mandel S, 2004) (Calabrese V, 2003)
Similarly, vitamin C, which acts synergistically with vitamin E in the inhibition of oxidation reactions, has been shown to act in a neuroprotective manner in various in vitro and in vivo experiments, when it is applied in the oxidized form such as dehydroascorbic acid. Other nutritional components which have shown antioxidative properties in animal models of Parkinson’s disease (PD) and in vitro experiments include selenium, flavonoids (essential compound in green tea, red wine, blueberries, etc.), vitamin B6, B12 and folate and the heat shock protein expression inducing curcium, a powerful antioxidant derived from the curry spice turmeric.


Role of oxidative stress in Parkinson’s Disease

The Association Between PD and OS

Oxidative stress (OS) is associated with many factors that are thought to be involved in the pathogenesis of Parkinson’s disease (PD). Mitochondrial damage may result from OS and, vice versa, mitochondrial impairment may enhance ROS/RNS release to the cytosol. Inflammation is always associated with high ROS/RNS generation (nitric oxide NO, hypochlorous acid HOCl, superoxide anion, hydrogen peroxide, etc.) as direct defense species and as signaling elements to induce a series of protecting genes. OS can contribute to protein misfolding and, when linked to an inability of the ubiquitin-proteasome system to degrade and remove them, such damage can result in the accumulation and aggregation of these abnormal proteins and induce what is termed proteolytic stress. Misfolding and aggregation of α-synuclein have been described in association with familial PD.
Oxidative stress (OS) is linked to 3 4-dihydroxy-phenylalanine (DOPA) and dopamine (DA) enzymatic and nonenzymatic metabolism with the formation of DOPA and DA quinones, a reactive species that can further react with endogenous compounds such as cysteine to form cytotoxic agents. OS is also associated with MAO activity, as well as in other enzyme activities present in the brain such as TH or tyrosinase, both of which hydroxylate tyrosine to DOPA, or the enzyme heme oxygenase-1 (HO-1) responsible for the degradation of heme into biliverdin, carbon monoxide (CO), and iron. All these enzyme activities can be involved in OS, which could lead to selective neuronal death in the substantia nigra and to Lewy body (LB) formation. *(LaVoie MJ, 2005)*

**Biomarkers of OS in PD**

Degeneration of dopaminergic neurons in the brain and the formation of the Lewy body (LB) are the hallmark events of Parkinson’s disease (PD). Oxidative stress (OS) is believed to contribute at least in part to these two incidents, and biomarkers that might be generated during the pathways of their formation are under investigation. It was assumed that, as a consequence of dopaminergic cell death, neuromelanin (NM) will be present not only in the brain but also in the plasma. Antibodies specific to NM have been found in the plasma of patients with PD, but it is not clear yet if the NM detected in plasma of PD patients is specific to the disease or common to other neurological disorders or even may arise from skin diseases. *(LaVoie MJ, 2005)*

In other studies, superoxide dismutase (SOD) activity was shown to decrease in the blood of advanced Parkinson’s disease (PD) patients vs. control subjects and a negative correlation was anticipated between this activity and disease duration. The level of oxidized pyrimidins and altered purine nucleotides in plasma of PD patients
vs. controls was shown to be elevated together with a higher incidence of DNA strand breaks, both of which can be generated via OS pathways. (Bostantjopoulou S, 1997), (Migliore L, 2001)

Munch et al. identified advanced glycated end products (AGEs) in substantia nigra (SN) of patients with incidental Lewy body disease, which is considered an early form of Parkinson’s disease (PD). The patients examined showed no clinical signs of PD, which suggests that AGEs are present in a very early stage of PD before symptoms are detected. AGEs are reactive ketones or aldehydes formed during the reaction of carbohydrates with the free amino groups in protein or during unsaturated fatty acid oxido-degradations. These intermediates are reactive species that can further lead to protein cross-linking and to protein dysfunction. The AGE-cross-linked proteins were detected by specific polyclonal antibodies raised against different AGE-protein adducts. (Munch G, 2000)

Evidence for an Association between DA Metabolism and OS

Various studies have pointed to a role of dopamine (DA) as one of the major factors influencing redox balance, ROS production, and oxidative stress (OS). The high rate of oxidative metabolism of DA by Monoamine oxidase (MAO) generating ammonia, hydrogen peroxide, and a highly reactive aldehyde metabolite (3,4-dihydroxyphenylacetaldehyde, DOPAL) in conjunction with the spontaneous autoxidation of DA to form quinones or semiquinones are major factors accelerating neurodegeneration. DA quinones are cytotoxic because of their interaction with the sulfhydryl group of cysteine forming various bioactive molecules, predominantly 5-cysteinyl-DA. (Miyazaki I, 2007)

In neuronal cell cultures, dopamine (DA) has been used as an inhibitor of brain mitochondria, as a precursor to 6-hydroxydopamine (6-OHDA) formation, and, as mentioned earlier, as a major source of quinone and semiquinone formation. Intrastriatal injections of DA caused dose-dependent increases in
neuronal degradation and in the amount of quinoproteins found in the rat brain. In a hemi-parkinsonian rat model of PD, treatment with L-DOPA, which caused an excess amount of DA outside the synaptic vesicles, caused increased DA turnover and quinoprotein formation in the damaged side and may exert neurodegenerative effect on the dopaminergic nerve terminal. The quinone formation and subsequent dopaminergic neuronal damage in vivo and in vitro can be slowed down or prevented through pretreatment with antioxidants, such as superoxide dismutase (SOD) and glutathione (GSH). *(Jana S, 2007), (Asanuma M, 2008)*

Amphetamine, methamphetamine and their analogues have been shown to release DA from dopaminergic nerve terminals by several different mechanisms, such as the reversal of the dopamine (DA) uptake transporter (DAT), or through interfering with the storage of DA in the vesicular stores. It has been shown that chronic administration or high doses of amphetamines can be extremely neurotoxic and cause a dramatic neurodegeneration, a reduction in the amount of TH-positive neurons, and a reduction in the amount of active DA in the brain. *(Afanas’ev II, 2001)*

Several hypotheses have been proposed to explain the neurotoxic effect of amphetamines, including adenosine triphosphate (ATP) depletion, mitochondrial inhibition, and toxin-induced oxidative stress (OS). In many studies of chronic amphetamine administration, a clear increase in OS was observed and tissue levels of lipid peroxidation and protein oxidation were elevated. Even after a single large dose of amphetamine, an increase in hydroxyl radical was observed, and an increase in malonaldehyde (MDA) was seen 7 days after administration. This increase in OS was abolished when animals were pretreated with the antioxidants N-acetylcysteine (NAC) and a-phenyl-N-tertbutylnitrone (PBN). In other experiments, animals were pretreated with antioxidants, such as ascorbate and vitamin E, before amphetamine, which resulted in reduced OS and fewer neuronal lesions. *(Jiang H, 2006), (Lotharius J, 2001)*

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The classic antipsychotic drugs (such as haloperidol), are mainly D2 dopaminergic receptor antagonists and have two different mechanisms by which they may increase OS. (i) By blocking presynaptic D2 receptors, which have an inhibitory effect on DA release they induce an increase in DA turnover in an attempt to overcome the postsynaptic receptor blockade. This increased turnover can yield an augmented amount of ROS production and quinone formation, thus increasing OS. (ii) The haloperidol pyridinium ion (HP+) metabolite, which is derived from haloperidol, is a potent inhibitor of mitochondrial complex I and can interfere with electron transport at both complexes I and II, causing an interruption in mitochondrial activity and an increase in the resulting ROS production. This situation is therefore another example of increased neuronal activity leading to OS and neuronal damage. *(Reinke A, 2004)*

**Mitochondrial Impairment and PD**

The first link between mitochondrial dysfunction and Parkinson’s disease (PD) was the finding of decreased activity of complex I in the substantia nigra (SN) of parkinsonian brain with a decrease in complex I protein level. A recent study that strongly implicates a defect in complex I with PD was carried out using mice with a knockout of respiratory chain proteins in their dopaminergic neurons. These animals show progressive impairment of motor function together with formation of intraneuronal inclusions and dopaminergic cell death. Inhibition of complex I by toxins such as 1-methyl-4-phenyl-1 2 3 6-tetrahydropyridine (MPTP), paraquat, or rotenone caused dopaminergic cell death or induced the formation of intracellular filamentous inclusions with α-synuclein protein as in the Lewy body (LB). *(Ekstrand MI, 2007), (Sherer TB, 2003)*
The critical role of α-synuclein, the major component of LB in PD, in mitochondrial impairment, and specifically in complex I deficiency, was evaluated in experiments with mice lacking the gene for α-synuclein. These mice were shown to be resistant to the toxic effects of the complex I inhibitor 1-methyl-4-phenyl-1\(\text{2}\)\(\text{3}\)\(\text{6}\)-tetrahydropyridine (MPTP) and did not show the pattern of DA neurodegeneration characteristic of MPTP-induced mitochondrial complex I inhibition. These findings indicate that in the absence of α-synuclein protein, the neurotoxin MPTP cannot inhibit complex I. (Dauer W, 2002)

Giasson et al. were able to show extensive accumulation of nitrated α-synuclein within the inclusion bodies of Parkinson’s disease (PD), dementia with LB, and in the major filamentous building blocks of these inclusion bodies. These nitration occur at the tyrosine aromatic ring residue of the α-synuclein protein and could be formed through different pathways such as the generation of NO, which in the presence of superoxide anion (O\(\text{2}\)\(^{-}\)) may form highly reactive peroxynitrite (ONOO\(^{-}\)). Oxidation of α-synuclein by either nitration or other ROS/RNS alters its protein structure and changes its physical properties, including decreased solubility, which may have an important role in the aggregation of α-synuclein in sporadic PD. (Giasson BI, 2000)

Neurotoxin-induced inhibition of complex I induces oxidative stress (OS) generation in a dose-dependent manner in isolated brain mitochondria, as detected by augmentation of hydrogen peroxide level. Injection of 1-methyl-4-phenyl-1\(\text{2}\)\(\text{3}\)\(\text{6}\)-tetrahydropyridine (MPTP) to mice causes oxidation of cardiolipin (phospholipids that bind the cytochrome c in the inner mitochondrial membrane) in ventral midbrain with the formation of phospholipids hydroperoxide, an established marker of OS, which was also obtained in mitochondria isolated from brain of mice treated with complex I inhibitors. Interestingly, the formation of oxidized phospholipids as a result of complex I inhibition was reduced by addition of an antioxidant known to convert superoxide anion to
hydrogen peroxide (similar to SOD). These results suggest that O2 is generated and contributes to the cardiolipin oxidation as a result of complex I inhibition by MPTP. (Perier C, 2005)

Advanced postmortem analysis of proteins from mitochondrial preparations obtained from frontal cortex of PD patients revealed a significantly increased level of keto-proteins in the complex I protein fraction compared with age-matched controls. This enhanced oxidation was mostly localized in a fraction of hydrophobic proteins thought to form the catalytic core of complex I. When proteins of complex I were exposed to exogenous ROS such as hydrogen peroxide to reproduce the type of damage to complex I proteins observed in the samples from PD patients, such an oxidative pattern was not formed but did form when NADH was used to transfer the electrons, suggesting that the ROS involved in the oxidation of complex I proteins are formed within the mitochondria and not from outside. (Keeney PM, 2006)

Mitochondrial impairment may result in complex I defect, increased free radical generation, and damage to macromolecules such as α-synuclein protein, which further accelerate mitochondrial dysfunction and free radical generation. This augmented superoxide anion O2 leakage, hydrogen peroxide accumulation, and protein nitration in conjunction with ubiquitin proteasomal dysfunction induces proteolytic stress, which results in protein misfolding, oxidation, and aggregation. The foregoing alteration at the molecular level results in tissue damage, alteration in mitochondria membrane permeability, damage to the respiratory chain, decreased ATP production, reduced membrane potential, formation of LB, apoptosis, and neuronal death. (Keeney PM, 2006)

Iron and OS

Free ferrous iron (Fe2+) is a potent catalyst for a variety of oxidative reactions, and its concentration is maintained at very low levels within cells. In particular, Fe2+ catalyzes the Fenton reaction,
in which hydrogen peroxide is converted to hydroxyl anion, and to hydroxyl free radical, which is among the most active ROS species. The potential toxicity of iron in the brain has been adequately demonstrated in earlier studies in which a dopaminergic lesion was created by direct infusion of iron to the brain of rats. *(Wesemann W, 1993), (Ben Shachar D, 1993)*

A number of studies have found high Fe levels in parkinsonian SNpc, as well as in the globus pallidus and dentate gyrus, although the levels in the substantia nigra pars reticulata (SNpr) are not increased. In addition, the ratio of Fe2+ to Fe3+ is increased in PD. Increased Fe-containing pigments are also found in basal ganglia areas in other neurodegenerative diseases, including Hallervorden Spatz disease, striato-nigral degeneration, and Huntington’s and Alzheimer’s diseases. *(Zecca L, 2001)*

Gerlach et al. described the possible sources of the increased Fe levels in parkinsonian SNpc. First, passage of Fe across the blood–brain barrier (BBB) could be increased. Evidence exists for a localized increase in BBB permeability in the mesencephalon of PD patients. Second, brain Fe uptake could be selectively increased in a particular brain area if transferrin receptor levels increased in that area; in fact, the opposite is the case for parkinsonian substantia nigra pars compacta (SNpc), although levels of lactoferrin receptors are increased. A third possibility could be transfer of Fe from areas of higher iron content to SN cells by axonal transport along connecting fiber tracts, but no evidence exists for such a possibility. The fourth possible cause is release of Fe from intracellular storage sites or reduction in iron binding by proteins such as neuromelanin (NM) and ferritin. The intracellular distribution of Fe is regulated by the iron regulatory proteins, iron regulatory protein (IRP1) and (IRP2). Targeted deletion of these proteins in mice leads to accumulation of cytosolic iron in axons and cell bodies, followed by severe neurodegeneration and loss of cell bodies, particularly in SN. *(Gerlach M, 2006), (Snyder AM, 2009)*
Several studies have assessed ferritin levels in parkinsonian SN tissue, with variable results, because of technical problems including the specificity of antibodies used and the type of ferritin (H or L) against which they were directed. The expression level of H-ferritin protein is low in neurons of substantia nigra pars compacta (SNpc), whereas high levels are seen in oligodendrocytes and microglia. L-Ferritin is absent from neurons but strongly expressed in oligodendrocytes. It is, therefore, apparent that determination of whole-tissue ferritin levels will yield minimal information on changes in iron storage in the target dopaminergic neurons of SNpc. Levels of ferritin mRNA also do not change in parkinsonian SN. The lack of change in transferrin receptors and ferritin in PD SNpc is suggestive of a role of NM in binding the increased Fe in the PD brain. 

(Snyder AM, 2009), (Hirsch EC, 2006)

Neuromelanin (NM) levels in individual DA neurons of substantia nigra pars compacta (SNpc) are decreased in PD, so that more Fe in parkinsonian SNc may be bound in a low-affinity, reactive form and therefore will increase the oxidative environment. Parkinsonian NM is also qualitatively different from normal NM. Faucheux et al. showed that redox activity of NM aggregates from PD brain positively correlated with severity of neuronal loss. In addition, however, NM released from degenerating neurons is broken down by H2O2 with the release of reactive iron and generation of toxic free radicals. Alterations in the Fe-binding properties of NM could therefore be an initiating factor in the dopaminergic neuron death in parkinsonian SN. (Snyder AM, 2009), (Hirsch EC, 2006)

**Iron chelators as neuroprotectants**

Considering the abundant evidence of increased toxic iron levels in parkinsonian SN, iron chelator treatment may be considered as a potential strategy in neuroprotective therapy of the early parkinsonian patient. Effective iron chelators must be capable of crossing the blood brain barrier (BBB), and must not damage the
physiological Fe pool, but should reduce excessive free Fe levels. A number of drugs have been described, although none are currently in clinical use in PD as such; future developments, however, are predicted in this area. Desferrioxamine (desferral) is a highly effective iron chelator that has been studied in animal experiments, but must be given directly to the brain, for example, by intracerebroventricular injection (i.c.v.). (Jiang H, 2006)

A BBB-permeable drug, VK-28 (5-[4-(2-hydroxyethyl) piperazine-1-ylmethyl]-quinoline-8-ol), was found to significantly protect against 6-OHDA-induced dopaminergic toxicity in low dosage, by systemic or i.c.v. administration. (Youdim MB, 2004)

Chelation of iron by the antibiotic cloquinol, or increased binding of tissue iron by over expression of ferritin, significantly reduced 1-methyl-4-phenyl-1 2 3 6-tetrahydropyridine (MPTP) toxicity in mice. As in many similar studies, these findings indicate that iron chelation can protect against dopaminergic neurotoxins, but such animal models do not reproduce the etiology of most PD cases. (Kaur D, 2003)

It is known that the immune system and its inflammatory response are not the initial cause of PD, but rather a consequence of damage, that is, tissue or cellular modifications in the CNS, such as neurodegeneration. Such threats activate the microglia, which respond through morphological changes in which the cells are converted to an amoeboid state with enlarged cytoplasmic processes capable of phagocytosis, migrate to the injured environment, alter gene expression, and release of inflammatory mediators such as cytokines, chemokines, ROS, and reactive nitrogen species (RNS). Excessive, chronic, or unregulated microglial activation may be harmful to neurons. The phagocytic activity of microglia is beneficial during neuronal development and in injury because of the effectiveness of this process to remove cellular debris and injured cells, but dysregulation or excessive activation and as a
consequence excessive ROS formation can lead to neuronal oxidative burden. (*Meredith GE, 2008*)

**Inflammation and PD**

The involvement of inflammation in neuronal death in PD has been observed post mortem in which an increase in the expression of the cyclooxygenase (COX) enzyme and of inflammatory mediators has been shown in the injured striatum. However, most of the data available have come from models of the disease, such as the lipopolysaccharide (LPS), 6-hydroxydopamine (6-OHDA), and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) models. In fact, the addition of LPS, which activates microglial cells, and induces the expression of cytokines in a pure dopaminergic culture, did not change the cell viability, although the addition of LPS to a culture consisting of both neurons and microglia caused an increase in neuronal death. (*Hartmann A, 2003*)

In vivo experiments demonstrated that a single intranigral injection of LPS caused no damage to GABAergic neurons but strongly injured dopaminergic neurons. Antiinflammatory therapies have provided a strong neuroprotective role in different kinds of illness and pathologies, and the use of nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin and other COX inhibitors, is known to confer at least partial protection against the neurodegeneration seen in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-OHDA models of PD. (*Villoslada P, 2008*)

In a large clinical trial, it was shown that users of ibuprofen (a common NSAID) had a 35% lower risk for PD; however, no other NSAID had the same results. (*Chen H, 2003*)

The involvement of the immune system and its activation in PD has been seen clearly post mortem and in vitro and in vivo models, but the precise mechanism by which activated microglia can worsen
dopaminergic neuronal degradation is still a mystery. Microglial activation can occur through many different pathways including interleukin-4 (IL-4), glucocorticoids, aggregated α-synuclein, and LPS. This microglial activation can accelerate neurodegeneration through various mechanisms, but central to all is the involvement of OS, either through the release of superoxide anions derived from NADPH oxidase, increased peroxynitrite production, or the nitration of α-synuclein. (*McGeer PL, 2008*)

**Enzyme Alterations in PD**

Heme oxygenase-1 (HO-1) expression was significantly higher in PD substantia nigra than that of control subjects. HO-1 is an inducible enzyme upregulated by several inducers including hydrogen peroxide and cytokines. HO-1 confers cytoprotection by the enhancement of the breakdown of pro-oxidant heme to form the antioxidants biliverdin and bilirubin, but under certain conditions the heme-derived end products, CO and especially iron, can exacerbate OS in cells and damage protein, unsaturated fatty acids, and DNA. (*Schipper HM, 2011*, *Piantadosi CA, 2006*)

In PD brains, enzyme immunoassay showed lower Tyrosine hydroxylase (TH) activity and reduced protein content. Although both TH protein and TH activity in the striatum were markedly decreased in PD brains as compared with those of the control brains, the molecular activity (activity per enzyme protein) was significantly increased. The increase in the molecular activity of residual TH in PD brains suggests that the remaining neurons compensate for the neuronal loss by increasing their DA release, to bring normal amounts of dopamine (DA) to the postsynaptic receptors, and therefore increase their tyrosine hydroxylation level. This overactivity of TH may further lead to increase in OS as a result of quinone formation. (*Okuno T, 2005*)
Inducible nitric oxide synthase (iNOS) is one of the NO-synthesizing enzymes that have been shown to induce dopaminergic neuronal loss. NO can react vigorously with superoxide anion to form a powerful RNS that may further degrade into other reactive intermediates. In iNOS-deficient mice, the toxic effect of MPTP treatment on dopaminergic neurons is eliminated, and the pharmacologic inhibition of Inducible nitric oxide synthase (iNOS) reduced the neuronal death caused by LPS both in vitro and in vivo. (Gao HM, 2002)

In the cerebrospinal fluid of PD patients, a marked increase in the concentration of nitrite was observed, but with the increase in the metabolite, there was an increase in the density of glial cells expressing Inducible nitric oxide synthase (iNOS) in the substantia nigra of PD patients, suggesting the involvement of NO as well as iNOS induction in the pathogenesis of the disease. (Chen J, 2006)

**Endogenous Antioxidant Alteration during PD**

Glutathione (GSH) is the major antioxidant in organs, and the ratio between GSH and the oxidized disulfide form (GSH/GSSG) has a major role in preserving the oxido-reduction homeostasis (the thiol balance). It is well documented that patients with PD have a decreased total GSH level in their brain without increased GSSG level. (Sian J, 1994)

The suppressed glutathione concentration is specific to the substantia nigra of PD and does not occur in other brain regions (cerebral cortex, globus pallidus, putamen) or in other neurodegenerative illnesses such as in multiple system atrophy. Furthermore, decreased glutathione (GSH) levels occur in the brain of patients with incidental LB disease before any change occurs in the complex I activity or in iron metabolism, which suggests that GSH depletion is an early event in PD progression. (Jenner P, 1992)
An approach of using a modified drug to overcome the obstacle of crossing the BBB was made by modifying cysteine to N-acetyl cysteine (NAC). NAC is a powerful thiol antioxidant that, when given systemically, passes the BBB and releases cysteine in the brain, elevating GSH. NAC increased dopaminergic neurons survival against MPTP toxicity and, following subcutaneous administration, it induced about 30% reduction of the dopaminergic lesion. *(Munoz AM, 2004)*

The link between Coenzyme Q-10 (Q10, ubiquinone) to PD was established by observations that (a) inhibition of mitochondrial complex I by MPTP caused parkinsonism in animals and humans (b) Q10 levels and complex I activity decreased whereas oxidized Q10 concentrations increased in blood of PD patients compared to age matched controls, and (c) supplementation of mice and monkeys with Q10 before MPTP treatment significantly attenuated the loss of nigral dopaminergic neurons. These observations encouraged the conduction of clinical trials to elevate Q10 concentration in blood as a treatment for PD progression in human, although efficacy in animal models has not predicted efficacy in humans. *(Sohmiya M, 2004), (Galpern WR, 2007)*

Melatonin (N-Methoxytryptamine) has been shown to scavenge ROS and RNS and to induce antioxidant enzyme activities such as those of superoxide dismutase (SOD), catalase, and glutathione peroxidase. It can donate an electron and be converted into a nitrogen centered radical, which may further scavenge superoxide and convert it into a stable compound. *(Reiter RJ, 2001)*

In PD patients, pineal activity and melatonin circulation were reduced. In MPP+-mediated parkinsonism in rats, melatonin reduced lipid peroxidation, protected nigral dopaminergic neurons, DNA fragmentation, and protein misfolding; in 6-OHDA-mediated parkinsonism, similar indole amine compounds also prevented the PD-like behavioral changes. Many derivatives of melatonin have been synthesized as potential drugs to overcome some of the disadvantages associated with melatonin, mainly its short
circulation half-life resulting from rapid catabolism and lack of selectivity at the target site. *(Suzen S, 2006)*

**Reduction of OS in Brain as a Methodology to Delay or Stop PD Progression**

Cumulative evidence emphasizes the role of OS in PD development and progression, which raised the hope that antioxidants may lower such risk. Major food antioxidants present in fruits and vegetables are polyphenols of flavonoid and nonflavonoid structure. Prospective cohort studies (PCS) were conducted correlating the effect of consumption of a diet rich in polyphenols on PD progression, such as that of Checkoway et al., which showed that consumption of green tea was associated with reduced risk for PD. *(Checkoway H, 2002)*

It was assumed that this protective effect may result from the ability of tea polyphenols to scavenge singlet oxygen, superoxide, hydroxyl radicals, and peroxides. Similarly, various correlations were carried out between consumption of polyphenols from other sources, such as extracts of blueberries or Ginkgo biloba, vs. parkinsonian symptom development. The conclusions from most of such experiments were that a diet rich in polyphenols such as catechin, epicatechin, anthocyanins, quercetin, and kaempferol had a beneficial effect on preventing development of neurological diseases including PD. Such clinical trials, when carried out using vitamin E, coenzyme Q-10, and glutathione, showed that vitamin E was not effective in slowing the progression of PD or in its prevention. Supplementation of vitamin E alone or in combination with selegiline was not found to reduce the probability of requiring levodopa therapy. *(Group TPS, 1993)*

In two large cohort studies covering 120,000 participants, the associations between risk of PD and use of vitamin E, vitamin C, carotenoids, or vitamin supplementation was examined. No
association was found between vitamin E or vitamin C intake and PD development. Meta-analysis studies on the effect of vitamin C, vitamin E, and b-carotene covering the period 1966 - 2005 on the risk of PD development revealed that b-carotene or vitamin C had no beneficial effects on the risk of developing PD, whereas a diet rich in vitamin E, as opposed to pure vitamin E, has some beneficial effect. *Etminan M, 2005*

Resveratrol is a well-known, nonflavonoid antioxidant present in grapes and red wine, and some of the protecting effects of red wine on the development of cardiovascular diseases have been attributed to the presence of resveratrol. Mice treated with 1-methyl-4-phenyl-1 2 3 6-tetrahydropyridine (MPTP) displayed severe neuronal loss whereas administration of MPTP in combination with resveratrol significantly protected mice from MPTP-induced motor coordination impairment and neuronal loss. *Sun AY, 2002*

Curcumin is a nonflavonoid polyphenol present in turmeric, curcumin possesses antiinflammatory and antioxidant effects. Administration of curcumin to a mouse model of glutathione (GSH) depletion restores the cellular GSH pool, significantly delays protein oxidation, and preserves mitochondrial complex I activity caused by glutathione depletion. Systemic administration of curcumin and its metabolite tetrahydrocurcumin reversed the MPTP-induced depletion of dopamine (DA), which was presumed to be the result of inhibition of the enzyme MAO-B. In a 6-OHDA model of PD, rats pretreated with curcumin exhibited protection of the number of TH-positive cells in the SN and of DA levels in the striatum. A similar effect was observed with naringenin but not with quercetin or fisetin. There are insufficient human clinical trials evaluating the beneficial effects of pure exogenous antioxidants, other than vitamin E, vitamin C, and b-carotene, on PD development; such clinical trials are urgently required. *Jagatha B, 2008*, *(Rajeswari A, 2008)*
The propargylamine MAO-B inhibitors selegiline and rasagiline have the potential to reduce OS by reducing DA oxidative deamination, and both drugs possess neuroprotective effects in preclinical studies, both in vivo and in vitro. The in vitro neuroprotective effect of the compounds is exerted at concentrations below those normally required for MAO inhibition and has been attributed to an intrinsic property of the drug molecules to improve cell resistance to a variety of stressors by increased expression of Bcl2, bcl-xL, SOD, and catalase, and by other mechanisms. When administered to patients, the drugs are used at doses that effectively inhibit MAO-B and so could potentially induce neuroprotection by (a) reducing DA catabolism and (b) increasing levels of DA, which induces release of neurotrophic factors. A previous multicenter study was not able to conclude that selegiline had a neuroprotective effect in humans, but recently rasagiline has been demonstrated to reduce the rate of disease progression in human PD patients. At present, however, it is impossible to conclude whether the indirect antioxidant effect of rasagiline is responsible for its neuroprotective effect in PD. *(Olanow CW, 2008)*

**Role of oxidative stress in Alzheimer’s disease (AD)**

**Energetic Metabolism Deficiency in Alzheimer’s Disease**

Blass and Gibson were among the first who prompted the notion that defective energy metabolism in AD was a fundamental component of the disease. A large amount of evidence implicates metabolic defects in AD, namely the reduced rate of brain metabolism as one of the best-documented abnormalities occurring early in the disease. Specific defects in energy metabolism in AD
brains have been reported in a number of positron emission tomography (PET) studies. (*Blass JP, 1999*)

Drzezga et al. reported a reduced glucose metabolic rate in the temporoparietal and posterior cingulate cortex in both AD and mild cognitive impairment (MCI) subjects, including the prefrontal cortex in those patients with definitive AD. Furthermore, AD patients with at least one - 4 allele of apolipoprotein E (ApoE) also presented reduced glucose metabolism levels in limbic and associative areas of the brain, supporting the idea that ApoE4 carriers are more prone to develop AD at an early age. These changes are evident even in young and presymptomatic ApoE4 carriers. An increase in oxygen consumption in comparison with glucose utilization in AD patients is also well documented. These cerebral abnormalities precede any evidence of functional impairment by neuropsychological testing or brain atrophy neuroimaging. (*Drzezga A, 2003*), (*Mosconi L, 2004*)

It has been reported that AD patients present atrophy in the vasculature, the major metabolic exchange surface of the brain. Furthermore, those patients present a reduced cerebral glucose transport activity. Although both cerebral blood flow and metabolism are decreased in AD, the oxygen extraction fraction is increased, suggesting that the reduction in cerebral blood flow is more than could be accounted for by the lowered state of metabolic demand. However, it is not clear whether the decreased glucose uptake in AD is due to lowered metabolic demand in response to pathology or decreased ability of the injured vasculature to uptake glucose. Taken together, these findings suggest a metabolic contribution of glucose metabolism in the early pathophysiology of AD. (*Perry G, 2003*), (*Cash AD, 2003*)

In vivo, postmortem and biopsy data suggest that during the evolution of AD, declines in brain synaptic activity and brain energy consumption occur. In the first stage of these declines, changes in synaptic structure and function reduce neuronal energy
demand and lead to potentially reversible down regulation of oxidative phosphorylation within neuronal mitochondria. However, during the evolution of AD, neurofibrillary tangles accumulate within neuronal cytoplasm associated with impairment of axonal transport of mitochondria between the cell nucleus and synapse. This situation leads to severe energy impairment and is associated with increased risk of cell death. *(Rapoport SI, 2003)*

The activity of a number of enzymes involved in intermediary metabolism is decreased in AD brains, notably the activity of glutamine synthetase, creatine kinase, aconitase, pyruvate dehydrogenase, and α-ketoglutarate dehydrogenase. *(Gibson GE, 2000)*

Bubber et al. tested whether impairments in tricarboxylic acid (TCA) cycle enzymes of mitochondria correlate with disability in AD brains. The authors observed significant decreases in the activities of the pyruvate dehydrogenase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase complexes. In contrast, the activities of succinate dehydrogenase (complex II) and malate dehydrogenase were increased. Furthermore, the authors observed that all the changes in TCA cycle activities (specifically that of pyruvate dehydrogenase complex) correlated with the clinical state, suggesting a coordinated mitochondrial alteration. These enzymes are highly susceptible to oxidative modification and are altered by exposure to a range of pro-oxidants. *(Gibson GE, 2005)*

The diminished activity of the pyruvate dehydrogenase complex yields reduced levels of acetyl CoA, which is the source of decrease of both the ApoE4-independent cholesterol level and the formation of neurosteroids. The reduced activity of acetylcholine transferase in the presynaptic cholinergic neuron reflects the diminished availability of acetylcholine. Degeneration of the cholinergic system correlates with the progress in disturbed mental capacities in Alzheimer’s patients. Another pathophysiological consequence of the markedly perturbed glucose metabolism is the fall of ATP
production from glucose by around 50% in the beginning of sporadic AD, with increasing tendency during its course.  
(Mulder M, 1998)

The most consistent defect in mitochondrial electron transport enzymes in AD has been a deficiency in cytochrome oxidase. There are several reports indicating a reduced cytochrome oxidase activity in AD platelets and in postmortem brain tissue from patients with AD, particularly in neurofibrillary tangle-bearing neurons.  
(Cardoso SM, 2004)

Previous studies have demonstrated perikaryal accumulation of cytochrome oxidase protein, immunolocalized to cytosol by immunoelectron microscopy in face of reduced numbers of intact mitochondria. Results suggest that enhanced degradation of mitochondria occurs in AD, leaving behind lysosomal detritus containing nonfunctioning mitochondrial components.  
(Castellani R, 2002)

Studies with cybrid cells demonstrated that deficits in cytochrome oxidase in AD platelets could be transferred to Rho0 cells, which retain the cytochrome oxidase deficit. Additionally, the resulting cybrid cells showed markedly increased free radical production, impaired intracellular calcium buffering, elevated basal cytosolic calcium concentration, and enhanced sensitivity to inositol 1,4,5-triphosphate-mediated calcium release. Altogether, these data indicate that mitochondria dysfunction is a relevant event occurring in AD pathophysiology. (Swerdlow RH, 1997)

Oxidative Stress in Alzheimer’s Disease

The classic definition of oxidative stress is the imbalance between generation of reactive oxygen species (ROS) and nitrogen species (RNS) and antioxidant defenses. Inherent in this definition is that
Thesis
oxidative stress is an unstable situation, for if there is net damage, viability of the systems decreases with time, leading to disequilibrium and death. However, this definition does not fit well in physiological situations or chronic diseases closely aligned to normal physiology, such as AD. Instead of radicals breaching defenses, they propose an altered homeostatic balance resulting from oxidant insult as characteristic of AD and likely other chronic degenerative diseases. Increased oxidative damage is a prominent and early feature of vulnerable neurons in AD. (Smith MA, 1997)

Over the past decade, oxidative-stress-associated modifications of biomacro-molecules has been described in association with the susceptible neurons of AD:
(1) DNA and RNA oxidation is marked by increased levels of 8-hydroxy-2-deoxyguanosine (8OHdG) and 8-hydroxyguanosine (8OHG). (Honda K, 2005)
(2) Protein oxidation is marked by elevated levels of protein carbonyl and widespread nitration of tyrosine residues. Moreover, cross-linking of proteins by oxidative processes may lead to the resistance of the lesions to intracellular and extracellular removal even though they are extensively ubiquitinated, and this resistance of neurofibrillary tangles to proteolysis might play an important role in the progression of AD. (Smith MA, 1998)
(3) Lipid peroxidation is marked by higher levels of thiobarbituric acid reactive substances (TBARS), malondialdehyde (MDA), 4-hydroxy-2-transnonenal (HNE), and isoprostanes and altered phospholipid composition. (Zhu X, 2004)

It is reported that HNE modifications of tau promote and contribute to the generation of the major conformational properties defining neurofibrillary tangles. Modification to sugars is marked by increased glycation and glycoxidation. Levels of these markers are initially elevated following some unknown triggering neuronal event, but these levels soon decrease as the disease progresses to advanced AD. (Liu Q, 2005), (Perry G, 2002)
In a study of oxidative damage in AD and normal aging, they found a strong inverse relationship between neuronal oxidative damage and neuronal size among cases of AD but not controls. In fact, neuronal size in cases of AD is inversely correlated with the duration of the disease. Previous studies found that during the progression of the disease, there is a significant decrease in the size of neurons in AD when compared to controls. Together, these data indicate that neuronal shrinkage and loss of larger (cholinergic) neurons can occur during the evolution of AD. Whereas the differences in neuron size are highly correlated with oxidative damage and duration of disease among the AD cases, the increase in size is not statistically different from control cases. Interestingly, and supporting the previous idea, we observed that neurons from cases of Down syndrome are significantly larger than control cases. Down syndrome models AD in lesion formation and markers of oxidative damage, yet these changes occur decades earlier. In addition, Down syndrome cases also experience neuronal loss and have similar genetic risk factors. (Perry G, 2002), (Nunomura A, 2001)

To clarify the relationship between neuronal size and AD, They determined the ApoE genotype of the control cases to identify those at risk of AD. The ApoE genotype predicts the age at onset of AD and neuropathological progression. Among the control cases aged 42–85, those patients displaying at least one ApoE4 allele had a cross-sectional area significantly larger than those that do not. They wanted to examine whether a similar relationship was seen for younger individuals (aged 20–40); their data was collected from the Cuyahoga County Coroner’s Office in Cleveland. After analyzing age-matched and young controls, they noted that age-matched controls displaying at least one ApoE4 allele had a neuronal cross-sectional area significantly larger that those without ApoE4 alleles. In contrast, young controls present no correlation between neuronal size and ApoE genotype. These findings further suggest that the ApoE4 allele may play a role during aging and disease progression that influences neuron size. The alteration of lipid or axonal
transport of ApoE4 carriers may be a cause of the accumulation of the organelles within neurons early in the disease, leading to a neuronal enlargement and then to neuronal shrinkage and death during the progression of AD. (*Cash AD, 2003*)

Altogether, these findings suggest that increased oxidative damage is not the terminal sequelae of the disease but instead plays an initial role and is inversely correlated with neuronal size. These results also suggest that damage does not mark further destruction by ROS and is instead marked by a broad array of increased cellular defenses. It can be argued that these defenses are responsible for the reduction of damage if we view AD in isolation. However, when seen in the context of other conditions where ROS are involved and damage is either limited or absent, such as Parkinson’s disease, this result leads us to consider whether oxidative damage noted in AD may be better thought of as homeostatic, i.e. that oxidative damage could initiate signal transduction pathways to manipulate cellular responses to stress, which is characterized by increased levels of ROS. (*Perry G, 2002*)

**Sources of Oxidative Stress in Alzheimer’s Disease (Mitochondria)**

Mitochondria are essential organelles for neuronal function because the limited glycolytic capacity of these cells makes them highly dependent on aerobic oxidative phosphorylation for their energetic needs. However, oxidative phosphorylation is a major source of endogenous toxic free radicals, including hydrogen peroxide (H2O2), hydroxyl (•OH), and superoxide (O−2 •) that are products of normal cellular respiration. (*Wallace DC, 1999*)

With inhibition of the electron transport chain, electrons accumulate in complex I and coenzyme Q, where they can be donated directly to molecular oxygen to give O−2 • that can be detoxified by the
mitochondrial manganese superoxide dismutase (MnSOD) to give H2O2 that, in turn, can be converted to H2O by glutathione peroxidase (GPx). However, O−2 • in the presence of (nitric oxide) NO•, formed during the conversion of arginine to citrulline by nitric oxide synthase (NOS), can originate peroxynitrite (ONOO−). Furthermore, H2O2 in the presence of reduced transition metals can be converted to toxic •OH via Fenton and/or Haber Weiss reactions, a process that they have specifically localized to neurofibrillary pathology in AD. If the amount of free radical species overwhelms the capacity of neurons to counteract these harmful species, oxidative stress occurs, followed by mitochondrial dysfunction and neuronal damage. Reactive species generated by mitochondria have several cellular targets including mitochondrial components themselves (lipids, proteins, and DNA). The lack of histones in mitochondrial DNA (mtDNA) and diminished capacity for DNA repair render mitochondria an easy target to oxidative stress events. *(Smith MA, 1997)*

Besides the key role of mitochondria in the maintenance of cell energy and generation of free radicals, these organelles are also involved in cell death pathways, namely apoptosis. There are three main apoptotic pathways leading to the activation of caspases, which converge onto mitochondria and are mediated through members of Bcl-2 family such as Bid, Bax, and Bad. The end result of each pathway is the cleavage of specific cellular substrates, resulting in the morphological and biochemical changes associated with the apoptotic phenotype. The first of these depends on the participation of mitochondria (mitochondrial pathway), the second involves the interaction of a death receptor with its ligand (death receptor pathway), and the third is triggered under conditions of endoplasmic reticulum (ER) stress (ER-specific pathway). *(Pereira C, 2004)*

Mitochondria in AD were studied in laboratory using in situ hybridization to mtDNA, immunocytochemistry of cytochrome oxidase, and morphometry of electron micrographs of biopsy
specimens to determine whether there were mitochondrial abnormalities in AD. They found that the neurons showing increased oxidative damage in AD also possess a striking and significant increase in mtDNA and cytochrome oxidase. Surprisingly, much of the mtDNA and cytochrome oxidase is found in the neuronal cytoplasm and, in the case of mtDNA, in vacuoles associated with lipofuscin, whereas morphometric analysis showed that mitochondria are significantly reduced in AD. They also observed an overall reduction in microtubules in AD compared to controls. Altogether, these data indicated that the abnormal mitochondrial turnover, as indicated by increased perikaryal mtDNA and mitochondrial protein accumulation in the face of reduced numbers of mitochondria, could be due to a defective microtubule system resulting in deficient mitochondrial transport. (Cash AD, 2003), (Hirai K, 2001)

Furthermore, They analyzed the ultrastructural features of vascular lesions and mitochondria in brain vascular wall cells from human AD, YAC, and C57B6/SJL transgenic positive Tg(+) mice overexpressing AβPP. They observed a higher degree of amyloid deposition, overexpression of oxidative stress markers, mtDNA deletion, and mitochondrial structural abnormalities in the vascular walls of human AD, Yeast artificial chromosome (YAC), and C57B6/SJL Tg (+) mice when compared to the respective controls. All the abnormalities observed occur before neuronal degeneration and amyloid deposition. (Aliev G, 2004)

Results indicate a clear involvement of oxidative stress, mitochondria dysfunction, and neuronal damage/death during AD evolution. In fact, an intricate interorganelle crosstalk was previously suggested by Ferri and Kroemer, who reviewed the participation of distinct organelles, namely the nuclei, lysosomes, ER, and Golgi, in the release of death signals that converged in mitochondria, the central executioner. (Ferri KF, 2001)
Redox-Active Metals (Iron And Copper)

In AD patients, overaccumulation of iron in the hippocampus, cerebral cortex, and basal nucleus of Meynert colocalizes with AD lesions, senile plaques, and neurofibrillary tangles. Iron is an important cause of oxidative stress in AD because it is found in considerable amount in the AD brain, and, as a transition metal, is involved in the formation of hydroxyl radicals via Fenton reaction. Furthermore, it has been reported that amyloid-β itself is a substrate for hydroxyl radicals. Amyloid-β extracted from postmortem AD brains present oxidative modifications such as carbonyl adduct formation, histidine loss, and dityrosine cross-linking, making this protein less water soluble and less susceptible to degradation by the proteases. Furthermore, it has been reported that amyloid-β deposition and AβPP cleavage and synthesis are promoted by the presence of iron. (Connor JR, 2001), (Rogers JT, 2002)

Huang and collaborators presented in vitro evidence that trace levels of zinc, copper, and iron are initiators of amyloid-β1-42-mediated seeding process and amyloid-β oligomerization, and these effects were abolished by chelation of trace metals. They reported that rRNA provides a binding site for redox-active iron and serves as a redox center within the cytoplasm of vulnerable neurons in AD in advance of the appearance of morphological change indicating neurodegeneration. (Huang X, 2004)

Data from one laboratory and others indicate that heme oxygenase-1 (HO-1) is induced in AD brains. HO-1 catalyzes the conversion of heme to biliverdin and iron. Biliverdin, in turn, is reduced to bilirubin, an antioxidant. Since HO-1 is induced in proportion to the level of heme, the induction of HO-1 suggests that there may be abnormal turnover of heme in AD. This idea is consistent with the mitochondrial abnormalities associated to AD, since it is well known that many heme-containing enzymes are found in mitochondria. (Smith MA, 1994), (Premkumar DR, 1995)
Ultrastructural studies concerning mitochondria suggest a high rate of mitochondrial turnover and redox activity in the residual body of lipofuscin, the lysosomes being probable sources of heme. In turn, the increase in heme induces synthesis of more HO-1, suggesting that mitochondrial turnover promotes oxidative stress via increase of redox-active iron. Furthermore, they observed that oxidized nucleic acids are commonly observed in the cytoplasm of the neurons that are particularly vulnerable to degeneration in AD. *(Hirai K, 2001), (Honda K, 2005)*

8OHG, a marker of nucleic acids oxidation, is likely to form at the site of •OH production, a process dependent on redox-active metal-catalyzed reduction of H2O2 together with cellular reductants such as ascorbate or O−2. Interestingly, the levels of 8OHG are inversely related to the extent of amyloid-β deposits being this oxidative marker found distant from the amyloid-β deposits, suggesting a complex interplay between amyloid-β and redox metal activity that may be critical to metal dynamics within the neuronal cytoplasm. A possible key element to these dynamics is mitochondria in neuronal cell body. *(Nunomura A, 1999)*

Copper can also participate in the Fenton reaction to generate ROS. Although conflicting results exist concerning the amount of copper and the formation of senile plaques, there is accumulating evidence that both iron and copper in their redox competent states are bound to neurofibrillary tangles and amyloid-β deposits. However, a recent study reported that cognitive decline correlates with low plasma concentrations of copper in patients with mild to moderate AD. *(Finefrock AE, 2003), (Pajonk FG, 2005)*

**Alzheimer’s disease lesions: cause or consequence of oxidative stress?**
At the time oxidative damage was established in AD, the putative source of ROS was supposed to be the lesions. However, evidence exists supporting the idea that instead of being the source, amyloid-β and hyperphosphorylated tau possess protective/antioxidant properties. Amyloid-β peptide Amyloid-β can be produced by numerous types of cells such as neurons, astrocytes, neuroblastoma cells, hepatoma cells, fibroblasts, and platelets, suggesting, along with its conserved sequence among different species, that this peptide should have an important function in normal cell development and maintenance. Yet, neurons and smooth muscle cells show the highest levels of expression. *(Atwood CS, 2003)*

An antioxidant role for amyloid-β in vivo is in agreement with recent data on the distribution of oxidative damage to AD neurons. 8OHG markedly accumulates in the cytoplasm of cerebral neurons in AD. Unexpectedly, an increase in amyloid-β deposition in cortex is associated with a decrease in neuronal levels of 8OHG, i.e. with decreased oxidative damage. Similar negative correlation between amyloid-β deposition and oxidative damage is found in patients with Down syndrome. Amyloid-β deposits observed in both studies mainly consist of early diffuse plaques, meaning that these diffuse amyloid plaques, may be considered as a compensatory response that reduces oxidative stress. *(Smith MA, 2000), (Nunomura A, 1999)*

The strong chelating properties of amyloid-β for zinc, iron, and copper explain the reported enrichment of these metals in amyloid plaques in AD and suggest that one function of amyloid-β is to sequester these metal ions. Chelation of transition metals in a redox-inactive form may theoretically serve to inhibit metal-catalyzed oxidation of biomolecules. Methionine at residue 35 in the amyloid-β sequence can both scavenge free radicals and reduce metals to their high-active low-valency form, thereby possessing both anti- and pro-oxidative properties. *(Dong J, 2003)*
Reduced metal ions are highly active oxidants and can catalyze further oxidation of biomolecules. For instance, they produce highly reactive hydroxyl radicals from H2O2, an important by-product of mitochondria electron transport chain. These data indicate that amyloid-β is a lipophilic metal chelator with a metal-reducing activity. However, an intricate combination of metal chelation, metal reduction, and radical scavenging can thus be expected to govern the overall activity of amyloid-β towards oxidation, which may basically embrace the full spectrum of anti and pro-oxidative effects. *(Cadenas E, 2000)*

In various biochemical studies, amyloid-β efficiently initiates oxidation of different biomolecules. It induces peroxidation of membrane lipids and lipoproteins, generates H2O2 and 4-HNE in neurons, damages DNA, and inactivates enzymes. In contrast, amyloid-β is one of the most important antioxidants of cerebrospinal fluid (CSF). However, amyloid-β-dependent oxidation processes require fibrillation, the presence of transition metals and methionine on residue 35. Indeed, amyloid-β must be present in a relatively high concentration (micromolar range), and the aggregation and fibrillation of amyloid-β occurs only if the peptide is “aged”. The presence of transition metals is a requisite for amyloid-β aggregation and its pro-oxidative activity. *(Kontush A, 2001), (Butterfield DA, 2002)*

The toxicity of amyloid-β is likely to be mediated by a direct interaction between this peptide and transition metals with subsequent generation of ROS. In this line, it has been shown that clioquinol, a metal-protein-attenuating compound that inhibits zinc and copper ions from binding to amyloid-β, is producing very encouraging results in the treatment of AD. *(Ritchie CW, 2003)*

It has been demonstrated that substituting methionine 35 by another amino acid abrogates or diminishes significantly the pro-oxidant action of amyloid-β. Methionine 35 can scavenge free radicals and reduce transition metals to their high-active low-valency form,
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thereby exhibiting both anti- and pro-oxidative properties. *(Butterfield DA, 2004)*

Increases in metal ion accumulation and oxidative stress in AD brain are associated with changes in the concentration of soluble and deposited amyloid-β. It has been shown that metabolic stress promotes AβPP expression and changes the metabolism of AβPP from the nonamyloidogenic to the amyloidogenic pathway. *(Atwood CS, 2003)*

Oxidative stress occurring in cytoplasm is inversely correlated with AD lesions. Furthermore, unpublished data from laboratory show that intracellular amyloid-β is inversely related to oxidative damage. Lue et al. found that in situ soluble amyloid-β levels are inversely correlated with synaptic loss. These observations suggest that the accumulation of amyloid-β in AD and probably Down syndrome and aging brains may be aimed at chelating metal ions to prevent oxidative events. *(Lue LF, 1999)*

**Tau Protein**

In the adult human brain, tau proteins are found essentially in neurons. Tau proteins bind microtubules through the microtubule-binding domains, and this assembly depends partially upon the degree of phosphorylation, since hyperphosphorylated tau is less effective than hypophosphorylated tau on microtubule polymerization. Besides the role in microtubule stabilization, tau has other functions, such as membrane interactions or anchoring of enzymes. *(Buee L, 2000)*

Among the 80 Ser/Thr residues on tau, at least 30 phosphorylation sites have been described, most of which occur on Ser-Pro and Thr-Pro motives. In fact, phosphorylation of Ser262, located in the first
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microtubule-binding domain, dramatically reduces the affinity of tau for microtubules in vitro. Nevertheless, this site alone is insufficient to abolish tau binding to microtubules. Thus, phosphorylation outside the microtubule-binding domains may also strongly influence tubulin assembly by modifying the affinity between tau and microtubules. By regulating microtubule assembly, tau has a role in modulating the functional organization of the neuron, particularly in axonal morphology, growth, and polarity. (Hamdane M, 2003)

In AD, hyperphosphorylated tau accumulates in neurons, aggregates into paired helical filaments, and loses its microtubule-binding and stabilizing functions, leading to neuronal degeneration. The abnormal phosphorylation of tau associated with AD may be related to either an increase in kinase activity (glycogen synthase kinase 3β, cyclin-dependent kinase-5, p42/44 MAP kinase, p38 MAPK, stress-activated protein kinases, and mitotic protein kinases) or a decrease in phosphatase activity (protein phosphatases 1, 2a, 2b). (Garcia ML, 2001)

However, there is evidence indicating that hyperphosphorylated tau exerts protective functions. It has been shown that oxidative stress and the modification of tau by-products of oxidative stress lead to protein aggregation (neurofibrillary tangles) and enable neurons to survive decades. (Takeda A, 2000)

Although the neurofilament-heavy subunit has a long half-life, the same extent of carbonyl modification is found throughout the normal aging process, as well as along the length of the axon, suggesting that oxidative-stress-modified molecules are under tight regulation. Neurofilament and tau proteins appear adapted to oxidative stress due to their high content of lysine–serine–praline (KSP) domains. Therefore, these molecules may work as a buffer by absorbing lipoxidation-derived and glycoxidation-derived aldehydes. (Wataya T, 2002)

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Since phosphorylation plays a pivotal role in redox balance, it is perhaps not surprising that oxidative stress, through activation of MAP kinase pathways, leads to phosphorylation. Changes such as MAP kinase and HO-1 may be but a few of the many responses that interrelate to lipid peroxidative modification. Seen as such, oxidative damage is no longer an end stage event, but rather a signal of an underlying change of state. (Zhu X, 2001)

Instead of viewing oxidative stress as the breach of antioxidant defenses, They argue that this seldom happens in chronic conditions, pathological and physiological, and that a better understanding occurs by viewing each circumstance as a different homeostatic balance in which ROS plays a key regulatory role. In early stages of AD, neuronal cells, despite showing increased oxidative damage, may actually be in homeostatic balance. If cells survive and function in the presence of high levels of oxidative stress, it is because critical systems of cells are not damaged. In this way, detection of increased oxidative damage in cells that survive must be associated with a commensurate increase in compensatory mechanisms such as amyloid-β deposition and hyperphosphorylated tau. (G.Qureshi, 2007)

However, with the progression of AD and the consequent increase of ROS levels, efficient removal of amyloid-β-metal complexes and, probably, hyperphosphorylated tau would be overtaken by their disproportionately high generation, resulting in an uncontrollable growth of plaques and neurofibrillary tangles and, consequently, an increase in reactive species generation. This would result in a feedback mechanism that could exacerbate plaque, neurofibrillary tangles growth, and reactive species generation, leading to a functional demise of neurons. (G.Qureshi, 2007)
Role of Oxidative Stress in Multiple Sclerosis (MS) and Amyotrophic Lateral Sclerosis (ALS)

The Role of Oxidative Stress in the Pathogenesis of Multiple Sclerosis:

Introduction

Pathologically, MS is characterized by perivenous infiltration of lymphocytes and macrophages into the CNS parenchyma, resulting in demyelinative lesions, termed plaques. These plaques are associated with oligodendrocyte death, axonal damage and even neuronal loss. The etiology of MS has not yet been fully elucidated, and it is attributed to both genetic and environmental causes. Genetic factors such as HLA-DR2 and DQ polymorphisms increase disease susceptibility. Interleukin-1 beta, interleukin-1 receptor, immunoglobulin Fc receptor genes, and apolipoprotein E genes (APO-E) have also been suggested as having a substantial effect on the susceptibility to MS. 

(Schrijver HM, 1999), (Evangelou N, 1999)

It is believed that immunological mechanisms have a central role in disease initiation and progression in MS. It is well known that proinflammatory cytokines, such as interferon γ and tumor necrosis factor β (TNF-β) released by activated TH-1 cells, up-regulate the expression of cell-surface molecules on neighboring lymphocytes and antigen-presenting cells. The binding of putative MS antigens, especially components of myelin such as myelin basic protein (MBP), myelin-associated basic glycoprotein (MOBP), myelin oligodendrocytes glycoprotein (MOG), proteolipid protein (PLP) and others, by the trimolecular complex, the T-cell receptor (TCR), and major histocompatibility complex (MHC) class II molecules on
antigen-presenting cells, may trigger either an enhanced immune response against the bound antigens or anergy. *(Stinissen P, 1997)*

Autoantibodies against MBP and MOG have been found in MS patients. In addition to the autoimmune response, oligodendrocyte death, axon damage, and neuronal loss also have been associated with the inflammatory attack on the CNS. *(Egg R, 2001)*

**Oxidative Stress and Inflammation**

It is well established that inflammation might raise ROS levels leading to OS. Demyelinative plaques in the CNS of MS patients are associated with an inflammatory reaction orchestrated by activated T cells, macrophages, and endogenous glial cells (astroglia and microglia). These cells produce a variety of proinflammatory and neurotoxic factors, including proinflammatory cytokines, of which interleukin (IL)-1α/β and tumor necrosis factor (TNF)-α/β play a predominant role. *(Saha RN, 2001)*

The proinflammatory cytokines, IL-1β and TNF-α, were found to inhibit the expression of myelin genes, MOG, CNPase, and PLP, in human primary oligodendrocytes through the alteration of cellular redox. These effects were blocked by the antioxidants N-acetylcysteine and pyrrolidine dithiocarbamate, indicating that they exert these effects through OS. *(Jana M, 2005)*

One of the most abundant sources of ROS, apart from the electron-transport chain of mitochondria, is the respiratory burst system of activated microglia. Microglia are the resident macrophage-like cells of the CNS. They play a pivotal role in the innate immune response of the CNS and are the first line of defense against microorganism invasion and injury. Microglia are extremely responsive to environmental and immunological challenges, and are the predominant cells producing inflammation-mediated OS in the CNS. Activated microglia release proinflammatory cytokines and chemokines, as well as proteases and reactive oxygen and nitrogen species. This system operates intermittently: when it is turned on,
large quantities of ROS, especially superoxide ions (O−2), are generated on the microglial external membrane, and then released into the surroundings. Other ROS includes hydroxyl radical (OH•), hydrogen peroxide (H2O2), peroxynitrite (ONOO), and nitric oxide (NO•), the latter produced by reactive astrocytes via cytokine-mediated induction of nitric oxide synthase. *(Dawson TM, 1998)*

A study demonstrated that LPS-activated microglia generate peroxynitrite that mediate oligodendroglial death. Up-regulation of inducible nitric oxide synthase (iNOS) and activation of superoxide-generating NADPH oxidase in microglia contribute to the formation of peroxynitrite and, thereby, the killing of pre-oligodendrocytes. Redox reactions are involved in the activity of matrix metalloproteinases (MMPs), which are important to T cell trafficking into the CNS. *(Li J, 2005)*

It is well established that extracellular proteolytic enzymes are implicated in the pathology of both MS and EAE and, in particular, the role of MMPs. Increasing evidence suggests that enhanced production of ROS activates the MAP kinases, c-Jun N-terminal protein kinase (JNK) and mitogen-activated protein kinase MAPK (p38). These phosphorylated intermediates at the stress-activated pathway induce the expression of matrix metalloproteinases (MMPs). The roles of MMPs in neuro inflammation include disruption of the blood–brain barrier (BBB) by the breakdown of the extracellular matrix around blood vessels, leading to inflammatory responses and pathological damages involved in the etiology of MS. *(Rosenberg GA, 2002), (Offen D, 2004)*

**Oxidative Stress in MS**

**Evidence of oxidative stress in EAE**

Studies suggest that glutamate plays a role in MS pathophysiology. Oligodendrocytes, the myelin-producing cell of the CNS, are highly vulnerable to glutamate excitotoxicity, mainly via the AMPA/kainate receptors, which have higher permeability to Ca2+.
Demyelinating lesions caused by excitotoxins can be similar to those observed in MS, causing histological damage. Moreover, treatment with AMPA/kainate receptor antagonists was found to ameliorate axonal damage and to improve the clinical score of EAE. (*McDonald JW, 1998*, *Smith T, 2000*)

During EAE, both blood-borne macrophages, as well as activated, resident microglial cells, are considered to be involved in inflammatory reactions in the CNS, resulting in neurological deficits. Oxygen and nitrogen free radicals generated by macrophages have been implicated as mediators of demyelination and axonal injury in both EAE and MS. (*Bo L, 1994*)

Free radicals can activate certain transcription factors, such as nuclear transcription factor-kappa B (NF-κB), which up-regulate the expression of many genes involved in EAE and MS, such as tumor necrosis factor-α (TNF-α), nitric oxide synthase (iNOS), intracellular adhesion molecule 1 (ICAM-1), and vascular-cell adhesion molecule 1 (VCAM-1). (*Barnes J, 1997*)

Ruuls et al. showed that macrophages and microglial cells, isolated from the CNS of Lewis rats with clinical signs of EAE, exhibited significantly elevated spontaneous and phorbol myristate acetate (PMA)-inducible levels of ROS compared to similar cells isolated from healthy controls, sham (complete Freund’s adjuvant, CFA)-immunized rats, as well as rats sacrificed before the manifestation of clinical signs of EAE. (*Ruuls SR, 1995*)

Using MOG-induced EAE, Espejo et al. found increased levels of oxidative stress, manifested as increased levels of inducible NO synthase, nitrotyrosine, and malondialdehyde. They also found an increase in tissue-protective antioxidant factors metallothionein I+II (MT-I+I). (*Espejo C, 2002*)
Penkowa et al. showed that MT-I+II deficient mice are more susceptible to EAE and that demyelination and axonal damage are significantly increased in MT-I+II deficient mice during EAE.  
*(Penkowa M, 2003)*

In a study, Espejo et al. noted the raised expression of metallothionein proteins in the peak clinical score and through recovery, implicating a role in the clinical recovery of EAE.  
*(Espejo C, 2005)*

**Evidence of oxidative stress in MS subjects**

Numerous studies of patients with MS have shown increased free radical activity, and/or deficiencies in important antioxidant enzymes compared with healthy controls. Generation of ROS in vivo has been inferred from the presence of lipid peroxidation products in the CSF and plasma of MS patients, the increase of free radical activity and decrease of major antioxidant enzymes, and the presence of 3-nitrotyrosine (a marker of peroxynitrite activity) in demyelinated lesions.  
*(LeVine SM, 1992), (Liu JS, 2001)*

Bizzozero et al. found increased carbonyl levels in both the white and grey matter of brains from MS patients, as compared with controls, indicating oxidative damage of CNS proteins in MS.  
*(Bizzozero OA, 2005)*

Karg et al. found increased lipid peroxidation, elevated levels of oxidized glutathione, and a reduction in the plasma vitamin E to lipid ratio during the active phase of MS. Reduced activity of the antioxidant enzymes, superoxide dismutase, and glutathione peroxidase was found in the red blood cells of MS patients.  
*(Karg E, 1999)*

In another study, oxidative damage in the CNS was provoked by the release of iron from injured cells and low levels of both enzymatic (glutathione peroxidase), and nonenzymatic antioxidants,
particularly ubiquinone and vitamin E in the plasma and lymphocytes. Examination of cerebrospinal fluid showed evidence of a significantly higher concentration of isoprostanes, an increase of malondialdehyde (MDA) and glutathione reductase activity, and a decrease of glutathione peroxidase activity. *(Syburra C, 1999)*

Koch et al. demonstrated that increased ROS formation occurs in all subgroups of MS. The highest production of ROS was found in patients with Primary Progressive Multiple Sclerosis (PPMS). Lipid peroxidation in leukocytes was not different in MS patients from that in healthy controls, but total antiradical activity in leukocytes was enhanced, indicating an up-regulation of cellular antioxidant defenses to withstand ROS-induced cell damage. *(Koch M, 2006)*

Ferretti et al. found that intracellular spontaneous ROS production in leukocytes from MS patients was higher with respect to cells from control subjects (p < 0.001). The addition of phorbol myristate acetate (PMA), a triggering agent, induced a higher formation of ROS both in leukocytes from MS patients and controls. However, the PMA-induced production of ROS was significantly higher in leukocytes from MS. Significant positive correlations were established between intracellular spontaneous or PMA-induced production of ROS in leukocytes isolated from MS patients and the clinical parameters used to evaluate disease disability, such as expanded disability status scale (EDSS), brain lesions evaluated by MRI, and visual evoked potential. *(Ferretti G, 2006)*

Vladimirova et al. found oxidative damage to DNA in plaques of MS brains induced by activated mononuclear cells. They also found higher production of ROS in mononuclear cells of MS patients, compared with controls. These elevated ROS and nitric oxide levels led to oxidative damage to DNA, including mitochondrial DNA, in association with inflammation in chronic active plaques. *(Vladimirova O, 1998)*
The most abundant source of ROS associated with CNS inflammation in MS is the respiratory burst of macrophages and microglial cells following their activation by proinflammatory cytokines. This source may explain oxidative damage to white matter, especially plaque areas in MS patients. However, evidence of oxidative damage to grey matter is not likely to occur as a result of the inflammatory process, since in MS, these areas are less prone to inflammation but rather from glutamate excitotoxicity.  

(Bizzozero OA, 2005)

Increased levels of glutamate have been detected in the CSF of patients with MS, and glutamate release has been shown to underlie axonal damage and oligodendrocyte cell death in MS lesions. During primate evolution, retrovirus elements were integrated into the human genome. (Werner P, 2001)

Antony et al. described up-regulation of syncytin, an endogenous retrovirus glycoprotein, in activated astrocytes and microglia in acute demyelinating plaques of MS patients. Syncytin expression in activated glia caused the release of ROS, leading to oligodendrocyte damage and death. Antioxidants prevented syncytin-induced toxicity to oligodendrocytes. (Antony JM, 2004)

Antioxidants as Possible Treatment for MS

The pathogenic role of oxygen and nitrogen free radicals in MS led to the recognition that antioxidants might prevent free-radical mediated tissue damage and inhibit some of the early proinflammatory events that lead to inflammation and tissue destruction in EAE and MS. However, the main obstacle and challenge in MS treatment are to introduce substances into the brain through the BBB. (Gilgun-Sherki Y, 2001)

Clinical Studies
Due to the encouraging findings in the EAE models, many authors suggested that dietary antioxidant intake, i.e. vitamin E or selenium, may help to inhibit disease progression. Indeed, Jensen et al. showed that supplementation with antioxidants (6.6 mg of sodium selenite, 2 gm of vitamin C, and 500 I.U. of vitamin E per day) increased and normalized the glutathione peroxidase activity and cellular content of linoleic acid in erythrocytes and hematogenous cells within 3 weeks, with no effect on MS severity. 

(\textit{Jensen C, 1986})

The only actual study examining the efficacy and safety of an antioxidant was that of Spitsin. Spitsin et al. showed that oral administration of inosine, the precursor of uric acid (UA), raised serum UA for at least one year without reported adverse events. Of the 11 patients given inosine, 3 showed some evidence of clinical improvement, and there was no sign of disease progression in the remaining patients. Gadolinium-enhanced lesions, observed in two patients before receiving inosine, could not be detected after either 10 or 12 months of inosine treatment. These interesting data provide evidence that serum UA levels can be readily manipulated, and that the benefit of UA should be further evaluated in a large cohort MS study. (\textit{Spitsin S, 2001})

\section*{The Role of Oxidative Stress in the Pathogenesis of Amyotrophic Lateral sclerosis (ALS)}

\section*{Introduction}

A major breakthrough in deciphering the molecular mechanisms underlying ALS was provided in 1993 by the observation that mutations in the gene coding for the antioxidant enzyme Cu, Zn superoxide dismutase (SOD1) are carried by one-fifth of fALS patients (i.e. 2\% of all ALS cases). This enabled the development of novel experimental models such as transgenic mice and cultured cells expressing mutant SOD1 (mutSOD1), and numerous studies
have been performed to investigate the toxic function of the mutant enzymes. (*Bendotti C, 2004*)

Increasing evidence indicates that cellular functions impaired as a consequence of the expression of mutSOD1 converge on pathways that could be activated in sporadic ALS by other toxic factors. Loss of neurons in ALS results from a complex interplay of oxidative injury, excitotoxic stimulation, aggregation and dysfunction of critical proteins, and genetic factors. (*Andersen PM, 2006*)

Even when due to a single gene defect, as in the case of SOD1 mutations, ALS is the result of a complex neurotoxic cascade that involves a molecular cross-talk between motor neurons and glia and between motor neurons and muscle. Indeed, the loss of motor neurons is associated with the activation of astrocytes and microglia, and, therefore, ALS must be regarded as not only a multifactorial disease but also a multisystemic disease, in which molecular signals are exchanged among different cell types and trigger a cascade of events leading to the degeneration of motor neurons. (*Sargsyan SA, 2005*)

**Neuroinflammation, Oxidative Stress, and ALS**

Similar to AD, microglial involvement is indicated to play an important role in ALS. About 10–20% of ALS cases are inherited, and, of the inherited cases, about 20% are caused by mutations in the gene encoding superoxide dismutase-1. The disease is considered to result from a deleterious gain of some cytotoxic function, rather than simply loss of dismutase activity. The search for the gain of function has led researchers to the investigation of possible mechanisms such as oxidative stress, impaired mitochondrial function, or excitotoxicity. (*Hand CK, 2002*)

Neuropathologically, ALS is characterized by degeneration and loss of motor neurons, gliosis, and intracellular inclusions in degenerating neurons and glia. A mouse model for ALS G93A-
SOD1 develops severe motoneuron disease between 100 and 120 days of age. Messenger RNA for multiple inflammatory cytokines, especially TNFα, IL1α, and IL1β and its principal receptor TNF-RI, are up-regulated in the spinal cords of these mice beginning at 80 days of age and continue to increase during the paralytic phase of the disease. Protein carbonylation also follows a similar pattern. The up-regulation of cytokines and protein carbonylation were found to precede the up-regulation of caspase genes and death receptor components and the onset of paralysis. (Hensley K, 2002)

Oxidative Stress and ALS

A role for ROS-mediated oxidative stress in ALS was proposed in many studies reporting the occurrence of other typical oxidation products (such as malondialdehyde, hydroxynonenal, oxidized proteins, DNA, and membrane phospholipids) both in sporadic and familial ALS patients and in several model systems as well. Furthermore, administration of several antioxidant molecules has been proven beneficial in a mouse model for fALS, indicating that oxidative stress is indeed a component of this pathology, although a generalized stress may be hardly considered specific for ALS, but rather a phenomenon preceding or accompanying neurodegeneration. (Carri MT, 2003), (Carri MT, 2006)

Inside the cells, mitochondria represent a preferential target for the noxious action of ROS, because the majority of cellular ROS is produced in these organelles and because mitochondrial functionality depends largely on membrane integrity. Indeed, there is substantial evidence of early mitochondria damage in both Sporadic amyotrophic lateral sclerosis (sALS) and familial amyotrophic lateral sclerosis (fALS) patients that might be attributed, at least in part, to intracellular oxidative stress. (Manfredi G, 2005)

Metabolic and morphological alterations of mitochondria have been observed in a variety of experimental models of ALS and in postmortem tissues from patients as well. Such damage may prelude
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to apoptotic cell death of motor neurons, which is likely to occur in ALS. In fact, ROS are very well-known inducers of cell death: they regulate early and late steps of apoptosis, and inhibition of ROS production also protects against apoptosis. *(Fleury C, 2002)*

A further support to the concept that oxidative stress plays a major role in ALS as in other neurodegenerative diseases is provided by the knowledge that, as mentioned above, mutations in the gene coding for the antioxidant enzyme SOD1 account for one-fifth of fALS cases. To date, there are more than 120 different SOD1 point mutations reported in fALS families (see www.alsod.org for an updated list) with various frequency in the population. Mutations are distributed in all five exons of the gene and result in alteration of amino acids scattered throughout the SOD1 structure: while some mutations affect the active site, others are positioned at the dimer interface, or inside β-strands or connecting loops. *(Rosen DR, 1993)*

Wild-type SOD1 (wtSOD1) is a very-well-characterized homodimeric enzyme present in virtually every cell type that binds zinc and copper ions, with the Cu atom playing the active role in the scavenging activity, i.e. the removal of superoxide and prevention of further generation of ROS. It has been demonstrated that a small fraction of wtSOD1, which was considered to be exclusively cytosolic, and of mutSOD1 associates with various mitochondria compartments, including the inner membrane, the intermembrane space, and the matrix, both in vitro and in vivo in the brain and spinal cord of mutSOD1-transgenic mice and in human spinal cord. Because of this localization, it has been proposed that mutSOD1 may be impairing cell function directly into mitochondria, but the mechanisms involved in such damage are only partly understood. *(Liu J, 2004), (Vijayvergiya C, 2005)*

In fact, it is generally considered that mutSOD1 acquires new noxious functions causing the pathological phenotype. However, it is not obvious which the toxic function shared by so many different mutSOD1s is. It has been proposed that the toxic function of
mutSOD1 may reside in the propensity to catalyze a novel pro-oxidant enzyme chemistry, consequent to alteration of the metal-binding active site. Imbalance of ROS metabolism might be elicited by fALS-SOD1 mutants because of their imperfect folding, possibly inducing a loosening of protein structure; in turn, this would result in modification of the active site and alteration of substrate specificity of the enzyme. However, wt-like mutants are very similar to wtSOD1 in their biophysical properties in vitro and, therefore, their toxic behavior in vivo is less obvious.

(Valentine JS, 2005)

SOD1 is an abundant component of many cell types, accounting for up to 1% of total cytoplasm proteins in some areas of the CNS. It has been suggested that, similar to what occurs in other neurodegenerative “conformational” diseases, formation of insoluble aggregates of misfolded mutSOD1 contributes to cell death in fALS. For instance, it is known that neurons and astrocytes of sALS patients contain cytoplasmic aggregates that show immunoreactivity for SOD1 and ubiquitin; similar inclusion bodies were also observed in SOD1-linked fALS patients.

(Bruijn LI, 1998)

Jonsson et al. have proposed that the motoneuron degeneration could be due to long-term exposure to misfolded aggregation-prone disulphide-reduced SOD1, which constitutes minute subfractions of the stable mutants and larger proportions of the unstable mutants. However, elevation of the heat shock protein 70 (Hsp70), a chaperone protein that is able to prevent mutSOD1 aggregate formation and toxicity in primary motor neurons in culture, does not effect toxicity in mouse models of familial amyotrophic lateral sclerosis. (Jonsson PA, 2006)

Pasinelli et al. have provided evidence of a direct link between SOD1 and an apoptotic pathway by demonstrating that both wtSOD1 and mutSOD1 bind the anti-apoptotic protein Bcl-2 in ALS models and patients, and it is known that cytochrome c had a
reduced association with the inner mitochondrial membrane in the brain of transgenic mice expressing G93A-mutSOD1.  
*(Pasinelli P, 2004)*

In other studies, it has been reported that aberrant macromolecular aggregates of enzymatically inactive mutSOD1 accumulate in the mitochondria matrix of brain, but not in liver. However, little is known about the properties of either wtSOD1 or mutSOD1 within the mitochondria as well as about the relationship of mitochondrial SOD1 with the antioxidative defense system of this organelle. At the same time, little is known about the mechanisms of toxicity to mitochondria of mutSOD1, which are the chemico-physical properties of such mutants in the mitochondria and whether protein aggregation and elevated oxidative stress are causally linked. They have demonstrated that each of the 12 different mutSOD1s with widely differing biophysical properties accumulates in mitochondria of motoneuronal cells to a much greater extent than wild-type SOD1, and that its reactive cysteine residues become oxidized. *(Vijayvergiya C, 2005)*

MutSOD1s proteins in the mitochondria tend to form high molecular-weight aggregates, and their presence causes a shift in the redox state of these organelles and results in impairment of respiratory complexes (96). Therefore, the “general toxic property” of all mutSOD1s seems to descend from their localization, and they have observed that this localization seems to arise upon glutathione-mediated modification of cysteine residues causing permanence of mutSOD1s into mitochondria in an oxidized, aggregate state. In turn, mutSOD1s exacerbate oxidative stress through impairment of the respiratory chain and by shifting the GSH/GSSG even more towards the oxidized form of glutathione. *(Ferri A, 2006)*

The common properties shared by the mutSOD1 proteins and not by wtSOD1 protein might only become apparent within a specific cellular compartment and mutSOD1s cause oxidative stress mainly through mitochondrial damage. Other molecules may then represent
direct targets/propagators of damage in fALS, such as those involved in mechanisms of intracellular signal transduction. For instance, they have observed that oxidative inactivation of protein phosphatase calcineurin occurs upon expression of mutSOD1 in human neuronal cells and transgenic mice and in lymphocytes from sALS and fALS patients. (*Ferri A, 2004*)

MutSOD1 expression also causes an increase in activity of transcription factor NF-κB in experimental model for ALS similar to that observed in glial cells from ALS patients and could result in induction of neuroinflammation via activation of the COX2 (cyclooxygenase 2) pathway. Mitogen-activated protein kinase (MAPK) seems also to play a crucial role in the “non-cell-autonomous” mechanism of motor neuron death in ALS. In fact, early and persistent activation of p38MAPK was observed in the motor neurons (and also in hypertrophic astrocytes and microglial cells during the progression of the disease) of transgenic mice expressing mutSOD1-G93A; in these cells, mutSOD1 also triggers up-regulation of TNFα, TNFα receptors, and other cytokines as well. (*Ferri A, 2002*)

In a study paper, Rothstein et al. reported that beta-lactam antibiotics, including penicillin and ceftriaxone, are potential therapeutic drugs to treat ALS by modulating the expression of glutamate transporter GLT1 via gene activation. As proposed by Ji et al. beta-lactam antibiotics have long been known as metal chelators, and their beneficial effect likely involves the ability to attenuate metal toxicity. In this context, it is worth mentioning that desferoxamine (Desferal), an FDA-approved iron chelator that is used for iron overload disease secondary to multiple transfusions, seems to have several potentially beneficial effects in ALS, including induction of hypoxia-inducible factor-1 (which would cause the transcription of Vascular endothelial growth factor (VEGF), erythropoietin, and other hypoxia-related genes) and is currently considered for ALS therapy. (*Rothstein JD, 2005*), (*Ji HF, 2005*)
Copper and iron: the devil’s pair?

Alterations in copper homeostasis may also interfere with iron homeostasis. One link between Cu and Fe metabolism is represented by the enzyme ceruloplasmin (Cp), the copper protein of the plasma. Cp is sensitive to alteration of copper levels, because when copper is not adequately incorporated into Cp at the rate of the protein synthesis, the protein is rapidly degraded. Cp has a major role in iron metabolism; in fact, upon oxidization of Fe(II) to Fe(III) by Cp, iron can enter its transport pathway via incorporation into Tf. In the genetic disease aceruloplasminemia, accumulation of iron in neuroglia and neurons occurs. This has prompted the hypothesis that upon Cu imbalance, Cp activity is affected, and this leads to iron accumulation and dismetabolism. *(Harris ZL, 1998)*

A decrease in serum-Cp-specific oxidative activity has been reported in only one investigation, in a limited sample of ALS patients. In the transgenic fALS-mice model, alteration of Cu metabolism via removal of CCS does not induce decrease of serum Cp. Furthermore, both hypoxia and copper treatment increased Cp mRNA levels in hepatoma cells through activation of hypoxia-inducible factor (HIF)-1. *(Torsdottir G, 2000)*

Another link connecting Cu and Fe metabolism has been established in the yeast Saccharomyces cerevisiae, in which lack of SOD causes a substantial increase in the Fe demand of the cell. Superoxide radical, if not detoxified appropriately, can inactivate enzymes containing Fe–S clusters by oxidizing one Fe and causing its release from the cluster. The Fe–S clusters constitute the core of some crucial enzymes, such as those operating in mitochondria respiratory chain, but also of the above-mentioned “IRE-IRP machinery.” If such a link between copper and iron exists in man, as suggested by studies in cell cultures, one could hypothesize that in...
ALS patients, an imbalance in ROS production could be responsible for damage of Fe–S clusters; in turn, this could cause inactivation of both the iron regulatory protein / iron-responsive element (IRE/IRP) machinery and of mitochondrial enzymes. *(De Freitas JM, 2000)*

That metal homeostasis is altered in ALS is indicated also by observations on the level of expression of metallothioneins, which is increased in the spinal cords from patients and in fALS transgenic mice in which changes in metallothioneins (MT) expression occur before the onset of motor deficits or significant motor neuron pathology. Furthermore, fALS mice reach the onset of clinical signs and death significantly earlier in response to the reduction of MT expression. *(Puttaparthi K, 2002)*

Till date, ALS is believed to be a multifactorial and multisystemic disease, but the question as to which is the primary alteration causing this disease is still actively debated. Neurodegeneration may arise by converging pathways, such as ROS-induced damage of critical molecular targets, accumulation of misfolded proteins, and triggering of neuroinflammation and apoptotic pathways. Many consistent observations support a role for metal-mediated oxidative stress as one of the mechanisms contributing to the pathogenesis of ALS. Intracellular oxidative stress may be primed by many different mechanisms, including the presence of mutant SOD1, alterations in copper-handling and copperresponsive genes such as angiogenin and VEGF, and iron mishandling. Most of this evidence comes from studies in experimental models, while data in patients are scarce and sometimes contradictory. Nonetheless, because of the potential feasibility of new therapeutic approaches aimed at the interception of metal-mediated toxicity in ALS (e.g. with metal chelators), further studies in this field may be worth pursuing. *(G.Qureshi, 2007)*
Role of oxidative stress in Friedreich’s ataxia (FRDA)

Friedreich’s Ataxia and Iron

Friedreich’s Ataxia (FRDA), a degenerative disease with autosomal recessive inheritance affecting 1 in 40,000 births, is characterized by progressive limb and gait ataxia, areflexia, pyramidal signs in the legs, and hypertrophic cardiomyopathy. The disease is caused by a GAA repeat expansion in the first intron of the nuclear encoded gene for the protein frataxin, resulting in deficiency of this mitochondrial protein. The long GAA repeats apparently interfere with transcription by forming aberrant DNA structures or by promoting repressive heterochromatin forms. (*Patel PI, 2001*)

The severity of FRDA appears to correlate with the degree of frataxin deficiency. Frataxin has been claimed to have several functions, including iron storage and protecting mitochondria from oxidative damage, but its best defined role is the delivery of iron to the machinery responsible for the synthesis of iron sulfur clusters (ISCs), which serve as prosthetic groups for a variety of enzymes, collectively termed iron sulfur cluster proteins (ISPs). Inefficient ISP formation in FRDA causes a combined aconitase and respiratory chain (complex I III) deficiency and leads to mitochondrial accumulation of labile iron, which in turn promotes oxidative damage. (*Schagerlof U, 2008*)

Much of the information on the biochemical consequences of frataxin deficiency has been obtained in studies with cellular models including genetically manipulated yeasts, fibroblasts isolated from patient skin, immortalized lymphoblasts from patients, or other cell
types in which frataxin was suppressed ectopically. Most studies in these models focused on a set of properties reflecting discrete changes in energy production and resistance to oxidative stress. 

(N. Gadoth, 2011)

It is generally agreed that frataxin-deficient cells show a marked reduction in mitochondrial membrane potential (MMP), respiration rate, and ATP levels, impaired antioxidant capacity manifested as increased levels of ROS, and signs of oxidative damage detectable as increased protein carbonylation and apoptotic index. 

(Lu C, 2007)

A study in which the biochemical and cellular effects of frataxin deficiency and the associated misdistribution of cell iron were comprehensively analyzed in a single system was recently published. Histopathological examination of biopsies and magnetic resonance imaging (MRI) studies of patients have shown that iron accumulates regionally in heart muscle, spinocerebellar tracts (dentate nuclei), and the spinal cord of FRDA patients, often colocalizing with structurally damaged areas. The iron detected histochemically is ferric, non-heme iron (usually ferritin or hemosiderin), based on Perl’s acid ferrocyanide staining of biopsies, fixed cells, or tissues. (Wilson RB, 2006)

In patients, the identification of iron accumulation in particular organs is done by MRI, based on the ability of clustered iron to cause a local nonhomogeneity in the magnetic field and thereby decrease the MRI signal of surrounding water. This effect leads to variations in the apparent transverse relaxation rate ($R2^*$), or its inverse, $T2^*$, the indices used to quantify MRI signal decay. Thus, assuming a homogeneous external magnetic field, changes in $R2^*$ reflect variations in local iron concentration. In FRDA patients, changes in $R2^*$ have been attributed to regional iron accumulation in the dentate nuclei of the cerebellum, with a possible, although less likely, signal contribution from fluctuating deoxyhemoglobin levels resulting from regional changes in blood perfusion.
FRDA as a Paradigm of Misdistribution of Cell Iron

The notion that iron is improperly distributed in frataxin-deficient FRDA cells was originally based on the observation that mitochondrial accumulation of redoxactive iron is accompanied by depletion of the cytosolic LIP. The latter has been inferred from the reduced levels of cytosolic iron regulatory proteins found in mammalian cells and the activation of the iron regulon in frataxin-deficient yeasts. Experimental in vivo support for this assumption was provided by a study of cardiomyopathy in conditional frataxin-knockout mice, where cytosolic iron depletion in cardiomyocytes was shown to be associated with an increased rate of total cellular iron uptake and accumulation in the mitochondria. (Whitnall M, 2008)

At the cellular level, labile iron accumulation in mitochondria and its concomitant depletion in the cytosol were observed in frataxin-deficient cells probed with fluorescent sensors of labile iron and ROS targeted to specific cell compartments. The cause of iron accumulation in mitochondria and iron depletion from the cytosol in frataxin-deficient cells has been assumed to be the failure to export processed forms of iron from the mitochondria, either in labile forms or “packaged” in heme or ISCs, but this has not been established. (Lill R, 2008)

The fact that frataxin deficiency causes an increase in mitochondrial LIP and a reduction in cytosolic LIP is consistent with the presumed function of frataxin and the interdependence between the two pools. However, more importantly, it provides an opportunity for assessing the redistribution of iron as a tool for correcting the cell properties impaired by the misdistributed metal. (Kakhlon O, 2008)
Restoration of Functions by a Siderophore in FRDA Models

Most of the experience with chelation has been gained from treating patients with systemic iron overload, namely, patients with hemosiderosis who show iron accumulation in all body fluids and tissues. The objective of such treatments is bulk removal of iron from the system. In contrast, diseases of regional iron accumulation are often accompanied by systemic or regional iron deficiency that affects specific iron-dependent processes. In these diseases, it may be essential to conserve iron and ideally even to render it available for metabolic reuse. In the case of FRDA, mere removal of excess iron from the mitochondria may not alleviate defects that stem from inadequate delivery of iron to the ISC synthesis machinery, which is also located primarily in the mitochondria. (Lill R, 2008)

Oxidatively stressed frataxin-deficient cells have been shown to be rescued by treatment with the coenzyme Q10 analogue idebenone, an antioxidant and electron donor to the respiratory chain, as well as the classical iron-depleting chelator deferrioxamine. (Jauslin ML, 2003)

An alternative therapeutic approach to FRDA is based on iron redistribution, which entails chelation of a particular (labile iron pool) LIP, relieving cells or mitochondria from foci of accumulated labile iron and transfer of the metal, directly or indirectly, to endogenous acceptors and possibly to other compartments inside or outside the cells [83]. Ideally, the ultimate acceptors of siderophore-redistributed iron should be iron-requiring proteins, such as (Iron-sulfur clusters) ISC-containing enzymes. The approach utilizes agents such as the oral chelator 1,2-dimethyl-3-hydroxypyrid-4-one (deferiprone, DFP). (Sohn Y S, 2008)

Two clinically used chelators, deferrioxamine and deferasirox, known for their higher iron-binding affinities and their reduced
capacity for donating iron to cellular acceptors, restored the impaired functions to a limited extent. The study demonstrated for the first time that a chemical agent can act as a “frataxin surrogate” in the reconstitution of ISP activity and the correction of the defective energetic parameters observed in frataxin deficient cells. Similar to frataxin, DFP could act in FRDA either by detoxification of LIP, thus slowing down oxidative destruction of Iron-sulfur clusters (ISCs), or by iron donation, facilitating ISC repair or synthesis, or both. (Kakhlon O, 2008)

Clinical Application of Siderophores in FRDA

The recognition of FRDA as a disease of regional misdistribution of iron that affects a plethora of cell properties restorable in vitro by siderophore treatment has led to an initial efficacy-tolerability Phase I II open study of DFP. Nine FRDA adolescents were selected, who had been treated for years with idebenone, as a possible cardioprotective agent, but who showed no neuromuscular improvements. The cohort completed a 6-month treatment series with 20 30 mg/kg/day DFP and was followed periodically for neurological and hematological parameters. Iron accumulation in dentate nuclei was assessed using R2* MRI. DFP treatment significantly and selectively reduced iron accumulation in cerebellar dentate nuclei, as well as improving the ICARS score in the youngest patients, without causing systemic iron deficiency or affecting the hematological status. (Boddaert N, 2007)

Possible Side Effects of Chelators and Siderophores

Any chelation treatment of individuals with normal metabolism, let alone those with compromised iron metabolism, must be carried out with moderation, particularly when applied over extended periods of time with chelators of high iron-binding affinity and tissue accessibility. This concern is exemplified by chelator treatment of
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Aceruloplasminemia, which is accompanied by anemia in the background of massive iron overload in the brain and liver. Deferrioxamine and deferasirox removed excess iron from the liver but aggravated the disease-related anemia. In non-iron-overloaded humans and in experimental animals, moderate chelation treatments appear to be tolerated, presumably because of enhanced iron recycling and absorption from the diet. *(Skidmore FM, 2008)*

Although chelators can potentially induce a state of intracellular iron deficiency in the organism, that deficiency can be compensated by increased iron acquisition from serum transferrin by upregulated iron-uptake mechanisms. Theoretically, the potential complications of overchelation may be less pronounced in treatments based on siderophores. *(Wessling M, 2006)*

An example of a less predictable side effect of chelation with DFP specifically is the risk of approximately 1-2% of iron-overloaded patients developing neutropenia/agranulocytosis. Although a slightly higher incidence was found in the only trial of DFP in FRDA patients published so far, the sample number was too small to draw definitive conclusions as to the significance of the observations. Irrespective, the neutropenia/agranulocytosis condition is readily reversible by suspending DFP administration. *(Forni GLB, 2008)*

One prophylactic maneuver to reduce the side effects of chelation could be the coadministration of an antioxidant. Although the validity of such an approach remains to be demonstrated clinically, idebenone, which is thought to enhance the antioxidant capacity of cells by compensating for deficient electron transport is already in common use in FRDA and has been claimed to be beneficial at high doses. The antioxidant coadministration strategy may also have additional benefits because antioxidants could also synergize with DFP by rendering iron more accessible to chelation (via its reduction) and relocation. *(Di Prospero NA, 2007)*
Therapeutic approaches by micronutrients, phytochemicals and antioxidants

Prevention and Treatment of Neurodegenerative Diseases by Spice-Derived Phytochemicals

- Multiple Sclerosis

Experimental autoimmune encephalomyelitis (EAE) is a demyelinating disease of the central nervous system widely accepted to be an animal model for multiple sclerosis. Inflammation plays a major role in neuropathological processes associated with neutrophilic infiltrates, such as EAE and traumatic injury of the brain. Whether curcumin may influence inflammation in the CNS through the modulation of the CXC chemokine, macrophage inflammatory protein (MIP)-2, has been investigated. Astrocytes prepared from the neonatal brains of mice were stimulated with lipopolysaccharides (LPS) in the presence or absence of various amounts of curcumin. The latter inhibited the LPS-induced induction of MIP-2 gene expression, the production of MIP-2 protein, and the transcription of MIP-2 promoter activity. Thus, curcumin potently inhibits MIP-2 production at the level of gene transcription and offers further support for its potential use in the treatment of inflammatory conditions of the CNS.

(Tomita M, 2005)

Mohamed et al. showed that Thymoquinone (TQ) inhibits the activation of NF-κB in the brain and spinal cord of rats with EAE. They showed that the treatment of rats with TQ prevents myelin basic protein-induced EAE. TQ inhibited the perivascular cuffing and infiltration of mononuclear cells in the brain and spinal cord, increased levels of red blood cells and GSH, and inhibited the
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activation of NF-κB in the brain and spinal cord, consistent with the clinical signs of the disease. *(Mohamed A, 2005)*

• Alzheimer’s Disease

It is not clear what causes the plaques associated with AD, but a variety of factors seem to be involved. Mutations or variants of the gene for the blood protein Apolipoprotein E are associated with the disorder, as are a variety of lifestyle factors, such as diet and drugs. The mere process of aging is a factor, hence the name “senile dementia.” Smoking, drinking, impaired nutrition, lack of exercise, and excessive exposure to the sun could be contributory factors. India is one of the developing countries where AD is less prevalent, even when adjusted for age. Although AD affects about 3.1% of Americans between 70 and 79 years old on average, only 0.7% of the people in India of that age are affected. Why India has the lowest rate and the United States the highest is not fully understood. *(Thakur MK, 2000)*

Oxidative damage and inflammation have been implicated in most age-related neurodegenerative diseases, including AD. Amyloid beta peptide (Abeta), a proteolytic fragment of the amyloid precursor protein, is a major component of the plaques found in the brains of AD patients. These plaques are thought to cause the loss of cholinergic neurons observed in the basal forebrain of AD patients. Curcumin has been shown to affect AD through numerous mechanisms. For instance, up-regulation of 75-kDa neurotrophin receptor (p75NTR), a nonselective neurotrophin receptor belonging to the death receptor family, has been reported in neurons affected by AD. The expression of p75NTR has been found to correlate with beta-amyloid sensitivity in vivo and in vitro, suggesting a possible role for p75NTR as an Abeta receptor. Human neuroblastoma cell lines were used to investigate the involvement of p75NTR in Abeta induced cell death. Abeta peptides bound to p75NTR, resulting in activation of NF-κB, a major mediator of inflammation. Blocking the interaction of Abeta with p75NTR using nerve growth factor or
inhibition of NF-κB activation by curcumin abolished Abeta-induced apoptotic cell death. These results suggest that p75NTR might be a death receptor for Abeta, and thus a possible therapy for AD. *(Kuner P, 1998)*

Kim et al. showed that curcumin protected neurons from Abeta (25–35)-induced apoptosis. They suggested that the hydroxy group at the para-position in curcumin is critical for the expression of biological activity. In addition to curcumin, shogoals from ginger *(Zingiber officinale)* were found to protect human neuroblastoma and normal human umbilical vein endothelial cells from Abeta (25–35) insult. *(Kim DS, 2002)*

Abeta1–40 has been shown to activate nuclear transcription factor early growth response-1 (Egr-1), which results in the increased expression of cytokines and chemokines in monocytes. Whether curcumin suppressed Egr-1 activation and the concomitant expression of chemokines was investigated. *(Giri RK, 2004)*

Curcumin inhibited the activation of Egr-1 DNA-binding activity, abrogated the Abeta-induced expression of cytokines [tumor necrosis factor (TNF)-alpha and interleukin (IL)-1beta] and chemokines (MIP-1beta, MCP-1, and IL-8) in monocytes, and suppressed MAP kinase activation and the phosphorylation of ERK-1/2 and its downstream target, Elk-1. Curcumin inhibited Abeta1–40-induced expression of CCR5, but not of CCR2b. This inhibition involved abrogation of Egr-1 DNA binding in the CCR5 promoter by curcumin. Finally, curcumin inhibited chemotaxis of THP-1 monocytes in response to chemoattractants. The inhibition of Egr-1 is one of the mechanisms by which curcumin could ameliorate the inflammation and progression of AD. Whether curcumin can protect against Abeta-induced damage in rats was examined. *(Frautschy SA, 2001)*

Lipoprotein carrier-mediated, intracerebroventricular infusion of Abeta peptides induced oxidative damage, synaptophysin loss, a
microglial response, and widespread Abeta deposits. Dietary curcumin (2000 ppm), but not ibuprofen, suppressed oxidative damage (isoprostane levels) and synaptophysin loss. Both ibuprofen and curcumin reduced microgliosis in cortical layers, but curcumin increased microglial labeling within and adjacent to Abeta-ir deposits. In a second group of middleaged female Sprague-Dawley rats, 500-ppm dietary curcumin prevented Abeta-infusion-induced spatial memory deficits in the Morris water maze and postsynaptic density-95 loss and reduced Abeta deposits. Because of its low side-effect profile and long history of safe use, curcumin may find clinical application for AD prevention. *(Frautschy SA, 2001)*

Yang et al. have investigated whether curcumin could bind and prevent the aggregation of Abeta in AD models. Under aggregating conditions in vitro, curcumin inhibited aggregation as well as disaggregating fibrillar Abeta40. Curcumin was a better Abeta40 aggregation inhibitor than ibuprofen and naproxen, and prevented Abeta42 oligomer formation and toxicity between 0.1 and 1 μM; curcumin also decreased Abeta fibril formation. The effects of curcumin did not depend on Abeta sequence but on fibril-related conformation. AD and Tg2576 murine brain sections incubated with curcumin revealed preferential labeling of amyloid plaques. In vivo studies showed that curcumin injected peripherally into aged Tg2576 mice crossed the blood-brain barrier (BBB) and bound the plaques. When fed to aged Tg2576 mice with advanced amyloid accumulation, curcumin labeled plaques and reduced amyloid levels and plaque burdens. Hence, curcumin directly binds small Abeta to block aggregation and fibril formation in vitro and in vivo. Low-dose curcumin effectively disaggregates Abeta as well as prevents fibril and oligomer formation, supporting the rationale for curcumin use in clinical trials on the prevention or treatment of AD. That curcumin can inhibit the formation of Abeta fibrils (fAbeta), as well as destabilize preformed fAbeta, has also been shown by other research groups. Ferulic acid was found to be less active than curcumin. *(Yang F, 2005)*
Curcumin is structurally similar to Congo red, and has been demonstrated to bind Abeta amyloid and prevent further oligomerization of Abeta monomers onto growing amyloid beta-sheets. Reasoning that the oligomerization kinetics and mechanisms of amyloid formation are similar in Parkinson’s disease (PD) and AD, the effect of curcumin on alpha-synuclein (AS) protein aggregation was examined. The in vitro model of AS aggregation was developed by treating purified AS protein (wild-type) with Fe3+ (Fenton reaction). It was observed that the addition of curcumin inhibited aggregation and increased alpha-synuclein (AS) solubility. Curcumin inhibits AS oligomerization into higher molecular weight aggregates and, therefore, should be further explored as a potential therapeutic compound for AD and related disorders. *(Pandey N, 2008)*

Another potential mechanism by which curcumin could mediate its effects is through modulation of p21-activated kinase (PAK). The PAK family of kinases are known to regulate actin filaments and the morphogenesis of dendritic spines through Rho family GTPases Rac and Cdc42. Active PAK has been shown to be markedly reduced in AD cytosol, accompanied by downstream loss of the spine actin-regulatory protein drebrin. Abeta oligomer was implicated in PAK defects. PAK was found to be aberrantly activated and translocated from cytosol to membrane in AD brains and in 22-month-old Tg2576 transgenic AD mice. Curcumin comparatively suppressed PAK translocation in aged Tg2576 transgenic AD mice and in Abeta42 oligomer-treated cultured hippocampal neurons. *(Ma QL, 2008)*

Ryu et al. evaluated radiolabeled curcumin as a potential probe for Abeta plaque imaging. Partition coefficient measurement and biodistribution in normal mice demonstrated that [18F]8 has a suitable lipophilicity and reasonable initial brain uptake. Metabolism studies also indicated that [18F]8 is metabolically stable in the brain and is a suitable radioligand for Abeta plaque imaging. *(Ryu EK, 2006)*
Another potential mechanism by which curcumin could exhibit activity against AD is through inhibition of gamma-secretase. To minimize the metal chelation properties of curcumin, Narlawar et al. synthesized curcumin-derived oxazoles and pyrazoles. The reduced rotational freedom and the absence of stereoisomers were thought to enhance the inhibition of gammasecretase. Accordingly, the replacement of 1,3-dicarbonyl moieties by isosteric heterocycles turned curcumin analogue oxazoles and pyrazoles into potent gamma-secretase inhibitors. They were potent inhibitors of gamma-secretase and displayed activity in the low micromolar range. (Narlawar R, 2007)

Another pyrazole derivative of curcumin, called CNB-001, was synthesized and found to exhibit far superior activity in neuroprotection when examined in cell culture assays for trophic factor withdrawal, oxidative stress, excitotoxicity, and glucose starvation, as well as toxicity from both intracellular and extracellular amyloids. (Liu Y, 2008)

In another study, the anti-amyloidogenic effects of dietary curcumin and its more stable metabolite, tetrahydrocurcumin (THC), were examined either when administered chronically to aged Tg2576 APPsw mice or acutely to LPS-injected wild-type mice. Despite dramatically higher drug plasma levels after THC compared to curcumin gavage, the resulting brain levels of parent compounds were similar, correlating with the reduction in LPS-stimulated iNOS, nitrotyrosine, F2 isoprostanes, and carbonyls. In both the acute (LPS) as well as chronic inflammation (Tg2576) models, THC and curcumin similarly reduced IL-1β. Despite these similarities, only curcumin was effective in reducing amyloid plaque burden, insoluble Abeta, and carbonyls. THC had no impact on plaques or insoluble Abeta, but reduced both tris-buffered saline (TBS)-soluble Abeta and pJNK. Curcumin (but not THC) prevented Abeta aggregation. The THC metabolite was detected in the brain and plasma of mice chronically fed the parent compound. These
data indicate that the dienone bridge present in curcumin, but not in THC, is necessary to reduce plaque deposition and protein oxidation in an Alzheimer’s model. Nevertheless, THC did reduce neuro-inflammation and the effects of soluble Abeta, which may be attributable to limiting JNK-mediated transcription. Thus, curcumin and THC display a very different activity profile against AD. *(Begum AN, 2008)*

- **Parkinson’s Disease**

Although the cause of dopaminergic cell death in PD remains unknown, the role of oxidative stress has been strongly implicated. Because of their ability to combat oxidative stress, spice-derived phenolic compounds continue to be considered as potential agents for long-term use in PD. Oxidative stress has been implicated in the degeneration of dopaminergic neurons in the substantia nigra (SN) of PD patients. An important biochemical feature of resymptomatic PD is a significant depletion of the thiol antioxidant GSH in these neurons, resulting in oxidative stress, mitochondrial dysfunction, and ultimately, cell death. *(Munch G, 1998)*

Treatment of dopaminergic murine neuronal cells with curcumin was found to restore depletion of glutathione (GSH) levels, protect against protein oxidation, and preserve the mitochondrial complex I activity that is normally impaired due to GSH loss. Using systems biology and dynamic modeling, researchers examined the mechanism of curcumin action in a model of mitochondrial dysfunction linked to GSH metabolism that corroborates the major findings. Thus, curcumin may also have therapeutic potential for neurodegenerative diseases involving GSH depletion-mediated oxidative stress. *(Mythri RB, 2007)*

The prevalence of PD is higher in men than in women. Although the reason for this gender difference is not clear, female steroid hormones or their receptors may be involved in the pathogenesis of PD. *(Shulman LM, 2007)*
It has been demonstrated that ligand-activated estrogen receptor beta suppressed dopaminergic neuronal death in an in vitro PD model that used 1-methyl-4-phenylpyridinium ions (MPP(+)). They showed that (similar to estrogen) MPP(+) treatment caused the up-regulation of C-jun N-terminal kinases (JNK) and dopaminergic neuronal death, which was blocked by curcumin. *(Sawada H, 2002)*

Selective damage to mitochondrial complex I within the dopaminergic neurons of the substantia nigra (SN) is the central event of PD. Peroxynitrite is one of the free radicals most likely mediating complex I damage. Peroxynitrite inhibits brain complex I mainly by 3-nitrotyrosine and nitrosothiol formation, but how these modifications alter the structure-function relation of complex I is unclear. Curcumin pretreatment protected brain mitochondria against peroxynitrite in vitro by direct detoxification and prevention of 3-nitrotyrosine formation, and in vivo by elevation of total cellular GSH levels. These results suggest a potential therapeutic role for curcumin against nitrosative stress in neurological disorders. *(Ebadi M, 2003)*

It has been shown that curcumin acts as a powerful free radical scavenger in vivo in the brain, and interferes with oxidative stress caused by the parkinsonian neurotoxin, 1-methyl-4-phenyl-1 2 3 6-tetrahydropyridine (MPTP). MPTP treatment caused a significant depletion in GSH and increased the specific activity of SOD, catalase in both the striatum and midbrain on the third and seventh days. Curcumin treatment blocked these changes. This provides direct evidence for the involvement of curcumin in neuroprotection against oxidative stress. *(Rajeswari A, 2006)*

Whether curcumin can be neuroprotective in the 6-OHDA model of PD was examined. Unilateral infusion of 6-OHDA into the medial forebrain bundle produced a significant loss of tyrosine hydroxylase (TH)-positive cells in the SN as well as decreased DA content in the
striata of the vehicle-treated animals. Rats pretreated with curcumin showed a clear protection of TH-positive cells in the SN and DA in the striata. The ability of curcumin to exhibit neuroprotection in the 6-OHDA model of PD may be related to its antioxidant capabilities and its ability to penetrate into the brain. *(Zbarsky V, 2005)*

Eugenol (derived from cloves) has also been examined as a means to prevent the progression of Parkinson’s disease. Experimental results indicate that demethylidiisoegenol is a potentially effective antioxidant and can protect rat brain homogenates and LDL against oxidation. Kabuto et al. showed that eugenol prevents 6-hydroxydopamine-induced DA depression and PLO inductivity in mouse striatum. *(Kabuto H, 2007)*

**Micronutrients and Antioxidants as Potential Therapies in Parkinson’s Disease:**

Mitochondrial defects and oxidative stress have emerged as common pathogenic causes for many diverse conditions and neurodegenerative disorders, including PD. While there are symptomatic therapies for PD, there are no effective treatments that can restore neuronal function or offer neuroprotection. Therefore, the use of micronutrients and antioxidants to improve mitochondrial function and prevent oxidant injury may be beneficial for neurodegenerative diseases. Animal models of PD have been useful in exploring pharmacological interventions, such as the metabolic modifiers creatine, coenzyme Q10 (CoQ10), lipoic acid, as well as the antioxidants Ginkgo biloba extract, N-acetyl-cysteine, nicotinamide, riboflavin, acetyl-carnitine, and resveratrol. *(Beal MF, 2003)*

CoQ10, also known as ubiquinone, serves as an acceptor of electrons from mitochondrial complexes I and II, potentially acts as an antioxidant, and is capable of regenerating α-tocopherol (vitamin E). It is a molecule that has been tested for the treatment of mitochondrial disorders and neurodegenerative diseases in a variety
of animal models and in clinical trials with patients having PD and Huntington’s disease. CoQ10 and CoQ9 levels were found increased in the nigrostriatal tract of mice 1 wk after acute treatment with MPTP. *(Dhanasekaran M, 2008)*

Administration of CoQ10 attenuated the loss of striatal dopamine and decreased tyrosine hydroxylase immunoreactivity in the striatum of aged mice treated with MPTP. CoQ10 has also been shown to be neuroprotective against other mitochondrial toxins, such as malonate, 3-nitropropionic acid, and rotenone. *(Cleren C, 2008)*

Lipoic acid is a coenzyme for pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. It functions as an antioxidant through the chelation of transition metals and the regeneration of endogenous antioxidants, such as ascorbic acid, glutathione, and α-tocopherol. Dihydrolipoic acid, which is the reduced product of lipoic acid, also interacts with CoQ. This interaction was shown to increase the antioxidant capacity of CoQ by reducing ubiquinone to ubiquinol, thus maintaining a normal ratio of reduced and oxidized CoQ following MPTP administration in mice. *(Gotz M, 1994)*

While the application of antioxidant micronutrients to cell and animal models of PD has produced encouraging results, extension of these treatments to clinical trials has produced variable findings. One of the most extensive clinical trials of antioxidants to treat PD was the Deprenyl and Tocopherol Antioxidative Therapy of Parkinsonism (DATATOP) study. Eight hundred patients presenting with early stages of PD were randomly assigned to receive both deprenyl and α-tocopherol, deprenyl with an α-tocopherol placebo, α-tocopherol with a deprenyl placebo, or two placebos. The end point of the trial was the onset of parkinsonian disability to the degree that levodopa therapy was needed. Deprenyl, also known as selegiline, is a monoamine oxidase type B inhibitor. α-Tocopherol is a biologically active component of vitamin E. At the end of 14 ± 6 mo of treatment and observation, the researchers
found that deprenyl was able to significantly delay the onset of PD symptoms, warranting levodopa therapy by a median time of 9 mo. Conversely, α-tocopherol did not delay the onset or severity of symptoms, and the combination of α-tocopherol and deprenyl did not provide any benefit above that achieved from deprenyl alone. *(Engl. J. study, 1993)*

Rasagiline is another selective monoamine oxidase type B inhibitor that has been successful in drug trials. In a series of large-scale studies, the Parkinson Study Group found that rasagiline slowed the progression of symptoms in individuals with early PD as measured by the Unified Parkinson’s Disease Rating Scale (UPDRS) and was more effective when administered immediately compared with after a 6-mo delay, showing that the benefit is not due to an immediate symptomatic effect but rather to an actual influence on disease progression. Additionally, rasagiline was able to potentiate the beneficial effects of levodopa on disease progression in PD patients with motor fluctuations who were receiving levodopa treatment. *(Arch. Neurol study, 2004), (Arch. Neurol study, 2005)*

In a study of eighty subjects with early PD, the Parkinson Study Group found that a dose of 1200 mg/d of CoQ10 reduced the rate of functional decline as measured by the UPDRS. However, this dose of 1200 mg/d of CoQ10 did not change the amount of time until disability requiring treatment with levodopa and lower doses of 300 and 600 mg/d of CoQ10 did not significantly improve PD symptoms. *(Shults CW, 2002)*

In a separate study, mid-stage PD patients who were administered 300 mg/d of CoQ10 did not differ from patients administered a placebo in progression of symptoms as measured by the UPDRS. *(Storch A, 2007)*

Pilot trials of the compound creatine have also been undertaken. Creatine, through its conversion to phosphocreatine, is responsible for ATP homeostasis and potentially has antioxidant properties.
While these small studies did not have the power to discriminate statistical significance, a definite trend of slower decline as measured by the UPDRS was noted with creatine treatment. However, creatine was not able to delay the amount of time needed before progression to dopaminergic replacement therapy. *(NET-PD study, 2008)*

Another small study produced slightly different results, where patients who were administered creatine did not exhibit any significant difference in overall UPDRS scores compared with controls. An improvement in mood did occur, however, as well as smaller dose increases in dopaminergic therapy. *(Bender A, 2006)*

**Food Antioxidants and Alzheimer’s Disease**

Epidemiological evidence linking nutrition to the incidence and risk for AD is rapidly growing. Certain nutritional deficiencies observed in patients with AD may suggest supplementation in specific macro- and micronutrients combined to the traditional drugs. These nutrients include omega-3 fatty acids, several B vitamins, and antioxidants such as vitamin E, vitamin C, and carotenoids.

- **B Vitamins**

Various studies support the potential beneficial effects of B vitamin [vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B6 (primarily pyridoxine), vitamin B12 (cobalamin), and folate (folic acid, tetrahydrofolate)] supplementation on neurocognitive function and gene expression in AD. Thiamine and riboflavin can be found in a great variety of foods, including whole grain cereals, vegetables, milk products, and liver. Thiamine is critically involved in glucose metabolism, and it is also implicated in oxidative stress (acting as a radical scavenger), protein processing, peroxisomal function, and gene expression. *(Gibson GE, 2007)*
Thiamine-dependent enzyme activities, such as pyruvate dehydrogenase and $\alpha$-ketoglutarate dehydrogenase, are diminished in AD, and the reductions in AD brain are well correlated with the extent of dementia. Although thiamine itself has not been shown to have dramatic benefits in AD patients, the available data are scarce. Further testing on developing more absorbable forms of thiamine or adding thiamine to tested treatments for the abnormality in glucose metabolism in AD may increase their efficacy. While the data on other compounds, including folate and cobalamin, are often conflicting with regard to whether levels in AD are significantly changed or if these compounds correlate with disease onset and severity, the levels are often reported to be lower in AD. These findings suggest that nutrition monitoring to at least keep the levels to within normal limits may be of worth. (Gibson GE, 2007)

- **Carotenoids**

Levels of many naturally occurring antioxidants have been shown to be decreased in AD patients. These include the class of antioxidants of carotenoids, including $\beta$-carotene and lutein, which protect polyunsaturated fatty acids from oxidation. Decreased levels have been shown to correlate with the severity of disease. Maintaining the levels of these compounds may at the least stabilize cognitive function. (Wang W, 2008)

- **Polyphenols**

Polyphenolic compounds protect plants against various biotic and abiotic stresses, and there are many pieces of evidence showing that their intake contributes to preventing and ameliorating certain diseases, including neurodegenerative disorders. These compounds present potent antioxidant and anti-inflammatory properties (reviewed by Pietta), and they are also capable of modulating cell signaling and enzyme activities (reviewed by Scalbert et al. and Ramiro-Puig et al.). Studies regarding brain health are mainly focused on polyphenolic compounds from green tea (catechins),
berry fruits (anthocyanins), curcumin, red wine (resveratrol), and, cocoa (procyanidins).
(Pietta PG, 2000), (Scalbert A, 2005)

- **Green Tea Catechins**

Epigallocatechin-3-gallate (EGCG) is the main polyphenol found in green tea, and its beneficial properties related to neuroprotection may be linked to its metal chelating, antioxidant, and cellsignaling modulatory properties. These multiple functions make EGCG a novel preventive and therapeutic approach to AD. Importantly, EGCG reduces Aβ and neurite plaque formation through the promotion of the non-amyloidogenic AβPP pathway. Thus, EGCG elevates α-secretase activity, specifically, tumor necrosis factor α-converting enzyme and ADAM10 (a desintegrin and metallo-protease 10), with the latter being critical for EGCG-mediated AβPP cleavage to sAPPα. EGCG also activates protein kinase C, a well-known pathway that leads to sAPPα release.
(Mandel SA, 2005), (Rezai-Zadeh K, 2005)

Weinreb et al. have shed light on the mechanisms of EGCG underlying its neuroprotective and neurorescue activities. Their transcriptomic study provides information about the effect of EGCG on the expression of several genes involved in neurite outgrowth, cell survival, and iron chelation. Although all these studies demonstrate a beneficial effect of catechins on AD, clinical trials are required to prove their effectiveness and safety in humans.
(Weinreb O, 2007)

- **Berry Fruits (Anthocyanins)**

Anthocyanins and/or proanthocyanidins are the main polyphenolic compounds found in berry fruits (blueberries, blackberries, cranberries, strawberries, etc.). Joseph and colleagues have provided an extensive knowledge of the neuronal effects of these compounds. Thus, long-term feeding with diets containing strawberry or
blueberry extracts (1–2% diet) improves age-related impairments in the cognitive and neuronal functions of rodents. 
*(Andres-Lacueva C, 2005)*

Anthocyanins can cross the brain barrier and reach areas associated with cognitive performance. Moreover, blueberry extract supplementation reduces ROS levels in the striata and enhances neurogenesis. With respect to AD, an in vitro study showed a protective effect of five berry extracts (blueberry, black currant, boysenberry, strawberry, and cranberry) on the putative toxic effects of Aβ. *(Joseph JA, 2004)*

In AD transgenic mice (AβPP and presenilin-1 mutations), long-term blueberry extract supplementation (2%) prevents deficits on the Y-maze performance test, although it does not affect Aβ deposits. As with green tea and other polyphenolic compounds, the action of blueberry extract goes beyond the radical scavenging capacity. Indeed, blueberry extract is capable of modulating cell-signaling pathways associated with cognitive function—for example, enhancement of the extracellular signal-regulated kinase activity and protein kinase Cγ activation. *(Joseph JA, 2003)*

- **Curcumin**

Curcumin (diferuloylmethane) is an active polyphenolic compound present in the herb Curcuma longa (commonly known as turmeric or curry powder). Traditionally, it has been used as a food spice, a cosmetic, and a natural therapeutic drug. In Asian folk medicine, curcumin is a well-described treatment for various illnesses (e.g., respiratory disorders, abdominal pain, swelling, etc.). Many of these therapeutic effects of curcumin have been confirmed in the last decades by research studies, and they appear to be linked not only to its potent antioxidant and anti-inflammatory properties but also to its ability to bind proteins and modulate the activity of various kinases (reviewed by Goel et al.). *(Goel A, 2008)*
Additionally, some studies have pointed out the potential benefits of this spice in neurodegenerative disorders. In AD transgenic mice, curcumin and its metabolite tetrahydrocurcumin reduce amyloid plaque burden, insoluble Aβ, reactive carbonyls, and inflammatory markers. *(Garcia-Alloza M, 2007)*

To date, clinical studies on AD are still forthcoming; however, an epidemiological study carried out in elderly Asians suggests better cognitive performance in curry consumers. Moreover, the extreme safety and tolerance of curcumin reported in humans (reviewed by Goel et al.) make it an exciting candidate for AD therapy. *(Goel A, 2008)*

- **Resveratrol**

Resveratrol (3,4′,5-trihydroxy-trans-stilbene) is a phytoalexin that is found in peanuts, red grape skins, and red wine. Research indicates that resveratrol may act as an antioxidant, promote nitric oxide production, inhibit platelet aggregation, and increase high-density lipoprotein cholesterol, thereby serving as a cardioprotective agent. *(Pallas M, 2008)*

Resveratrol is an SIRT-1 activator that has been shown to stimulate mitochondrial biogenesis and deliver health benefits in rodents and increase longevity and protects against neurodegenerative and neurotoxic insults in animal models. *(Anekonda TS, 2006)*

Resveratrol has been shown to be protective against kainate-induced seizures and against brain injury due to ischemia/reperfusion in a gerbil model. Similarly, resveratrol is protective in Parkinson’s disease models, such as after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine MPTP treatment, and has been found to protect neurons in Huntington’s disease models, such as from 3-nitropropionic acid treatment. *(Kumar P, 2006)*
With regard to AD, studies demonstrate that resveratrol reduced neurodegeneration in the hippocampus and prevented learning impairment in an inducible p25 transgenic mouse model of AD and tauopathies and that consumption of Cabernet Sauvignon attenuated Aβ neuropathology in the Tg2576 mouse model of AD. ([Ho L, 2009])

- **Cocoa**

Cocoa’s beneficial effects on brain health have recently become an object of interest given the potent antioxidant, anti-inflammatory, and iron-chelating properties of its polyphenolic fraction (mainly composed of epicatechin, catechin, and procyanidins). Thus, a serving size of certain cocoa-derived products provides more phenolic antioxidants than beverages and fruits, such as tea and blueberries, traditionally considered to be high in antioxidants. ([McShea A, 2008])

Cocoa intake has been shown to improve cerebral blood flow in humans, and this may have a positive impact in aging and cerebrovascular diseases, such as stroke and dementia, in which endothelial function is impaired. ([Francis ST, 2006])

Regarding AD, it has been found that cocoa extract, epicatechin, and catechin reduce the toxic effects of Aβ in vitro through membrane and mitochondrial protective mechanisms. Although no studies on AD animal models have been performed yet, oral administration of cocoa extract (100 mg/kg/day) has been shown to attenuate nigrostriatal dopaminergic cell loss in a murine model of Parkinson’s disease induced by the infusion of the neurotoxin 6-hydroxidopamine. ([Datla KP, 2007])
SUMMARY

Neurodegenerative diseases are characterized by progressive dysfunction and death of cells that frequently affect specific neural systems, implying some form of selective vulnerability. Morphologically, neuronal loss is associated with gliosis and, frequently, with misfolding and aggregation of proteins leading to the relentless accumulation of abnormal extracellular and intracellular filamentous deposits in specific cell types, mainly neurons and glia, representing the core features/hallmarks of many neurodegenerative disorders.

There are common pathogenic mechanisms underlying many diverse neurodegenerative disorders. They include:
1. abnormal protein dynamics with protein misfolding, defective protein degradation, and aggregation.
2. oxidative stress (OS) and formation of free radicals/reactive oxygen species (ROS).
3. impaired bioenergetics and mitochondrial dysfunctions.
4. fragmentation of neuronal Golgi apparatus (GAs).
5. disruption of cellular/axonal transport.
6. actions and mutations of molecular chaperones.
7. dysfunction of neurotrophins.

All these mechanisms are interrelated in complex vicious circles finally leading to cell death, the basic molecular cascades of which are still to be elucidated.

In healthy aerobes, there is a balance between the production of ROS and antioxidant defenses. In health, the cell has become well equipped to cope with the normal production of ROS. Indeed,
continuous low concentrations of ROS induce expression of antioxidant enzymes and related defense mechanisms. A large body of evidence has been accumulated that living organisms have not only adapted to a coexistence with free radicals but have developed various mechanisms for the advantageous use of free radicals in various physiological functions.

The various neurodegenerative diseases (diseases in which neurons degenerate and die) have a variety of different symptoms, affect different parts of the brain, and have different causes. They have in common impaired mitochondrial function, increased oxidative damage, defects in the ubiquitin-proteosome system, presence of abnormal aggregated proteins, changes in iron metabolism, and some involvement of excitotoxicity and of inflammation. It seems likely that all these events are involved in a vicious cycle and that any of them could initiate neuronal cell death, rapidly recruiting the others to its destructive purpose. Oxidized proteins are usually removed by the proteosome. Inhibition of the proteosome allows abnormal proteins to accumulate and produces OS, but how this is done is still unclear. Finally, ROS-producing agents could initiate neurodegeneration, because ROS damage mitochondria, cause rise in Ca2+, and may inhibit proteosome function. The iron content of most brain areas increases with age, and iron and other metals promote the aggregation of several proteins. How do neurons die in these various diseases? Sometimes they die by necrosis, as in excitotoxicity, and sometimes, probably, by apoptosis. However, as more studies are done, the role of intermediate types of cell death, with features of both necrosis and apoptosis, is becoming more prominent.

The central nervous system is particularly vulnerable to oxidative stress (OS) as it utilizes large amounts of dioxygen but harbors relatively poor concentrations of antioxidants and related enzymes. Moreover, it contains a very high amount of polyunsaturated lipids, the most vulnerable biomacromolecule to oxidation.
Role of oxidative stress in Parkinson’s Disease

OS is associated with many factors that are thought to be involved in the pathogenesis of PD. Mitochondrial damage may result from OS and, vice versa, mitochondrial impairment may enhance ROS/RNS release to the cytosol. Inflammation is always associated with high ROS/RNS generation (nitric oxide NO, hypochlorous acid HOCl, superoxide anion, hydrogen peroxide, etc.) as direct defense species and as signaling elements to induce a series of protecting genes. OS can contribute to protein misfolding and, when linked to an inability of the ubiquitin-proteasome system to degrade and remove them, such damage can result in the accumulation and aggregation of these abnormal proteins and induce what is termed proteolytic stress. Misfolding and aggregation of a-synuclein have been described in association with familial PD.

Degeneration of dopaminergic neurons in the brain and the formation of the LB are the hallmark events of PD. OS is believed to contribute at least in part to these two incidents, and biomarkers that might be generated during the pathways of their formation are under investigation. It was assumed that, as a consequence of dopaminergic cell death, NM will be present not only in the brain but also in the plasma. Antibodies specific to NM have been found in the plasma of patients with PD, but it is not clear yet if the NM detected in plasma of PD patients is specific to the disease or common to other neurological disorders or even may arise from skin diseases.

It is known that the immune system and its inflammatory response are not the initial cause of PD, but rather a consequence of damage, that is, tissue or cellular modifications in the CNS, such as neurodegeneration. Such threats activate the microglia, which
respond through morphological changes in which the cells are converted to an amoeboid state with enlarged cytoplasmic processes capable of phagocytosis, migrate to the injured environment, alter gene expression, and release of inflammatory mediators such as cytokines, chemokines, ROS, and RNS. Excessive, chronic, or unregulated microglial activation may be harmful to neurons. The phagocytic activity of microglia is beneficial during neuronal development and in injury because of the effectiveness of this process to remove cellular debris and injured cells, but dysregulation or excessive activation and as a consequence excessive ROS formation can lead to neuronal oxidative burden.

Cumulative evidence emphasizes the role of OS in PD development and progression, which raised the hope that antioxidants may lower such risk. Major food antioxidants present in fruits and vegetables are polyphenols of flavonoid and nonflavonoid structure. Prospective cohort studies (PCS) were conducted correlating the effect of consumption of a diet rich in polyphenols on PD progression, such as that of Checkoway et al., which showed that consumption of green tea was associated with reduced risk for PD.

**Role of oxidative stress in Alzheimer’s Disease**

Over the past decade, oxidative-stress-associated modifications of biomacro-molecules has been described in association with the susceptible neurons of AD:

1. DNA and RNA oxidation is marked by increased levels of 8-hydroxy-2-deoxyguanosine (8OHdG) and 8-hydroxyguanosine (8OHG).
2. Protein oxidation is marked by elevated levels of protein carbonyl and widespread nitration of tyrosine residues. Moreover, cross-linking of proteins by oxidative processes may lead to the resistance of the lesions to intracellular and extracellular removal even though they are extensively ubiquitinated, and this resistance
of neurofibrillary tangles to proteolysis might play an important role in the progression of AD.

3) Lipid peroxidation is marked by higher levels of thiobarbituric acid reactive substances (TBARS), malondialdehyde (MDA), 4-hydroxy-2-transnonenal (HNE), and isoprostanes and altered phospholipid composition.

Besides the key role of mitochondria in the maintenance of cell energy and generation of free radicals, these organelles are also involved in cell death pathways, namely apoptosis. There are three main apoptotic pathways leading to the activation of caspases, which converge onto mitochondria and are mediated through members of Bcl-2 family such as Bid, Bax, and Bad. The end result of each pathway is the cleavage of specific cellular substrates, resulting in the morphological and biochemical changes associated with the apoptotic phenotype. The first of these depends on the participation of mitochondria (mitochondrial pathway), the second involves the interaction of a death receptor with its ligand (death receptor pathway), and the third is triggered under conditions of endoplasmic reticulum (ER) stress (ER-specific pathway).

Iron is an important cause of oxidative stress in AD because it is found in considerable amount in the AD brain, and, as a transition metal, is involved in the formation of hydroxyl radicals via Fenton reaction.

There is accumulating evidence that both iron and copper in their redox competent states are bound to neurofibrillary tangles and amyloid-β deposits. However, study reported that cognitive decline correlates with low plasma concentrations of copper in patients with mild to moderate AD.

There is evidence indicating that hyperphosphorylated tau exerts protective functions. It has been shown that oxidative stress and the modification of tau by-products of oxidative stress lead to protein
aggregation (neurofibrillary tangles) and enable neurons to survive decades.

With the progression of AD and the consequent increase of ROS levels, efficient removal of amyloid-β-metal complexes and, probably, hyperphosphorylated tau would be overtaken by their disproportionately high generation, resulting in an uncontrollable growth of plaques and neurofibrillary tangles and, consequently, an increase in reactive species generation. This would result in a feedback mechanism that could exacerbate plaque, neurofibrillary tangles growth, and reactive species generation, leading to a functional demise of neurons.

The Role of Oxidative Stress in the Pathogenesis of Multiple Sclerosis:

It is well established that inflammation might raise ROS levels leading to OS. Demyelinative plaques in the CNS of MS patients are associated with an inflammatory reaction orchestrated by activated T cells, macrophages, and endogenous glial cells (astroglia and microglia). These cells produce a variety of proinflammatory and neurotoxic factors, including proinflammatory cytokines, of which interleukin (IL)-1α/β and tumor necrosis factor (TNF)-α/β play a predominant role.

Studies suggest that glutamate plays a role in MS pathophysiology. Oligodendrocytes, the myelin-producing cell of the CNS, are highly vulnerable to glutamate excitotoxicity, mainly via the AMPA/kainate receptors, which have higher permeability to Ca2+. Demyelinating lesions caused by excitotoxins can be similar to those observed in MS, causing histological damage. Moreover, treatment with AMPA/kainate receptor antagonists was found to ameliorate axonal damage and to improve the clinical score of EAE.

Numerous studies of patients with MS have shown increased free radical activity, and/or deficiencies in important antioxidant
enzymes compared with healthy controls. Generation of ROS in vivo has been inferred from the presence of lipid peroxidation products in the CSF and plasma of MS patients, the increase of free radical activity and decrease of major antioxidant enzymes, and the presence of 3-nitrotyrosine (a marker of peroxynitrite activity) in demyelinated lesions.

The most abundant source of ROS associated with CNS inflammation in MS is the respiratory burst of macrophages and microglial cells following their activation by proinflammatory cytokines. This source may explain oxidative damage to white matter, especially plaque areas in MS patients.

Increased levels of glutamate have been detected in the CSF of patients with MS, and glutamate release has been shown to underlie axonal damage and oligodendrocyte cell death in MS lesions.

The pathogenic role of oxygen and nitrogen free radicals in MS led to the recognition that antioxidants might prevent free-radical mediated tissue damage and inhibit some of the early pro-inflammatory events that lead to inflammation and tissue destruction in EAE and MS.

The Role of Oxidative Stress in the Pathogenesis of Amyotrophic Lateral sclerosis:

Mutations in the gene coding for the antioxidant enzyme Cu, Zn superoxide dismutase (SOD1) are carried by one-fifth of fALS patients (i.e. 2% of all ALS cases). This enabled the development of novel experimental models such as transgenic mice and cultured cells expressing mutant SOD1 (mutSOD1), and numerous studies have been performed to investigate the toxic function of the mutant enzymes.
Similar to AD, microglial involvement is indicated to play an important role in ALS. About 10–20% of ALS cases are inherited, and, of the inherited cases, about 20% are caused by mutations in the gene encoding superoxide dismutase-1.

A role for ROS-mediated oxidative stress in ALS was proposed in many studies reporting the occurrence of other typical oxidation products (such as malondialdehyde, hydroxynonenal, oxidized proteins, DNA, and membrane phospholipids) both in sporadic and familial ALS patients and in several model systems as well. Furthermore, administration of several antioxidant molecules has been proven beneficial in a mouse model for fALS, indicating that oxidative stress is indeed a component of this pathology, although a generalized stress may be hardly considered specific for ALS, but rather a phenomenon preceding or accompanying neurodegeneration.

SOD1 is an abundant component of many cell types, accounting for up to 1% of total cytoplasm proteins in some areas of the CNS. It has been suggested that, similar to what occurs in other neurodegenerative “conformational” diseases, formation of insoluble aggregates of misfolded mutSOD1 contributes to cell death in fALS.

Rothstein et al. reported that beta-lactam antibiotics, including penicillin and ceftriaxone, are potential therapeutic drugs to treat ALS by modulating the expression of glutamate transporter GLT1 via gene activation.

ALS is believed to be a multifactorial and multisystemic disease, but the question as to which is the primary alteration causing this disease is still actively debated. Neurodegeneration may arise by converging pathways, such as ROS-induced damage of critical molecular targets, accumulation of misfolded proteins, and triggering of neuroinflammation and apoptotic pathways. Many consistent observations support a role for metal-mediated oxidative
stress as one of the mechanisms contributing to the pathogenesis of ALS. Intracellular oxidative stress may be primed by many different mechanisms, including the presence of mutant SOD1, alterations in copper-handling and copper responsive genes such as angiogenin and VEGF, and iron mishandling. Most of this evidence comes from studies in experimental models, while data in patients are scarce and sometimes contradictory. Nonetheless, because of the potential feasibility of new therapeutic approaches aimed at the interception of metal-mediated toxicity in ALS (e.g. with metal chelators), further studies in this field may be worth pursuing.

Prevention and Treatment of Neurodegenerative Diseases by Spice-Derived Phytochemicals

- Multiple Sclerosis:

Experimental autoimmune encephalomyelitis (EAE) is a demyelinating disease of the central nervous system widely accepted to be an animal model for multiple sclerosis. Inflammation plays a major role in neuropathological processes associated with neutrophilic infiltrates, such as EAE and traumatic injury of the brain. Whether curcumin may influence inflammation in the CNS through the modulation of the CXC chemokine, macrophage inflammatory protein (MIP)-2, has been investigated. Astrocytes prepared from the neonatal brains of mice were stimulated with LPS in the presence or absence of various amounts of curcumin. The latter inhibited the LPS-induced induction of MIP-2 gene expression, the production of MIP-2 protein, and the transcription of MIP-2 promoter activity. Thus, curcumin potently inhibits MIP-2 production at the level of gene transcription and offers further support for its potential use in the treatment of inflammatory conditions of the CNS.

- Alzheimer’s Disease

Kim et al. showed that curcumin protected neurons from Abeta (25–35)-induced apoptosis. They suggested that the hydroxy group at
the para-position in curcumin is critical for the expression of biological activity. In addition to curcumin, shogoals from ginger (Zingiber officinale) were found to protect human neuroblastoma and normal human umbilical vein endothelial cells from Abeta (25–35) insult.

Abeta1–40 has been shown to activate nuclear transcription factor early growth response-1 (Egr-1), which results in the increased expression of cytokines and chemokines in monocytes. Whether curcumin suppressed Egr-1 activation and the concomitant expression of chemokines was investigated.

- Parkinson’s Disease

Treatment of dopaminergic murine neuronal cells with curcumin was found to restore depletion of GSH levels, protect against protein oxidation, and preserve the mitochondrial complex I activity that is normally impaired due to GSH loss. Using systems biology and dynamic modeling, researchers examined the mechanism of curcumin action in a model of mitochondrial dysfunction linked to GSH metabolism that corroborates the major findings. Thus, curcumin may also have therapeutic potential for neurodegenerative diseases involving GSH depletion-mediated oxidative stress.

Micronutrients and Antioxidants as Potential Therapies in Parkinson’s Disease:

Mitochondrial defects and oxidative stress have emerged as common pathogenic causes for many diverse conditions and neurodegenerative disorders, including PD. While there are symptomatic therapies for PD, there are no effective treatments that can restore neuronal function or offer neuroprotection. Therefore, the use of micronutrients and antioxidants to improve mitochondrial function and prevent oxidant injury may be beneficial for
neurodegenerative diseases. Animal models of PD have been useful in exploring pharmacological interventions, such as the metabolic modifiers creatine, coenzyme Q10 (CoQ10), lipoic acid, as well as the antioxidants Ginkgo biloba extract, N-acetyl-cysteine, nicotinamide, riboflavin, acetyl-carnitine, and resveratrol.

**Food Antioxidants and Alzheimer’s Disease**

Epidemiological evidence linking nutrition to the incidence and risk for AD is rapidly growing. Certain nutritional deficiencies observed in patients with AD may suggest supplementation in specific macro- and micronutrients combined to the traditional drugs. These nutrients include omega-3 fatty acids, several B vitamins, and antioxidants such as vitamin E, vitamin C, and carotenoids.

**DISCUSSION**

The various neurodegenerative diseases (diseases in which neurons degenerate and die) have a variety of different symptoms, affect different parts of the brain, and have different causes. They have in common impaired mitochondrial function, increased oxidative damage, defects in the ubiquitin-proteosome system, presence of abnormal aggregated proteins, changes in iron metabolism, and some involvement of excitotoxicity and of inflammation. It seems likely that all these events are involved in a vicious cycle and that any of them could initiate neuronal cell death, rapidly recruiting the others to its destructive purpose.

Oxidized proteins are usually removed by the proteosome. Inhibition of the proteosome allows abnormal proteins to accumulate and produces OS, but how this is done is still unclear.
Finally, ROS-producing agents could initiate neurodegeneration, because ROS damage mitochondria, cause rise in Ca²⁺, and may inhibit proteosome function. The iron content of most brain areas increases with age, and iron and other metals promote the aggregation of several proteins. How do neurons die in these various diseases? Sometimes they die by necrosis, as in excitotoxicity, and sometimes, probably, by apoptosis. However, as more studies are done, the role of intermediate types of cell death, with features of both necrosis and apoptosis, is becoming more prominent.

Increased levels of oxidative damage to DNA, lipids, and proteins have been detected in postmortem tissues from patients with PD, AD, ALS, PSP, and related disorders, and at least some of these changes may occur early in disease progression. Markesbery et al. (2005) stated that recent studies showed that lipid peroxidation is an early event in the brain in amnestic MCI suggesting that oxidative damage occurs early in the pathogenesis of AD.

Most common free radicals are reactive oxygen (ROS) & reactive nitrogen (RNS) species such as: Superoxide (O₂⁻), Hydroxyl (OH⁻), Hydroperoxyl (HO₂⁻), Peroxyl (RO₂⁻), Alkoxyl (RO⁻), Carbonate (CO₃⁻), Carbon dioxide (CO₂⁻), Chlorine (Cl⁻), Nitric oxide (NO.), Nitrogen dioxide (NO₂⁻).

Effects of free radicals can be positive or negative. Positive effects including normal cellular oxidation-reduction (redox) status, immune function (neutrophils & macrophages use ROS to destroy engulfed microorganisms), intracellular signaling (serve as second messengers) activation of some enzymes, drug detoxification & muscle contraction. Negative Effects of excessive production of free radicals can damage lipids, proteins and DNA leading to cell death by necrosis or apoptosis. ROS damage membranes by oxidizing lipids leads to failure in mitochondrial function due to damage of
mitochondrial membranes (mitochondria are the primary source of ROS) may trigger apoptosis. ROS alter proteins biological functions (such as enzymes activities), or proteins turnover by carbonylation of proteins or by reactions with proteins SH groups. ROS produce several changes in mitochondrial & genomic DNA e.g., base alterations & single strand breaks. ROS can affect many biological systems, therefore, oxidative stress may be central to aging & neurodegenerative diseases.

The brain is uniquely vulnerable to oxidative damage because of many reasons: first, the brain is intolerant for blood flow interruptions with limited regeneration -although neurogenesis and gliogenesis can be stimulated. Circuit-based functions of the brain allow small deficits to have huge impact. The brain is aging sensitive and with Ca-dependant processes. Sayre et. al (2005) stated that the central nervous system is particularly vulnerable to oxidative stress (OS) as it utilizes large amounts of dioxygen but harbors relatively poor concentrations of antioxidants and related enzymes. Moreover, it contains a very high amount of polyunsaturated lipids, the most vulnerable biomacromolecule to oxidation.

Munoz et. al (2004) stated that an approach of using a modified drug to overcome the obstacle of crossing the BBB was made by modifying cysteine to N-acetyl cysteine (NAC). NAC is a powerful thiol antioxidant that, when given systemically, passes the BBB and releases cysteine in the brain, elevating GSH. NAC increased dopaminergic neurons survival against MPTP toxicity and, following subcutaneous administration, it induced about 30% reduction of the dopaminergic lesion.

PD is diagnosed on the basis of neurological symptoms including bradykinesia, rest tremor, postural instability, muscle rigidity,
flexed posture, and freezing of gait. There are several parkinsonian states in addition to primary Parkinson’s disease: secondary parkinsonism (e.g., caused by drugs, tumors, or toxins), parkinsonism-plus syndromes (with additional complicating neurodegenerative conditions), and heredodegenerative diseases (including Alzheimer’s and Huntington’s disease). Many incorrect diagnoses are made on the basis of neurological signs only, and the completely correct diagnosis can only be made at autopsy, when the presence of the characteristic neuronal inclusion bodies (Lewy bodies, LB) is confirmed. The difficulty of diagnosis is a factor that must always be considered in reviewing the relationship between clinical findings and mechanisms. Braak et. al (2003) stated that Most research on etiology of the disease is aimed at understanding the cause of primary parkinsonism, a condition in which 5-10% of the cases have been shown to have a genetic basis, with currently more than ten genes described as causing the disease, and the remaining 90% of cases without known genetic involvement, or other known cause, and referred to as idiopathic parkinsonism.

Braak also stated that a major development in recent years has been the hypothesis that Parkinson’s disease may in fact be caused by a prion-like infective agent. This proposal was based on the finding that LB exist in peripheral neuronal structures, for example, olfactory tract and intestinal nerve plexus, in the earliest stages of the disease, and show a gradual ascent within the central nervous system (CNS), reaching the cerebral cortex in the most advanced stages.

Mitochondrial defects and oxidative stress have emerged as common pathogenic causes for many diverse conditions and neurodegenerative disorders, including PD. While there are symptomatic therapies for PD, there are no effective treatments that can restore neuronal function or offer neuroprotection. Therefore, the use of micronutrients and antioxidants to improve mitochondrial
function and prevent oxidant injury may be beneficial for neurodegenerative diseases.

Instead of viewing oxidative stress as the breach of antioxidant defenses, we argue that this seldom happens in chronic conditions, pathological and physiological, and that a better understanding occurs by viewing each circumstance as a different homeostatic balance in which ROS plays a key regulatory role.

In early stages of AD, neuronal cells, despite showing increased oxidative damage, may actually be in homeostatic balance. If cells survive and function in the presence of high levels of oxidative stress, it is because critical systems of cells are not damaged. In this way, detection of increased oxidative damage in cells that survive must be associated with a commensurate increase in compensatory mechanisms such as amyloid-β deposition and hyperphosphorylated tau. However, with the progression of AD and the consequent increase of ROS levels, efficient removal of amyloid-β-metal complexes and, probably, hyperphosphorylated tau would be overtaken by their disproportionately high generation, resulting in an uncontrollable growth of plaques and neurofibrillary tangles and, consequently, an increase in reactive species generation. This would result in a feedback mechanism that could exacerbate plaque, neurofibrillary tangles growth, and reactive species generation, leading to a functional demise of neurons.

There are three Defense mechanisms against free radicals. First is Cellular antioxidants including: Superoxide dismutase (SOD), Cu-Zn SOD (in cytosol, SOD1), Mn-SOD (in mitochondria, SOD2), EC-SOD (Cu-Zn SOD, SOD3), Glutathione peroxidase (GPX), Glutathione reductase (GR), Catalase (CAT), Glutathione (GSH), Glucose-6-phosphate dehydrogenase, GSSG-S-transferase, Thioredoxin and Thioredoxin reductase and Heme oxygenase. Second mechanism are Major dietary antioxidants: Vitamin E (8 different isomers), Vitamin A (α, β and γ carotenes), Vitamin C
(ascorbate), Selenium (for GPX activity), Copper (for Cu-Zn SOD activity), Zinc (for Cu-Zn SOD activity and protects SH groups), Manganese (for Mn-SOD activity), α-lipoic acid, Phytochemicals (flavanoids, lignans, phenols). Third mechanism is Transition metals which are tightly bound to various proteins that prevent them from reacting with peroxides to form free radicals. These Metal-Binding Proteins include: Ceruloplasmin, Transferrin, Haemoglobin, Myoglobin, Cytochrome oxidases, Ferritin, Lactoferrin, Metallothionein.

Iron is a powerful promoter of free radical damage, able to catalyze generation of highly reactive hydroxyl, alkoxyl, and peroxyl radicals from H2O2 and lipid peroxides, respectively. Although most iron in the brain is stored in ferritin, “catalytic” iron is readily mobilized from injured brain tissue. As a result of a loss of iron homeostasis, the brain becomes vulnerable to iron-induced OS. Jiang et. al (2006) stated that iron chelator treatment may be considered as a potential strategy in neuroprotective therapy of the early parkinsonian patient. Effective iron chelators must be capable of crossing the BBB, and must not damage the physiological Fe pool, but should reduce excessive free Fe levels. A number of drugs have been described, although none are currently in clinical use in PD as such; future developments, however, are predicted in this area.

Antiinflammatory therapies have provided a strong neuroprotective role in different kinds of illness and pathologies, and the use of nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin and other COX inhibitors, is known to confer at least partial protection against the neurodegeneration seen in the MPTP and 6-OHDA models of PD. Chen et. al (2003) stated that In a large clinical trial, it was shown that users of ibuprofen (a common NSAID) had a 35% lower risk for PD; however, no other NSAID had the same results.
Etminan et al. (2005) stated that in two large cohort studies covering 120,000 participants, the associations between risk of PD and use of vitamin E, vitamin C, carotenoids, or vitamin supplementation was examined. No association was found between vitamin E or vitamin C intake and PD development. Meta-analysis studies on the effect of vitamin C, vitamin E, and b-carotene covering the period 1966 - 2005 on the risk of PD development revealed that b-carotene or vitamin C had no beneficial effects on the risk of developing PD, whereas a diet rich in vitamin E, as opposed to pure vitamin E, has some beneficial effect.

Ji et al. (2005) stated that beta-lactam antibiotics have long been known as metal chelators, and their beneficial effect likely involves the ability to attenuate metal toxicity. In this context, it is worth mentioning that desferoxamine (Desferal), an FDA-approved iron chelator that is used for iron overload disease secondary to multiple transfusions, seems to have several potentially beneficial effects in ALS, including induction of hypoxia-inducible factor-1 (which would cause the transcription of VEGF, erythropoietin, and other hypoxia-related genes) and is currently considered for ALS therapy.

Curcumin (diferuloylmethane) is an active polyphenolic compound present in the herb Curcuma longa (commonly known as turmeric or curry powder). Traditionally, it has been used as a food spice, a cosmetic, and a natural therapeutic drug. Many of these therapeutic effects of curcumin have been confirmed in the last decades by research studies, and they appear to be linked not only to its potent antioxidant and anti-inflammatory properties but also to its ability to bind proteins and modulate the activity of various kinases. (reviewed by Goel et al.(2008)). Mythri et al (2007) also stated that treatment of dopaminergic murine neuronal cells with curcumin was found to restore depletion of GSH levels, protect against protein oxidation, and preserve the mitochondrial complex I activity that is normally impaired due to GSH loss. Using systems biology and dynamic modeling, researchers examined the mechanism of curcumin action in a model of mitochondrial dysfunction linked to
GSH metabolism that corroborates the major findings. Thus, curcumin may also have therapeutic potential for neurodegenerative diseases involving GSH depletion-mediated oxidative stress.

Levels of many naturally occurring antioxidants have been shown to be decreased in AD patients. These include the class of antioxidants of carotenoids, including β-carotene and lutein, which protect polyunsaturated fatty acids from oxidation. Decreased levels have been shown to correlate with the severity of disease. Maintaining the levels of these compounds may at the least stabilize cognitive function.

Cocoa intake has been shown to improve cerebral blood flow in humans, and this may have a positive impact in aging and cerebrovascular diseases, such as stroke and dementia, in which endothelial function is impaired. Datla et. al (2007) stated that it has been found that cocoa extract, epicatechin, and catechin reduce the toxic effects of Aβ in vitro through membrane and mitochondrial protective mechanisms. Although no studies on AD animal models have been performed yet, oral administration of cocoa extract (100 mg/kg/day) has been recently shown to attenuate nigrostriatal dopaminergic cell loss in a murine model of Parkinson’s disease induced by the infusion of the neurotoxin 6-hydroxidopamine.

**RECOMMENDATIONS**

(I)- Instead of viewing oxidative stress as the breach of antioxidant defenses, we argue that this seldom happens in chronic conditions, pathological and physiological, and that a better understanding
occurs by viewing each circumstance as a different homeostatic balance in which ROS plays a key regulatory role. In early stages of AD, neuronal cells, despite showing increased oxidative damage, may actually be in homeostatic balance. If cells survive and function in the presence of high levels of oxidative stress, it is because critical systems of cells are not damaged.

(2)- All factors leading to pathological cascades – redox imbalance, influences of specific genes and mitochondrial dysfunction – seem to be closely linked and interconnected in a way that disturbance in each one of the systems has a deleterious influence on the other, promoting its dysfunction with the final consequence of neuronal death. Therapeutic strategies should, therefore, focus on influencing these contributing factors in all diseases.

(3)- Concerning redox imbalance, efforts are needed to develop therapeutic strategies to prevent the deleterious effects of ROS either by directly scavenging or by triggering protective mechanisms inside the cell. Several agents exerting antioxidative influence by modulating cellular energy metabolism in animal models seem promising as neuroprotective agents. These include coenzyme Q10, creatine, Ginkgo biloba, nicotinamide, acetyl-l-carnitine as well as Redox Imbalance 193. Also, the recently developed subtype-selective inhibitors of iNOS and nNOS could exert a neuroprotective influence by diminishing OS.

(4)- Diet is becoming increasingly important as there is evidence that patients may influence disease development and progression by adapting specific dietary concepts. The role of nutritionally administered iron has been supported by an epidemiological study revealing that iron taken in the highest quartile compared with those in the lowest quartile leads to an increased risk of PD. The vitamins, tocopherol (vitamin E) and vitamin C are potent free-radical scavengers.
(5)- Effort should be taken to find premotor markers. Because of the knowledge, that neurodegeneration starts long before clinical diagnosis may be made, based on motor deficits. As neuroprotection in healthy subjects with good motor functions should be harmless and free of side effects, it might be useful to recommend a diet based on antioxidative capacities of many nutritional components. This could contain adequate amounts of fruits and vegetables, green tea and supplementation of antioxidative vitamins.

(6)- Focusing on recently reported modifiable risk factors for AD, we recommend maintaining a low-calorie diet as well as intellectual and physical activities for the prevention of AD. As Most of the known genetic, medical, environmental, and lifestyle-related factors for AD are associated with increased oxidative stress.

(7)- An efficient strategy would be the use of multiple antioxidants in the treatment of AD, PD, and ALS. , it is conceivable that the therapeutic regimen used so far (e.g. one or two antioxidants) might not be sufficient to halt the neuropathologic process. In this regard, it is important to point out that one possible advantage of the use of extracts of fruits, vegetables, or beverages (such as red wine, green tea, or ginkgo biloba) in the treatment of neurodegenerative disorders, is that they often contain multiple antioxidant compounds which can potentate each other. Consistent with this line of reasoning, it has recently been shown that a complex anti-aging dietary supplement composed of 31 ingredients, most of them with antioxidant activity.

(8)- It is important to start the therapeutic intervention at an early stage of the disease process. It was found that the extent of neuronal loss in AD and PD patients during the early period of the disease
may not be as great as initially thought, because many dysfunctional neurons may be able to recover. In this regard, it is interesting to note that some epidemiological studies have shown that dietary habits can influence the incidence of neurodegenerative disorders. It was found that a diet rich in vitamin E can reduce the risk for PD.

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