Oxidative stress in neurodegenerative diseases

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Abstract

Reactive oxygen species are constantly produced in aerobic organisms as by-products of normal oxygen metabolism and include free radicals such as superoxide anion (O₂⁻) and hydroxyl radical (OH•), and non-radical hydrogen peroxide (H₂O₂). The mitochondrial respiratory chain and enzymatic reactions by various enzymes are endogenous sources of reactive oxygen species. Exogenous reactive oxygen species -inducing stressors include ionizing radiation, ultraviolet light, and divergent oxidizing chemicals. At low concentrations, reactive oxygen species serve as an important second messenger in cell signaling; however, at higher concentrations and long-term exposure, reactive oxygen species can damage cellular macromolecules such as DNA, proteins, and lipids, which leads to necrotic and apoptotic cell death. Oxidative stress is a condition of imbalance between reactive oxygen species formation and cellular antioxidant capacity due to enhanced ROS generation and/or dysfunction of the antioxidant system. Biochemical alterations in these macromolecular components can lead to various pathological conditions and human diseases, especially neurodegenerative diseases. Neurodegenerative diseases are morphologically featured by progressive cell loss in specific vulnerable neuronal cells, often associated with cytoskeletal protein aggregates forming inclusions in neurons and/or glial cells. Deposition of abnormal aggregated proteins and disruption of metal ions homeostasis are highly associated with oxidative stress. The main aim of this review is to present as much detailed information as possible that is available on various neurodegenerative disorders and their connection with oxidative stress. A variety of therapeutic strategies designed to address these pathological processes are also described. For the future therapeutic direction, one specific pathway that involves the transcription factor nuclear factor erythroid 2-related factor 2 is receiving considerable attention.

Key Words: oxidative stress; neurodegenerative diseases; reactive oxygen species; therapy; reviews

INTRODUCTION

Free radicals are molecules with at least one unpaired electron in the outermost shell; they are highly reactive due to the presence of unpaired electron. Any free radical involving oxygen can be referred to as a reactive oxygen species (ROS)⁴. Since ROS are common outcome of normal aerobic cellular metabolism, in-built antioxidant system of body plays its decisive role in prevention of any loss due to ROS overproduction. Oxidative stress arises as a result of an imbalance between the production of ROS and the biological system’s ability to detoxify the reactive intermediates⁵. Oxidative stress has been implicated in the progression of Alzheimer’s disease (AD), Parkinson’s disease (PD) and other neurodegenerative diseases. Oxidative stress leading to free radical attack on neural cells contributes calamitous role to neurodegeneration. Toxicity of ROS contributes to protein misfolding, glia cell activation, mitochondrial dysfunction and subsequent cellular apoptosis⁶. However, the systems in place to cope with biochemistry of oxidative stress are complex, and this complexity provides a number of therapeutic targets. Recognition of upstream and downstream antioxidant therapy has been proved an effective tool in alteration of neuronal damage as well as novel metal-protein attenuating compound (MPAC). Furthermore, therapeutic approaches aiming at nuclear factor erythroid 2-related factor 2 (Nrf2) transcriptional pathway have shown promise in clinical studies. This review presents detailed information on oxidative stress and its connection with neurodegenerative diseases. The therapeutic strategies designed to address these diseases are also described.

ROS GENERATION

Free radicals, with at least one unpaired electron in the outermost shell, is highly reactive⁷. The most common reported cellular free radicals are hydroxyl (OH•), superoxide (O₂⁻), nitric oxide (NO•), nitrogen dioxide (NO₂•), peroxyl (ROO•) and ...
lipid peroxyl (LOO•). Molecules such as hydrogen peroxide (H₂O₂), ozone (O₃), singlet oxygen (1O₂), hypochlorous acid (HOCl), nitrous acid (HNO₂), peroxynitrite (ONOO⁻), dinitrogen trioxide (N₂O₃), lipid peroxide (LOOH), while not considered free radicals, can easily lead to free radical reactions in living organisms[5]. Cells exposed to environment fortified with oxygen continuously generate oxygen free radicals. ROS includes oxygen-related free radicals and reactive species[6], and they are produced as a result of aerobic metabolism. Formation of ROS can occur in two ways: enzymatic and non-enzymatic reactions. Enzymatic reactions generating free radicals include those involved in the mitochondrial respiratory chain, phagocytosis, prostaglandin synthesis and the cytochrome P450 system[7]. For example, the superoxide radical is generated via several cellular oxidase systems such as 5,10-methylenetetrahydrofolate reductase oxidase, xanthine oxidase, peroxidases. ROS can also be produced from non-enzymatic reactions of oxygen with organic compounds as well as those initiated by ionizing radiations. The non-enzymatic process can also occur during oxidative phosphorylation (i.e., aerobic respiration) in the mitochondria[6]. ROS is generated from either endogenous or exogenous sources. Endogenous free radicals are generated from immune cell activation, inflammation, mental stress, excessive exercise, ischemia, infection, cancer and aging. Exogenous ROS result from air and water pollution, cigarette smoke, alcohol, heavy or transition metals (Cd, Hg, Pb, Fe, As), certain drugs (cyclosporine, tacrolimus, gentamycin, bleomycin), industrial solvents, cooking (smoked meat, used oil, fat) and radiation[9-12]. After penetrated into the body by different routes, these exogenous compounds are decomposed or metabolized into free radicals. Generation of ROS can occur in various organelles, mitochondria is the main source of ROS production[13-14]. The generation of mitochondrial ROS is a consequence of oxidative phosphorylation, a process that occurs in the inner mitochondrial membrane and involves the oxidation of reduced form of nicotinamide-adenine dinucleotide to produce energy. Mitochondrial electron transport involves four-electron reduction of O₂ to H₂O. However, during mitochondrial electron transport, a one-electron reduction of O₂ results in superoxide (O₂•⁻). Superoxide anion is detoxified by the mitochondrial manganese superoxide dismutase to yield H₂O₂, and H₂O in the presence of reduced transition metals can also be converted to hydroxyl radical (OH•). For the ROS is not originated from mitochondria, peroxisomal β-oxidation of fatty acids was considered to be a second source of oxygen radicals. This reaction generates H₂O₂ as a by-product. Peroxisomes are organelles responsible for degrading fatty acids as well as other molecules[14]. Phagocytic cells are another important source of oxidants; these cells defend the central nervous system (CNS) against invading microorganisms and clear the debris from damaged cells by an oxidative burst of nitric oxide, H₂O₂, and O₂. Finally, cytochrome P450 enzymes in animals are one of the first defenses against natural toxic chemicals from plants. In addition, the generation of ROS in living systems is closely linked with the participation of redox-active metals such as iron and copper. As a general principle, the chemical origin of the majority of ROS is the direct interactions between redox-active metals and oxygen species via reactions such as the fenton and haber-weiss reaction. Free iron (Fe²⁺) reacts through the Fenton reaction with H₂O₂, leading to the generation of very reactive and damaging hydroxyl radicals. Superoxide can also react with ferric iron in the Haber-Weiss reaction leading to the production of Fe³⁺, which then again affects redox cycling[15]. Apart from direct ROS generation, indirect pathway involves calcium activation with metallo-enzymes such as phospholipases, nitric oxide synthase. Calcium stimulates the tricarboxylic acid cycle and enhances electron flow into the respiratory chain; it also stimulates the nitric oxide synthase and subsequently promotes nitric oxide generation, which would inhibit respiration at complex IV. These events would enhance ROS generation from Q cycle. Calcium is an important signaling molecule and it is required for many cellular responses and cell-cell communication. Thus, any disruption of calcium homeostasis may disrupt the cellular physiology[16].

**BENEFICIAL AND DELETERIOUS ACTIVITIES OF ROS**

At low or moderate concentrations, ROS are necessary for the maturation process of cellular structures and can act as weapons for the host defense system, supporting cell proliferation and survival pathways. Indeed, phagocytes (neutrophils, macrophages, monocytes) release free radicals to destroy invading pathogenic microbes as part of the body’s defense mechanism against disease[7]. Other beneficial effects of ROS involve their physiological roles in the function of a number of cellular signaling systems. ROS signaling can affect cellular energetics by acutely regulating adenosine-triphosphate production via activation of uncoupling proteins[17]. Moreover, ROS are required for transduction growth signals via certain receptor tyrosine kinases[18]. Specific example includes nitric oxide, which is an intercellular messenger for modulating blood flow, thrombosis, and neural activity[19]. Nitric oxide is also important for nonspecific host defense, and for killing intracellular pathogens and tumors. Another beneficial activity of free radicals is the induction of a mitogenic response[18]. In brief, ROS at low or moderate levels are vital to human health. When produced in excess, these highly reactive radicals can start a pathological chain reaction, like dominoes[20], damaging all components of the cells, and leading to a progressive decline in physiological function[21]. This will generate a phenomenon called oxidative stress. Oxidative stress is
a deleterious process that can seriously alter the cell membranes and other structures such as proteins, lipids, lipoproteins, and deoxyribonucleic acid (DNA).[3, 6] Oxidative stress can arise when cells cannot adequately destroy the excess of free radicals formed. In other words, oxidative stress is caused by an imbalance between the production of reactive oxygen and a biological system’s ability to detoxify the reactive intermediates. Furthermore, in the CNS, ROS production has been linked to another key feature of neurodegenerative diseases, such as accumulation of protein aggregates, increase in intracellular free Ca2+, release of excitatory amino acids, autophagy and apoptosis, and all mechanisms play a critical role in the pathogenesis of many neurological disorders, such as PD, AD and amyotrophic lateral sclerosis[22]. For example, ROS overproduction within mitochondria can lead to oxidative damage to mitochondrial proteins, membranes, and mitochondrial DNA, finally resulting in mitochondrial injury[23]. ROS affect the heme-containing cytochrome c oxidase I molecule of complex IV of the respiratory chain, as well as induce additional damage to complex I, II, and III components[24]. Mitochondrial oxidative damage leads to the release of cytochrome c into the cytosol resulting in apoptosis. Increased permeability makes the inner membrane permeable to small molecules. Despite a large amount of scientific evidence supporting oxidative stress as a pathogenic factor in these diseases, human also experience with antioxidant neuroprotectants[25]. The body counteracts oxidative stress by producing antioxidants, either naturally generated in situ (endogenous antioxidants), or externally supplied through foods (exogenous antioxidants). The role of antioxidants is to neutralize excess of free radicals, protecting the cells against their toxic effects, and to contribute to disease prevention. However, overproduction of ROS which could not be fully neutralized can cause oxidative damage to biomolecules, and eventually leading to many chronic diseases, such as atherosclerosis, cancer, diabetics, rheumatoid arthritis and degenerative diseases.

OXIDATIVE STRESS IN NEURODEGENERATIVE DISEASES

Neurodegenerative diseases are clinically characterized by their insidious onset and chronic progression, and are pathologically characterized by progressive dysfunction and death of cells that frequently affect specific neural system. 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 deposit in specific cell types, representing the core features/hallmarks of many neurodegenerative disorders. While many brain neurons can cope with a rise in oxidative stress, there are selected populations of neurons that are vulnerable to increase oxidative stress, this phenomenon in neurodegenerative conditions is called selective neuronal vulnerability[25]. Selective neuronal vulnerability refers to the differential sensitivity of neuronal populations in the CNS to stresses that cause cell injury or death and lead to neurodegeneration. For example, neurons in the entorhinal cortex, hippocampal CA1 region, frontal cortex, and amygdala are the populations of neurons most sensitive to the neurodegeneration associated with AD. In PD, dopaminergic neurons of the substantia nigra are the primary neurons undergoing cell death[26]. Amyotrophic lateral sclerosis is characterized by the degeneration of, primarily, spinal motor neurons, but also cortical and brain stem neurons[27]. The fact that specific brain regions exhibit differential vulnerabilities to oxidative stress in various neurodegenerative diseases is a reflection of the specificity in the etiology of each disease, and it is possible that the selected cells involved in the pathology of neurodegenerative diseases may share a common increased vulnerability to the detrimental effects of oxidative stress. Neuronal cells are highly sensitive to oxidative stress, because (1) their large dependence on oxidative phosphorylation for energy as compared with other cells; (2) they are exposed to high oxygen concentration, utilizing about 20% of respired oxygen, even though the brain represents only 5% of the body weight. Under physiological condition, 1–2% of consumed O2 is converted to ROS, leading to oxidative stress, and this percentage goes up dramatically in aged brain[28]; (3) they are enriched in metal ions, which accumulated in the brain as a function of age and can be a potent catalyst for oxidative species formation; (4) they are rich in polyunsaturated fatty acids that are prone to oxidation; (5) they contain relatively poor concentrations of antioxidants and related enzymes. The brain is lower in antioxidant activity in comparison with other tissues, for example, about 10% of the liver. Under normal conditions, cells are capable of counteracting the oxidant insults by regulating their homeostatic balance. However, during the progression of age-related neurodegenerative conditions, the capacity of cells to maintain the redox balance decreases, leading to the accumulation of free radicals, mitochondrial dysfunction, and neuronal injury. It is widely accepted that oxidative stress increases during aging[29], and it can be considered as an important age-dependent factor making the neuronal systems more susceptible to several neurodegenerative diseases such as AD and PD.

EVIDENCE OF OXIDATIVE STRESS IN NEURODEGENERATIVE DISEASES

Oxidative overload in the neuronal microenvironment causes oxidation of lipids[30], proteins[31] and DNA[32] and generates many by-products such as peroxides, alcohols, aldehydes, ketones and cholesterol oxide[33]. (1) Lipid oxidation. Unsaturated lipids are particularly susceptible to oxidative modification and lipid peroxidation is a
sensitive marker of oxidative stress. Lipid peroxidation is the result of attack by radicals on the double bond of unsaturated fatty acids to generate highly reactive lipid peroxy radicals that initiate a chain reaction of further attacks on other unsaturated fatty acids. The chain reaction leads to the formation of breakdown products including 4-hydroxy-2, 3-nonenal (HNE) and F₂-isoprostanones[34-37]. HNE is able to modify proteins, resulting in a multitude of effects, including inhibition of neuronal glucose and glutamate transporters, inhibition of Na⁺⁻K⁺-ATPases, activation of kinases and dysregulation of intracellular calcium signaling, that ultimately induce an apoptotic cascade mechanism[38-40]. These findings, together with the recent demonstration that HNE is cytotoxic to neurons and that it impairs the function of membrane proteins including the neuronal glucose transporter 3, indicate that HNE is a characteristic marker and a toxin leading to neurodegeneration[41]. (2) Protein oxidation. ROS mediated oxidation of protein side-chains and resulted in the introduction of hydroxyl groups or in the generation of protein based carbonyls[42]. Carbonyl groups are introduced in proteins by oxidizing amino acid residue side-chain hydroxyls into ketone or aldehyde derivatives[43]. Carbonyl groups can also be introduced in proteins by direct oxidation of lysine, arginine, proline and threonine residues, or from the cleavage of peptide bonds by the α-amidation pathway or by the oxidation of glutamyl residues[44]. Measurement of protein carbonylation is thought to be a good estimate for the extent of oxidative damage of proteins associated with various conditions of oxidative stress[45-47]. (3) DNA oxidation. DNA bases are vulnerable to oxidative stress damage involving hydroxylation, carboxylation and nitration[48-50]. DNA and RNA oxidation is marked by increased levels of 8-hydroxy-2-deoxyguanosine and 8-hydroxyguanosine[51-53]. It is now widely accepted that oxidative damage is responsible for DNA strand breaks and this is consistent with the increased free carbonyls in the nuclei of neuronal cells in neurodegenerative diseases. (4) Glycoxidation. Advanced glycation end products (AGEs), which are formed by a non-enzymatic reaction of sugars with long lived protein deposits, are also potent neurotoxins and proinflammatory molecules. A cascade of reactions results thereafter in the formation of AGEs, which are composed of irreversibly cross-linked heterogeneous protein aggregates[54]. Accumulation of extracellular AGEs in neurodegenerative diseases is caused by an accelerated oxidation of glycated proteins (“glycoxidation”)[55].

**Protein aggregation and oxidative stress in neurodegenerative diseases**

Abnormal interactions between proteins that result in aberrant intracellular and extracellular deposition of self aggregating misfolded proteins with formation of high-ordered insoluble fibrils are common pathological hallmarks of multiple neurodegenerative disorders. Although the pathogenicity of protein aggregates remains uncertain[56], 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 prototypical derivatives of misfolded proteins may play a pathogenic role[57-58]. The exact mechanisms of abnormal folding are not fully understood; however, speculations lead to the presumption that genetic and environmental factors (especially oxidative stress) are involved[59]. Aberrant proteins, the result of inherited or acquired amino acid substitution or damage, especially oxidative modification, cannot fold correctly and will be trapped in misfolded conformations. Growing evidence supports the hypothesis that oxidative stress, combined with protein aggregation, triggers a cascade of events leading to cell death in multiple neurodegenerative diseases. Because proteins modified by oxidative reactive species tend to form aggregates, and highly oxidized and cross-linked proteins may act as endogenous inhibitors of proteasomal activity. Since the proteasome represents the major proteolytic machinery for the removal of oxidized and misfolded protein[60], inhibition of the proteasome or decreasing proteasomal activity will result in an accumulation of abnormal proteins[61]. Therefore, timely removal of oxidatively damaged proteins is of critical importance to maintain normal cellular homeostasis and viability. If homeostasis is not restored, cells ultimately undergo apoptotic or necrotic cell death. To prevent cytotoxicity induced by oxidized proteins, normal proteasome-dependent degradation is essential for cells to cope with oxidative stress[62]. In a vicious cycle, proteasomal dysfunction can lead to decreased degradation of misfolded proteins, resulting in accumulation of oxidized proteins and subsequent protein aggregation. Protein aggregates can then feedback to further inhibit proteasome activities, stimulate reactive species formation, and lead to cytotoxicity and human pathologies. Such phenomena have been implicated in many oxidative stress-associated disorders, including neurodegenerative diseases[63-64].

**Metal ions and oxidative stress in neurodegenerative diseases**

Metals are known to play a fundamental role in numerous essential metabolic processes of living systems. Homeostasis of metal ion is maintained through tightly regulated mechanism of uptake, storage, and secretion[65]. In the brain, the movement of metals across the blood brain barrier is highly regulated, and there is no passive flux of metals from the circulation to the brain[66]. Since the generation of free radicals is closely linked with the participation of redox-active metals, the disruption of metal homeostasis may lead uncontrolled metal-mediated formation of deleterious free radicals participating in pathogenesis of neurodegenerative disease. While iron, copper, and zinc are being increasingly implicated in interaction with...
major protein components of neurodegenerative diseases, this is not merely due to increased exposure to metals but rather because a breakdown in the homeostasis mechanisms that compartmentalize and regulate metals\textsuperscript{[87]}. The ability of metal ions to accept and donate electrons can lead to the formation of ROS, which may trigger the oxidative stress, therefore contributing to disease and perhaps aging itself\textsuperscript{[88-89]}. Increasing age is a dominant risk factor associated with the neurodegenerative diseases. Several studies in mice have shown that one of the consequences of normal aging is a rise in the levels of copper and iron in brain tissue\textsuperscript{[70-71]}. The brain is an organ that concentrates metal ions and recent evidence suggests that a breakdown in metal homeostasis is a key factor in a variety of age-related neurodegenerative diseases. Impaired iron metabolism is a hallmark in several neurodegenerative diseases such as PD and AD, multiple sclerosis, amyotrophic lateral sclerosis, and neuroferritinopathies. In the case of PD and AD, iron has been shown to play a key role in neuronal fate: depending on the extent and intensity of the oxidative stress caused by the increase in the labile iron pool, it affects transcriptional activity and signaling cascades that could participate in neuronal survival or death\textsuperscript{[72]}. Furthermore, in several age-dependent neurodegenerative disorders, the proteins might abnormally present Cu\textsuperscript{+2} or Fe\textsuperscript{+3} ligands for inappropriate reaction with O\textsubscript{2}, examples include β-amyloid in AD, α-synuclein in PD, Cu-Zn superoxide dismutase in amyotrophic lateral sclerosis. These proteins might have some aspect of their function subserved by these metal ions, which normally occupy higher-affinity, embedded, redox-shielded binding sites. As metal concentration rises in the brain with age, the probability increases that a redox-competent, low-affinity metal-binding site will recruit a metal ion from the normally redox-silent cellular pool. In this manner, proteins such as Aβ can harness endogenous biometals to foster the release of inappropriate redox activity and ROS generation.

**Oxidative stress in AD**

AD is the most common neurodegenerative disease in elderly people. It is characterized by progressive memory deficits, cognitive impairment and personality changes. Pathological features in AD are loss of neurons and synapses in the neocortex, hippocampus and other sub-cortical regions of the brain\textsuperscript{[73]}. The main histological features are extracellular protein deposits called senile Aβ-amyloid plaques and intraneuronal neurofibrillary tangles\textsuperscript{[73]}. Oxidative stress plays a major role in AD, believed to be stronger than in other neurodegenerative diseases\textsuperscript{[24]}. In addition, an accumulation of misfolded protein in the aging brain results in oxidative and inflammatory damage, which in turn leads to energy failure and synaptic dysfunction\textsuperscript{[75]}. Oxidative damage in AD exhibited through increased levels of DNA oxidation products like 8-hydroxydeoxyguanosine in mitochondria and nucleus\textsuperscript{[76]}. Protein carbonyls and 4-HNE are also found to be increased in brain tissues\textsuperscript{[77]}. Elevated levels of oxidized, nitrated and glycosylated proteins are found in plaques, helical filaments and cerebrosinal cord fluid from AD patients\textsuperscript{[78-80]}. AGEs are found to accumulate in Aβ and neurofibrillary tangles and it could be shown that AGEs induce the release of various potentially neurotoxic inflammatory mediators such as nitric oxide, IL-1 and tumor necrosis factor-α\textsuperscript{[81]}. The activity of the proteasome is also impaired, as hyperphosphorylated tau that is heavily ubiquitinated forms cross-linked aggregates and inhibits the proteasome\textsuperscript{[82-83]}. Transition metals are abnormally distributed in AD as well, studies revealed a marked association between redox-active iron and both Aβ-rich senile plaques and neurofibrillary tangles\textsuperscript{[84]}. It is well known that AD is characterized by Aβ accumulation in senile plaques, and Aβ deposition has also been demonstrated to participate in a positive feedback loop, where oxidative stress leads to increased Aβ generation, and, conversely, the mechanism of Aβ polymerization generates oxidative stress which in turn enhances Aβ production\textsuperscript{[85]}. Additionally, Aβ has been characterized as a metalloprotein, which is able to bind transition metals (e.g., zinc, iron, copper) via three histidine (positions 6, 13, and 14) and one tyrosine (position 10) residues located in the hydrophilic N-terminal part of the peptide\textsuperscript{[86-87]}. Binding of Cu\textsuperscript{+2} and Fe\textsuperscript{+3} produce toxic chemical reaction, alter oxidation state both the metals, produce H\textsubscript{2}O\textsubscript{2} catalytically in presence of transition metals. The H\textsubscript{2}O\textsubscript{2} can initiate a number of different events, including Fenton reactions to form toxic hydroxyl radicals and calcium dysregulation. As calcium is pivotal in signal transduction, it can induce further production of ROS and elicit an excitotoxicity response. In health, soluble Aβ is not present in the cortical synapse. In AD, soluble oxidized Aβ accumulates within the synapse, at which the high Zn\textsuperscript{+2} concentrations precipitate the copper/iron-metallated Aβ, creating a reservoir of potentially toxic Aβ. Augmented metal ions concentrations and oxidative stress have been found to correlate with changes in the concentration of both soluble and deposited Aβ\textsuperscript{[88]}. When Aβ interacts with these metals, the peptide aggregates, forming toxic oligomers and ultimately amyloid plaques. The toxicity of oligomers is elicited through interactions with the glutamatergic receptors such as the N-methyl-D-aspartate receptor. Interestingly, the metal-dependent generation of ROS by Aβ may be a good target for therapeutics\textsuperscript{[89]}. MPACs such as clioquinol (CQ, 5-chloro-7-iodo-8- hydroxyquinoline) and a copper/zinc ionophore (PBT2) seek to inhibit metal interactions with Aβ and prevent the subsequent formation of ROS and facilitate neuroprotective signaling.

**Oxidative stress in PD**

PD is characterized by the loss of dopaminergic neurons of the substantia nigra, and the deposition of intracellular inclusion bodies\textsuperscript{[90-91]}. It is the most common movement disorder in elderly people and the second most common...
neurodegenerative disease. PD is associated with the appearance of round, intracytoplasmic proteinaceous inclusions Lewy bodies. Several cellular components have been found in Lewy bodies, including synphilin-1, α-synuclein and others [92]. A characteristic feature of the neurons within the substantia nigra is the age-dependent accumulation of neuromelanin, and these neuromelanin-containing cells are most likely to be lost in PD. Neuromelanin is a dark brown pigment that accumulates metal ions, particularly iron. Significant evidence shows enhanced oxidative stress in PD, because markers of oxidative damage to biological structures, such as lipid, protein, and DNA oxidation are found to be increased in PD. Proteasomal function is affected in the substantia nigra in patients with sporadic PD [93], suggesting that the ubiquitin-proteasome pathway as well as the lysosomal enzyme is defective in PD [94]. Furthermore, proteins in Lewy bodies are generally oxidized, nitrated and contain products from lipid peroxidation that all promote aggregation [95]. Especially selective tyrosine nitration of α-synuclein may play a role in fibril formation of unfolded native synuclein and decrease the rate of degradation by the proteasome. Defective mitochondrial function like an impaired complex I activity is also revealed in Parkinson’s brain tissue, and inhibitors of mitochondrial complex-I lead to aggregation of α-synuclein in vitro as well as in animal models [96–97]. PD is typically associated with an increased iron content of the substantia nigra. Recent studies demonstrated raised iron levels in individual dopaminergic neurons of the substantia nigra. Moreover, accumulation of iron into mitochondria might lead to oxidative stress damaging iron-sulphur cluster-containing proteins [98]. Iron-mediated cellular destruction is mediated primarily via reactive oxygen or/nitrogen species induced oxidative stress. Furthermore, these pathogenic mechanisms appear to be closely interlinked to the cascade of events leading to cellular death [99]. Dopamine coordinates metals such as Cu²⁺ and Fe³⁺, reduces the oxidation state of the metals, and subsequently engenders production of H₂O₂, setting up conditions for Fenton chemistry [100]. Furthermore, studies have showed synthetic melamins were produced by incubating dopamine with Cu²⁺ and Fe³⁺ [101]. At low iron concentrations, melamins are known to have antioxidant properties, but at higher metal loads melamins are pro-oxidant. The oxidative stress associated with PD could be the result of a breakdown in the regulation of dopamine (neuromelanin)/iron biochemistry. Studies have showed that α-synuclein has a role in modulating the activity of dopamine. The mutation of α-synuclein associated with familial PD impairs vesicular storage of dopamine in the cytoplasm and subsequent generation of ROS through its interaction with iron [102]. In the presence of iron and under conditions of oxidative stress α-synuclein will aggregate and form deposits. In addition to the regulation of dopamine by α-synuclein, studies have shown a direct interaction of α-synuclein with metal ions, leading to protein aggregation.

Therapeutic options targeting oxidative stress in neurodegenerative diseases

**Antioxidants**

Antioxidants are exogenous or endogenous molecules which act against any forms of oxidative stress and its associated ill effects on the cellular system. They could neutralize ROS and other kinds of free radicals to inhibit oxidative stress. Many foods we consume contain a variety of antioxidant supplements, including flavonoids and phenolic compounds, lipoic acid (thiotic acid), ubiquinone and idebenone, β-carotene and vitamin C [103]. These natural antioxidants prevent oxidation of proteins, lipid peroxidations and prevent generation of ROS, thus act as an upstream therapeutic barrier to oxidative stress. One consequence of ROS generation is the initiation of excitotoxicity, which is modulated through the over-activation of glutamate receptors. Drugs that target these receptors are efficient upstream approaches in treating neurodegenerative diseases. For example, Memantines slows the development of AD and is of modest benefit to patients in the moderately severe to severe range of the disease by targets the N-methyl-D-aspartate receptor [104]. One of important futuristic upstream therapeutic aspect that can regulate oxidative stress to protect neuronal cells from death is vaccination against potential toxic protein formed in different types of neuronal disorders. A promising example is β-amyloid vaccination in AD that prevents plaque formation and subsequent neuron inflammation [105]. Downstream antioxidant activity functions as coverage for post oxidative stress events. For example, ginkgo biloba extracts (EGb 761), have been found to possess excellent antioxidant properties that restrict β-amyloid toxicity after plaque formation [106].

**MPACs**

MPACs are molecules that compete with the target protein for the metal ions. Since the breakdown in metal homeostasis in neurodegenerative diseases leads to tissue saturation with metal, the intention of the MPAC is to disrupt an abnormal metal-protein interaction, to achieve a subtle repartitioning of metals and a subsequent normalization of metal distribution [107]. The prototypic MPAC is clioquinol. CQ is able to bind a range of metal ions with moderate affinity, that is, in the nM range. CQ was then given orally in a blinded study to Tg2576 transgenic mice, the results showed a 49% decrease in brain Aβ burden compared with non-treated controls after 9 weeks of treatment [108]. Moreover, the effect of oral CQ treatment in a randomized, double-blind, placebo-controlled pilot Phase II clinical trial [109] of moderately severe AD patients was evaluated. The results showed a statistically significant prevention of cognitive deterioration during a 36-week period in the more severely affected patients. There was also a significant decline in plasma Aβ₄₂ in the CQ group.
compared with an increase in the placebo group. A novel second generation MPAC PBT2 has been synthesized that has higher solubility and increased blood-brain barrier permeability as compared with CQ. When tested in the APP/PS1 transgenic mouse model of AD, PBT2 decreased soluble interstitial Aβ within hours; this was accompanied by improved cognitive performance. In addition, there were significant decreases in insoluble Aβ load and tau phosphorylation. The randomized, double-blind, placebo-controlled Phase II clinical trial demonstrated reduced cerebrospinal fluid levels of Aβ42 and improved cognitive performance in patients taking PBT2[111].

**Concluding remarks and future strategies-targeting Nrf2 pathway**

Accumulating data suggests that oxidative stress is involved in the pathogenesis of neurodegenerative diseases, and that antioxidant administration may be useful in the prevention and treatment of neurodegenerative diseases. To obtain efficacy in delaying diseases progression, the candidate antioxidant must be given as early as possible, before irreversible neuronal loss. It also should be tailored to the precise oxidative stress physiology, e.g. the type of ROS involved, the place of generation, and the severity of the damage. The chosen antioxidant should also be able to penetrate the blood-brain barrier after systemic administration in order to attain a critical therapeutic level within the CNS. Cellular protection against oxidative stress-induced toxicities is provided by two types of antioxidants: (1) direct antioxidants, which are redox active, short-lived, are sacrificed in the process of their antioxidant actions and need to be replenished or regenerated, and may evoke pro-oxidant effects; and (2) indirect antioxidants, that may or may not be redox active. Indirect antioxidants activate the Kelch like-ECH-associated protein 1 (Keap1)/Nrf2/antioxidant response element pathway resulting in transcriptional induction of a battery of cytoprotective proteins (also known as phase 2 enzymes) that act catalytically, are not consumed, have long half-lives, and are unlikely to evoke pro-oxidant effects[112]. Indirect antioxidants act through the augmentation of cellular antioxidant capacity by enhancing gene expression[113]. Nrf2, an important stress-responsive transcription factor of the “cap-and-collar” β-leucine zipper family, is widely activated in response to stimuli, such as oxidative and reactive species. The activation of Nrf2 leads to upregulation of an entire array of genes that impart protection, as a result, this pathway has been identified as a promising therapeutic target for neurodegenerative diseases where oxidative stress and neuro-inflammation occur[114]. Eventually, this effect influences the physiological, biochemical, and/or cellular processes that inactivate free radicals or that prevent free radical-initiated chemical reactions[115]. The role of Nrf2 is now considered instrumental to several neurodegenerative disorders[116]. Under normal or unstripped conditions, Nrf2 is tethered in the cytoplasm by another protein called Keap1. Keap1 acts as a substrate adaptor protein for Cullin 3-based ubiquitination, which results in degradation of Nrf2 and, under normal conditions; Nrf2 has a halflife of only 20 minutes. Oxidative stress disrupts critical cysteine residues in Keap1, resulting in a disruption of the Keap1-Cullin 3 ubiquitination system and a build-up of Nrf2 in the cytoplasm. Unbound Nrf2 is then able to translocate into the nucleus, where it heterodimerizes with a small Maf protein and binds to the antioxidant response element in the upstream promoter region of many anti-oxidative genes, where it initiates their transcription. Activation of Nrf2 results in the induction of many cytoprotective proteins. These include heme oxygenase-1, nicotinamide adenine dinucleotide phosphate hydratenucleotide NAD(P)H quinone oxidoreductase 1, glutathione S-transferase and glutamatecysteine ligase[117-118]. In response to oxidative stress, Nrf2 normally translocates from the cytoplasm into the nucleus and transactivates expression of genes with antioxidant activity. Despite this cellular mechanism, severe oxidative damage is not uncommon in AD and PD. Intense mechanistic investigations in this arena have revealed that Nrf2 expression is abundant in both the nucleus and the cytoplasm of neurons in normal hippocampus with predominant expression in the nucleus. However, in AD, Nrf2 was predominantly cytoplasmic rather than nuclear in hippocampal neurons and was not a major component of beta amyloid plaques or neurofibrillary tangles. In contrast, the magnitude of expression of nuclear Nrf2 was much stronger in PD nigral neurons, but it was cytoplasmic centric in substantia nigra from normal Alzheimer’s patients. Such observations suggest that Nrf2-mediated transcription is not robust in neurons in AD despite the presence of oxidative stress. But in PD, despite a stronger nuclear localization of Nrf2, the impact of Nrf2 may be inadequate to protect neurodegeneration. Because of this differential Nrf2 expression, it can be considered as a potential therapeutic target for conditions that are sensitive to free radical damage[89]. Pre-clinical and clinical studies of the therapeutic potential of phytochemicals that activate the Nrf2/antioxidant response element pathway in several different neurodegenerative disorders are in progress[119].

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