

The Role of Oxidative Stress in Neurodegenerative Diseases

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Oxidative stress is induced by an imbalanced redox states, involving either excessive generation of reactive oxygen species (ROS) or dysfunction of the antioxidant system. The brain is one of organs especially vulnerable to the effects of ROS because of its high oxygen demand and its abundance of peroxidation-susceptible lipid cells. Previous studies have demonstrated that oxidative stress plays a central role in a common pathophysiology of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. Antioxidant therapy has been suggested for the prevention and treatment of neurodegenerative diseases, although the results with regard to their efficacy of treating neurodegenerative disease have been inconsistent. In this review, we will discuss the role of oxidative stress in the pathophysiology of neurodegenerative diseases and *in vivo* measurement of an index of damage by oxidative stress. Moreover, the present knowledge on antioxidant in the treatment of neurodegenerative diseases and future directions will be outlined.

Key words: Oxidative stress, Reactive oxygen species, Neurodegenerative disease, Alzheimer's disease, Parkinson's disease, Antioxidant

INTRODUCTION

Neurodegenerative diseases, as a heterogeneous group of disorders, are characterized by slowly progressive losses of neurons [1, 2]. The etiology of neurodegenerative diseases has not yet been fully elucidated, however increased oxidative stress has been suggested as one of the potential common etiology in various neurodegenerative diseases. Cumulative oxidative stress may

induce cellular damage, impairment of the DNA repair system [3], and mitochondrial dysfunction, all of which have been known as key factors in acceleration of aging process and the development of neurodegenerative disorders [2, 4, 5]. For these reasons, there have been continuing efforts to find the agents that can protect against oxidative damage and potentially treat neurodegenerative diseases. In this review, we focus to discuss the fundamental pathophysiological pathway of oxidative stress to the development of neurodegenerative diseases, especially in Alzheimer's disease (AD) and Parkinson's disease (PD). In addition, we will outline the present knowledge of available evidence in the prevention and treatment of neurodegenerative diseases and future directions for the potential of antioxidant supplementation with enhanced efficacy.

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CHARACTERISTICS OF REACTIVE OXYGEN SPECIES

Types of reactive oxygen species (ROS)

Oxygen is susceptible to radical formation due to two unpaired electrons present in the outer electron shell [6]. Reactive oxygen species (ROS) are defined as a group of reactive molecules derived from oxygen [7], which are generally short-lived and highly reactive because of their unpaired valence electrons [5]. ROS include, but are not limited to free radicals (superoxide, O_2^-), hydroxyl radical ($\cdot OH$), or non-radicals (hydrogen peroxide, H_2O_2) (Fig. 1) [2, 7, 8].

O_2^- is suggested to play a gateway role in ROS production. O_2^- may be transformed into the more stable form of H_2O_2 by superoxide dismutases (SOD) (Fig. 2). It may also be protonated to form HO_2^- . H_2O_2 may have potential to generate highly reactive hydroxyl radicals $\cdot OH$ [5], while it can further be divided into H_2O and O_2 by catalase, glutathione peroxidase, and other peroxidases [3] (Fig. 2). $\cdot OH$ is known to be one of the most reactive ROS that are mainly responsible for the cytotoxic effects of ROS [7]. $\cdot OH$ can be generated from H_2O_2 and O_2^- and is catalyzed by iron ions through the Fenton reaction that refers to Fe^{2+} -mediated decomposition of H_2O_2 [9].

Generation of ROS in brain

Cellular ROS are usually generated by both exogenous and endogenous sources [3, 10]. Exogenous sources of ROS generation include ultra violet (UV), ionizing radiation, drugs whose mechanism of action is mediated via ROS production. Environmental toxins and chemicals may also produce ROS as a by-product of their metabolism [10]. Endogenous production of ROS is mediated by mitochondrial and non-mitochondrial ROS-generating enzymes including nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Nox), xanthine oxidase (XO), cytochrome P450 from endoplasmic reticulum (ER), and flavin oxidases from peroxisomes [3, 10]. The major sources of ROS production are the mitochondrial respiratory chain and Nox systems.

Mitochondrial ROS production

The mitochondrion is the primary source of ROS production in the majority of cells. Under normal physiological condition, up to 2% of the total cellular mitochondrial O_2 consumption may be related to the generation of ROS including O_2^- [11-13]. Multiple ways of mitochondrial ROS productions have been proposed, which are mainly modulated by the mitochondrial respiratory chain complexes [12-14]. The mitochondrial electron transport

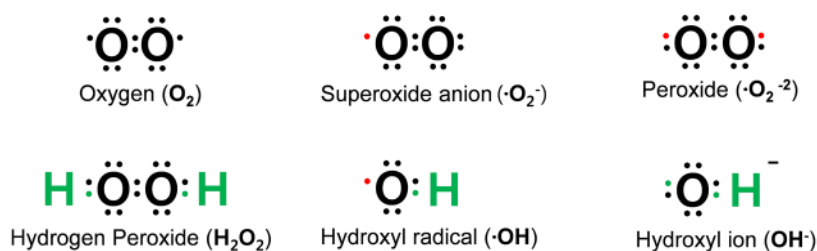


Fig. 1. Common reactive oxygen species (ROS). The consecutive reduction of oxygen through adding electrons cause the formation of a variety of ROS, which include superoxide (O_2^-), hydroxyl radical ($\cdot OH$), hydroxyl ion (OH^-) and hydrogen peroxide (H_2O_2). The red dot indicates an unpaired electron.

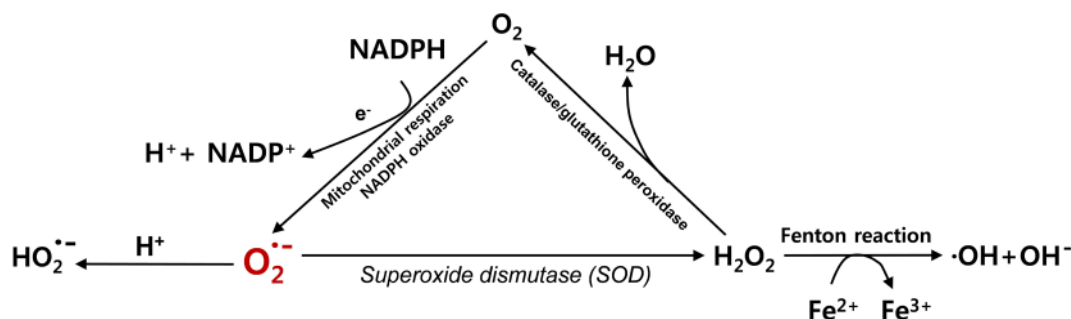


Fig. 2. Generation of ROS. The superoxide (O_2^-) is generated from O_2 as a by-product of respiratory chain complex in the mitochondria or by NADPH oxidase. By superoxide dismutase (SOD), the superoxide (O_2^-) can be transformed into hydrogen peroxide (H_2O_2), which can be further transformed to a number of other ROS such as hydroxyl radicals ($\cdot OH$) and hydroxyl anions (OH^-).

chain (ETC) consists of five multi-subunit complexes including NADH-coenzyme Q (CoQ) reductase (NADH dehydrogenase, Complex I), succinate dehydrogenase (Complex II), coenzyme Q-cytochrome c reductase (Complex III), cytochrome C oxidase (Complex IV), and ATP synthase (Complex V) [3, 10].

Complex I is responsible for ROS production of O_2^- [7, 15] and facilitates electron transfers from NADH to CoQ. During this step, protons are also translocated from the matrix to the intermembrane space [7]. Complex II is involved in the reduction of CoQ and is known to be involved in producing low levels of O_2^- [16, 17]. Complex III, on the other hand, is involved in the generation of O_2^- in the intermembrane space. The generation of O_2^- is especially enhanced when the electron transfer is reduced with the increased membrane potential [7].

Interestingly, the capacity of these enzymes to produce ROS may vary among the organs or during disease conditions [18]. For instance, Complex I appears to contribute to the production of most of O_2^- in the brain, while Complex III is considered as the primary source of O_2^- in the heart and lung [18]. In addition, within mitochondria, ETC Complex I and III are regarded as the main producers of O_2^- [3]. ROS productions from Complex I is approximately one-half of those from complex III in healthy state [3], while Complex I exerts the primary role in ROS productions under pathological conditions ranging from accelerated aging to neurodegenerative diseases [9].

NADPH oxidases (Nox)

Nox, a transmembrane enzyme complex, is known to be another important endogenous source of O_2^- production as the result of the catalyzing the electron transfer from NADPH to oxygen [19, 20]. Nox is found highly in phagocytes (neutrophils, eosinophils, monocytes and macrophages, called as Phox or NOX2) as well as in the endothelium of cardiovascular tissue [20, 21]. Until now, seven Nox isoforms have been identified in mammalian cells including Nox1 to Nox5 and dual oxidases (Duox1 and Duox2) [21]. Each Nox isoform has unique cellular localization, regulation, and function [3, 22]. For instance, Nox4 and Nox2 are abundant, whereas Nox1 is less in endothelial cells [23, 24]. In contrast, Nox1 and Nox4 are the more highly expressed isoforms in vascular smooth muscle cell than Nox2 [25]. Nox2, which is mainly expressed in phagocytes and produces large amounts of ROS, can help kill the foreign organisms as a part of the immune defense system [5, 20]. On the other hand, Nox produces relatively less ROS at a slow and sustained rate in cardiovascular tissue and exerts a role as intracellular signaling molecules [5]. Previous studies have reported that Nox4 is one of the most common isoforms in vascular structures [22, 26, 27]. In addition, contrary to Nox1 and

Nox2, Nox4 is fundamentally active in the cardiovascular systems [28, 29] and the primary source of H_2O_2 production rather than O_2^- production [30, 31].

Xanthine oxidases (XO)

XO and xanthine dehydrogenase (XDH) are inter-convertible forms of xanthine oxidoreductase [32]. XO is responsible for the catabolism of purines by converting hypoxanthine to xanthine and xanthine to uric acid [33]. XO donates electrons to oxygen and subsequently generate O_2^- and H_2O_2 within the cell [34]. In normal conditions, the enzyme is present as a form of XDH [2]. The involvement of XO in ROS-mediated diseases has been proposed with the increased level of intracellular calcium when energy status of the cell decreases and transmembrane gradients are disrupted in such a case of ischemic injury. Increased intracellular calcium may lead to the irreversible conversion of XDH into XO that catalyzes the oxidation of hypoxanthine to xanthine [2, 35]. Particularly during reperfusion, oxygen is reduced to its radical forms, H_2O_2 and O_2^- , in the presence of XO [2, 34, 35]. Increased ROS levels may contribute to further tissue damage.

ROS production in the endoplasmic reticulum

The endoplasmic reticulum (ER) is the membrane-based intracellular organelle that is primarily related to protein folding and lipid biosynthesis [10, 36]. ER may generate ROS by following two mechanisms [36]. First, under normal conditions, proper formations of disulfide bonds and protein folding take place in the ER for the stability and maturation of the membrane, which is driven by endoplasmic reticulum oxidoreduction-1 (ERO-1) and protein disulfide-isomerase (PDI) [36]. As electrons are transferred from protein thiol to oxygen by ERO-1 and PDI [36], ROS can be produced as a byproduct. Second, ROS is also produced by protein misfolding particularly in cases of glutathione (GSH) depletion [36]. Oxidized thiols are repaired to interact with ERO-1 and PDI by glutathione (GSH) [36]. These steps would initiate repetitive cycles of breakage and formation of disulfide bond within ER lumen, which more ROS is generating as a byproduct [36, 37]. Therefore, proteins which have multiple disulfide bonds may be more vulnerable to producing ROS [36]. Since GSH is used to reduce incorrectly formed disulfide bonds particularly under oxidizing environment, the level of GSH is further decreased and more ROS can be generated in this situation [30]. Furthermore, because the process involved in oxidative protein folding in the ER may be highly energy dependent, it should be noted that depletion of adenosine triphosphate (ATP) caused by protein misfolding may elicit oxidative phosphorylation in mitochondria and consequently produce more ROS [36, 38].

ROS production in peroxisomes

Peroxisomes are present in most eukaryotic cells and participate in multiple metabolic pathways including fatty acid oxidation, phospholipid biosynthesis, amino acid catabolism, and oxidative part of the pentose phosphate pathway [10, 39]. Under normal physiologic conditions, oxygen consumption in the peroxisomes may lead to the production of H_2O_2 , but not O_2^- . Peroxisomes is one of the organelles that can generate the majority of H_2O_2 using a number of different oxidases such as acyl-CoA oxidases, D-aspartate oxidase, and urate oxidase, which makes hydrogen to be transferred to O_2 from their respective substrates [10]. In addition, peroxisomes also contain catalases that can decompose H_2O_2 and help maintain the balance between the production and removal of ROS [39, 40]. When peroxisomes are damaged and the amount of catalases decreases, increased H_2O_2 release into the cytosol can contribute to oxidative stress in the cell [40].

Antioxidant pathway

Cellular ROS levels may be reduced through the defense mechanisms of antioxidant enzymes and small-molecule antioxidants [2]. O_2^- radical can be inactivated by SOD to produce H_2O_2 . Then H_2O_2 may further be removed by the action of glutathione peroxidases, catalase, and peroxiredoxins [41].

Superoxide dismutase (SOD)

SOD plays a significant role in catalyzing the breakdown of highly reactive O_2^- to less reactive H_2O_2 and oxygen [33]. Cytosolic copper/zinc-SOD (SOD1), mitochondrial manganese SOD (SOD2), and extracellular SOD (SOD3) are three distinct isoforms of SOD that have been identified. SOD1 and SOD2 are mainly involved in the elimination of O_2^- in the cytosol and mitochondria, respectively [33].

Glutathione peroxidases (GPX)

GPX contains a family of multiple isoenzymes which catalyze the reduction of H_2O_2 and lipid peroxides utilizing GSH as an electron donor [2, 33]. GPX is located in both cytosol and mitochondria. In mammals, there are five different isoforms of selenium-dependent glutathione peroxidases (GPX1-4 and 6) and three non-selenium congeners (GPX 5, 7 and 8) that have cysteine instead of selenocysteine [42]. Antioxidant function of GPXs depends on each isoform and location in the cells; GPX1 exists universally in the cytosol and mitochondria, GPX2 does in the epithelium of intestine, and GPX3 does in the plasma [42]. It is noteworthy that GPX1 has been regarded as one of the major antioxidant enzymes in the brain, which is expressed predominantly in microglia but not in neurons [41]. Studies have suggested that upregulation of

GPX1 could be one of the protective responses against neuronal injury [41].

Catalase

Catalase is responsible for the conversion of H_2O_2 to water and oxygen using either iron or manganese as a cofactor [2, 33, 43]. Catalase is located in peroxisomes and also found in the cytoplasm and mitochondria [2]. The role of catalase is minor at low levels of H_2O_2 , but becomes increasingly important at higher levels of H_2O_2 [2].

Peroxiredoxins (PRX)

PRX are thiol-specific peroxidases that catalyze the reduction of H_2O_2 as well as other organic hydroperoxides and peroxynitrite [33, 44, 45]. Among the six PRX isoforms, PRX1, 2, and 4 are present in the cytoplasm as well as in the nuclei. In addition, PRX1 is also expressed in the mitochondria and peroxisomes, while PRX4 is found in the lysosomes [44]. PRX3 is exclusively localized in the mitochondria [44, 46], whereas PRX5 is found in the mitochondria, cytoplasm, nuclei, and peroxisomes [47]. All PRX utilize a conserved active-site cysteine residue in order to directly reduce peroxide [48]. Since PRX are abundant in eukaryotic cells, constitute approximately more than 1% of cellular proteins, and show high reactivity, PRX are responsible for the reduction of up to 90% of mitochondrial H_2O_2 and almost 100% of cytoplasmic H_2O_2 [48-51].

Glutathione (GSH)

GSH, a tripeptide synthesized from glutamate, cysteine, and glycine, exerts protective function of cell survival against oxidative stress [2, 33]. In the brain, *in vivo* GSH is produced by the consecutive actions of two enzymes; γ dipeptide of γ -glutamylcysteine is formed by γ -glutamylcysteine synthetase, using glutamate and cysteine as substrates. And this dipeptide is further combined with glycine by the catalyzing action of glutathionine synthetase to synthesize GSH [52].

GSH is involved in the following two types of reactions; Firstly, GSH, in its reduced form, is known to non-enzymatically react with ROS such as O_2^- and $\cdot OH$ for the removal of ROS [2, 53]. Secondly, GSH is the electron donor for the reduction of peroxides in the GPX reaction [54]. Reaction with ROS firstly oxidizes GSH, which generates glutathione disulfide, the final product of GPX reactions. GSH can be regenerated from glutathione disulfide by the reaction with glutathione reductase that transfers electrons from NADPH to glutathione disulfide [54, 55].

Several studies have reported that GSH is involved in inhibiting apoptotic cell death [32, 56] and DNA damage in cells following

oxidative stress [56, 57].

Vitamin E

Vitamin E is a lipid-soluble antioxidant that can attenuate the effects of peroxide and protect against lipid peroxidation in cell membranes [2, 33].

Vitamin C

Vitamin C is a water-soluble antioxidant, which is involved in the removal of free radicals by electron transfer and also acts as a cofactor for antioxidant enzymes [3, 33].

Physiological functions of ROS

Low to moderate levels of ROS are critical in cellular signaling and pro-survival pathways [3, 5, 40, 58]. For instance, Nox-derived ROS play a role in cellular signaling related to the cardiovascular systems [22] and those in phagocytes (Nox2-derived) are involved in defense mechanisms of the immune system against foreign organisms [20]. Furthermore, the increased level of Nox-derived ROS activates important survival pathways, such as mitogen-activated protein kinase (MAPK) pathways [5]. The MAPK, the serine/threonine-specific protein kinases, represents the major redox-regulated signaling molecules in the cardiovascular systems [59]. It also modulates various cellular activities including gene expression, mitosis, proliferation, migration, cell survival, and apoptosis [5, 58, 60].

ROS can also activate transcription factors that regulate cellular responses to ROS [5]. Increased ROS may therefore promote antioxidant defense processes. An example is NF-E2-related factor 2 (Nrf2), which is one of major redox-sensitive transcription factors. It is activated by ROS and modulates the expression of several antioxidant enzymes including SOD, PRX, GPX, and heme oxygenases [61, 62]. A suppressor protein, Kelch-like ECH-associated protein 1 (Keap1), which is anchored in the cytoplasm, prevents the translocation of Nrf2 to the nucleus and keeps Nrf2 inactive under normal conditions [62]. Increased ROS production disrupts binding between Keap1 and Nrf2, allowing transcription by activation of Nrf2 [5, 63]. Nuclear factor-kappa B (NF- κ B) would be another pro-survival transcription factor that may be activated by ROS [5]. NF- κ B is normally present in the cytoplasm as an inactive state by the action of a NF- κ B inhibitor. Moderate levels of ROS may induce the phosphorylation and degradation of a NF- κ B inhibitor and result in activation of NF- κ B [64]. The activated NF- κ B transcribes anti-apoptotic proteins and inhibits caspase-dependent cell death pathways [5, 65]. In contrast, high levels of ROS may contribute to inactivation of NF- κ B by inhibiting its binding to DNA, attenuate pro-survival pathway, and

consequently promote apoptosis [66]. In this regard, the role of NF- κ B activation in a survival response to apoptosis depends on the amount of ROS formation [65].

Oxidative stress: excessive accumulation of ROS

In a healthy condition, the production of ROS is balanced by various antioxidant systems [2, 33]. Oxidative stress is a condition of imbalance between ROS production and antioxidant defenses, resulting in excessive accumulation of ROS [33, 67]. Oxidative stress may be related to cell membrane damage from lipid peroxidation, changes in protein structure and function due to protein oxidation, and structural damage to DNA [2].

As the brain is one of the most metabolically active organs in the body, it is vulnerable to oxidative stress particularly because of the following reasons. First, the brain has a high oxygen demand, which constitutes 20% of the body oxygen consumption. Second, the redox-active metals such as iron or copper exist abundantly in the brain and they are actively involved to catalyze ROS formation. Third, the high levels of polyunsaturated fatty acids are found in the brain cell membranes and react as substrates for lipid peroxidation [68]. Fourth, there are relatively low levels of GSH in the brain, which plays a role of endogenous antioxidant in the elimination of ROS [69].

OXIDATIVE STRESS AND NEURODEGENERATIVE DISEASES

Alzheimer's disease

Alzheimer's disease (AD), as one of the most common neurodegenerative diseases, is characterized by progressive neuronal loss and accumulation of proteins including extracellular amyloid plaques (A β) and intracellular tau tangles (neurofibrillary tangles, NFT) [70]. It has been suggested that oxidative imbalance and resultant neuronal damage may play a critical role in the initiation and progression of AD [71]. The excessive accumulation of ROS in patients with AD may induce mitochondrial dysfunction, however, the origin of increased ROS production and the exact mechanisms underlying the disruption of redox balance still remain elusive [72].

The accumulation of A β seems to increase oxidative stress and lead to mitochondrial dysfunction and energy failure [2] even in early stage of AD [73]. Previous studies have implicated that A β -induced oxidative imbalance may increase the levels of the byproducts related to lipid peroxidation (e.g. 4-hydroxynonal, malonaldehyde), protein oxidation (e.g. carbonyl) and DNA/RNA oxidation (e.g. 8-hydroxydeoxyguanosine and 8-hydroxyguanosine). In contrast, decreased levels of antioxidants (e.g. uric acid, vitamin C and E) or antioxidant enzymes (e.g. superoxide dismutase, catalase etc.) have been found in patients

with AD [71, 72]. In addition, AD transgenic mouse models expressing mutant amyloid precursor protein (APP) and presenilin-1 (PS-1) have shown increased levels of H₂O₂ and peroxidation of proteins and lipids, implying that A β may enhance oxidative stress in AD [72, 74].

Oxidative stress can also aggravate the production and aggregation of A β and promote the phosphorylation of tau protein, which could induce a vicious cycle of pathogenesis in AD [72]. Numerous previous studies have proved that oxidative stress also promotes the production of A β . It was reported that the defects in antioxidant defense mechanisms caused increased oxidative stress and, further facilitated A β depositions in transgenic mice with APP mutation [72]. Moreover, the deletion of cytoplasmic/zinc SOD in the Tg2576 APP overexpressing AD mouse model was related to increases in the oligomerization of A β and aggravated memory dysfunction [72, 75]. It has been hypothesized that oxidative stress may decrease the activity of α -secretase, promote the expression and activity of β and γ -secretase, and then lead to the enhanced production of A β [76].

There is emerging evidence to suggest the relationship between oxidative stress and tau pathology. It has been reported that cells with overexpressed tau protein showed increased vulnerability to oxidative stress, and it may be due to the depletion of peroxisomes [72, 77]. Furthermore, transgenic mouse models expressing mutant (P301S and P301L) tau proteins showed reduced NADH-ubiquinone oxidoreductase activity and mitochondrial dysfunction, both of which were associated with increased ROS production [78-80].

A β is accumulated primarily in the extracellular regions but also found in different subcellular areas including the ER and Golgi apparatus [81]. Interestingly, previous studies on AD have shown that the accumulation of A β has also been observed in the mitochondria [81], which may affect mitochondrial respiratory function, increase ROS production, and change mitochondrial membrane potentials in various brain regions [81]. A β -induced mitochondrial dysfunction has been suggested to inhibit the efficient production of ATP and increase the generation of ROS in AD [72, 82].

There are several evidences that suggest mitochondrial dysfunction in AD. First, reduced energy metabolism of the brain has frequently been observed in AD [71]. A previous study has also reported that decreased cerebral glucose metabolism in AD was associated with reductions in neuronal expression of genes that encode subunits of the mitochondrial electron transport chain [71]. Second, the activities of key enzymes of oxidative metabolism including α -ketoglutarate dehydrogenase complex, pyruvate dehydrogenase complex, and cytochrome oxidase were reduced in patients with AD [71]. They were significantly correlated with

the clinical severity and the senile plaque [83]. Reduced Complex IV activity has also been observed in the mitochondria of the hippocampus and platelets in patients with AD [2]. Third, A β -induced mitochondrial dysfunction contributes to impairment in calcium homeostasis [71]. This process is observed subsequently with the result in increased calcium overload and decreased reuptake of calcium [84]. The accumulation of mitochondrial calcium may be related to increased ROS production and the opening of permeability transition pore (PTP) [2]. It may be responsible for the translocation of pro-apoptotic molecules from the mitochondria to the cytosol and apoptosis. Increases in intracellular calcium can be measured indirectly by assessing the activity of calmodulin-dependent kinase and calpain. Interestingly, increased activity in calmodulin-dependent kinase and calpain has been observed in the early stage of AD [71]. Finally, increased oxidative damage to mitochondrial DNA, which may lead to more mutations of mitochondrial DNA, has been reported in patients with AD [71].

Parkinson's disease

Parkinson's disease (PD) is a neurodegenerative disorder characterized by selective neuronal loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc) and decreased DA levels in the nigrostriatal DA pathway in the brain [85, 86]. Although the exact mechanism still remains unclear, oxidative stress has been considered as one of major pathophysiological mechanisms underlying PD [86, 87].

Previous studies have found the reduced activity in Complex I of the respiratory chain in SNc of patients with PD, which may contribute to the generation of excessive ROS and, in turn, induce apoptosis [86-88]. Mitochondrial Complex I deficiency in the frontal cortex, fibroblasts, and blood platelets have also been reported in patients with PD [86]. Furthermore, the relationship between mitochondrial dysfunction and PD is supported by the findings that the Complex I inhibitors, such as 1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine (MPTP) and its metabolite, 1-methyl-4-phenylpyridinium (MPP⁺), may exert cytotoxic effects on the DA neurons, resultong in clinically parkinsonian phenotype, and induce nigral degeneration with cytoplasmic α -synuclein [1]. It has also been reported that genetic mutations in proteins including α -synuclein, parkin, and phosphatase and tensin homolog-induced putative kinase (PINK) were linked to the familial forms of PD [86]. Mutations of these genes have been known to affect mitochondrial function and increase oxidative stress [86].

Increasing evidence has supported that the accumulation of point mutations and deletions in mitochondrial DNA may be associated with Complex I defect or mitochondrial dysfunction

in PD [89]. Mitochondrial DNA has been well known to encode 13 proteins, mitochondrial transfer RNA, and ribosomal RNA including subunits from electron transport chain. Point mutations or deletions in mitochondrial DNA encoding Complex I subunit have been reported in patients with PD [89-91].

Changes in the antioxidant molecules have also been reported even in the early stage of PD [89]. For example, the levels of GSH, a major antioxidant molecule, have been reduced in the SNc of PD although this finding is not specific for PD [92]. In addition, higher levels of iron were observed in the SNc of PD in comparison with that of controls, which may arise from dysfunctional transport of iron to the mitochondria in DA neurons of PD [93,94]. It has also been reported that iron levels were increased in DA neurons in PD, which may allow easier interaction of ferrous iron with H₂O₂ and enhance production of highly toxic hydroxyl radicals (OH) [89]. High levels of iron in the SNc, therefore, may potentially exert harmful effects on the survivals of DA neurons

EVALUATION OF OXIDATIVE STRESS IN NEURODEGENERATIVE DISEASES

Measurement of oxidative stress in peripheral blood

Since oxidative stress may be a common pathophysiological mechanism underlying various neurodegenerative diseases, several surrogate markers for oxidative stress or antioxidant activity, including circulating lipid peroxides, GSH, and vitamins C and E, have been assessed in peripheral blood [69, 95-98]. A previous study has shown that AD patients demonstrated the decreased peripheral levels of vitamins A, C, and E [97] along with lower activities of SOD and glutathione peroxidase [95]. The levels of GSH in plasma have been suggested as a significant predictor of cognitive functions in patients with AD, implying the relationship between lower plasma levels of GSH and more severe cognitive impairment [99]. Although the results have been inconsistent, the activity of SOD in erythrocyte would be altered in patients in PD [100-103]. Increased SOD activity may contribute to the protection mechanism against enhanced production of O₂⁻ relating to neurodegenerative diseases [104].

Measurement of oxidative stress in human brain

Although there are various peripheral biomarkers for oxidative stress, it is still challenging to assess oxidative stress in the *in vivo* human brain. To date, the following measurements have been attempted and used to assess oxidative stress in the human brain.

Magnetic Resonance Spectroscopy

Glutathione (GSH): GSH is currently the only antioxidant

which can be measured using ¹H magnetic resonance spectroscopy (MRS) [105]. Until recently, the assessment of GSH in the human brain has been quite challenging since the concentration of GSH is lower in the human brain than those of other metabolites including N-acetyl aspartate, creatine, and choline. In addition, it is difficult to distinguish between the spectral proximity of resonance peak in GSH and those in other metabolites such as glutamate with MRS [105, 106]. For example, two protons from β-CH₂ of GSH-cysteine resonate at 2.93 and 2.97 ppm, which largely overlap with those of creatine (3.03 ppm) and aspartate (2.82 ppm) [105]. Therefore, in order to enhance GSH signal and to acquire reliable signals from the nuclei, specific spectral editing techniques such as MEScher-GARwood-PRESS (MEGA-PRESS) [107] would be required to measure GSH levels in the brain. The MEGA-PRESS technique in combination with additional editing pulse of 180° in the original PRESS pulse sequence [108] can help separate GSH-cysteine signals from other signals, especially the creatine signals in the brain [109]. Using this technique, a recent study has reported that the concentration of GSH was reduced in patients with AD, particularly in hippocampus and frontal cortices [108, 110]. These reductions were correlated with global cognitive functions [108, 110].

Vitamin C (Ascorbic acid): The concentration of vitamin C is approximately 1.0 mM in the human brain, which can be detectable with the use of MRS [105]. However, it is also difficult to measure vitamin C with ¹H MRS due to the similarity between the resonance of vitamin C (3.73, 4.01 and 4.50 ppm) and that of glutamate (3.75 ppm) [105]. The MEGA-PRESS editing could also help measure vitamin C in the human brain [111]. A few previous studies using ¹H-MRS with the MEGA-PRESS have measured the levels of vitamin C in the human brain [105, 109, 111].

Positron Emission Tomography

[⁶²Cu] diacetyl-bis (N⁴-methylthiosemicarbazone) ([⁶²Cu] ATSM): [⁶²Cu] diacetyl-bis (N⁴-methylthiosemicarbazone) ([⁶²Cu] ATSM) is a radiotracer for positron emission tomography (PET) that can measure intracellular over-reductive state [112]. This PET tracer has previously been used to detect an over-reductive state from myocardial ischemia or hypoxia associated with malignant tumors [113]. [⁶²Cu] ATSM could be accumulated in the brain regions in an over-reductive state through the reduction of Cu (II) to Cu (I) [114, 115]. Recently, regional oxidative stress which was mainly caused by mitochondrial dysfunction in patients with PD has been visualized through the application of this tracer in one study. In this study, the deposition of [⁶²Cu] ATSM was observed in the striatum in patients with PD [113], suggesting regional over-reductive state induced by

mitochondrial dysfunction.

Electron paramagnetic resonance (EPR) spectroscopy

EPR spectroscopy has been considered as one of possible methods to detect and characterize the radicals *in vivo* [116]. The basic concepts of EPR are analogous to those of nuclear magnetic resonance (NMR). However, EPR uses paramagnetic sample such as one or more unpaired electrons, instead of the spins of atomic nuclei, for excitation. The absorptions of the electromagnetic radiation may occur usually within the microwave frequency range of electromagnetic spectrum [116, 117], and thus depend on the types of paramagnetic species that are present in a magnetic field. However, EPR spectroscopy could not directly detect the radicals due to their short half-life compared to the EPR time scale [118]. Therefore, to compensate for this problem, a stable compound is usually utilized to trap the radical and enable a radical to be detectable [116]. Despite of great interest in EPR spectroscopy over years, one of the major reasons why it has not been commonly used is probably low sensitivity especially at the concentrations of free radicals typically found in the biological system [118]. Further studies are needed to apply EPR to human clinical studies.

ANTIOXIDANT TREATMENT FOR NEURODEGENERATIVE DISEASES

Clinical studies with antioxidant therapy in neurodegenerative diseases

Antioxidants are divided into endogenous or exogenous agents. The human endogenous antioxidant molecules include various enzymes such as SOD, GPX and catalase as well as non-enzymatic molecules (e.g. uric acid, GSH, and ascorbic acid), precursors of antioxidants (e.g. N-acetyl cystein) and cofactors of antioxidants (e.g. selenium and coenzyme Q 10) [119]. The exogenous antioxidants could be natural (e.g. N-acetyl cysteine, NAC) or synthetic (e.g. α -Lipoic Acid) [120]. The most commonly used antioxidants for clinical applications include vitamin E (the important scavenger of lipid peroxidation in the brain), vitamin C (intracellular reducing molecule), NAC (acting as a precursor of GSH), and coenzyme Q10 (transporter of electrons from complexes I and II to III in the ETC).

Although the initial results of the efficacy of antioxidants in the animal studies have been promising, the majority of the clinical trials in humans have shown negative results in terms of their efficacy for neurodegenerative diseases. A summary of clinical studies for neurodegenerative diseases is presented in Table 1 [121-137].

Possible reasons for little efficacy of antioxidants in treating neurodegenerative diseases

The following explanations may address why the current clinical trials have not yet found the potential antioxidants, which would effectively treat neurodegenerative diseases.

First, antioxidant therapy could not decrease oxidative stress in patients with neurodegenerative diseases potentially due to insufficient dose of antioxidants, unsuitable timing for therapy, or inappropriate duration of treatments [138]. On each related issue, the actual challenge could be how to evaluate the exact effects of antioxidants on the levels of a particular ROS at its proper action sites [138]. It is also important to evaluate the magnitude of therapeutic effects of antioxidants on alterations in levels of a particular ROS at its presumptively proper action sites [138]. In that sense, the development of biomarkers to better assess ROS is critical for the development of novel antioxidant therapeutic approach of neurodegenerative diseases.

The second point is that the oxidative damage may not be the primary cause, which contributes to the pathophysiology of the neurodegenerative diseases. An increase in oxidative damage could occur during the progression of disease, but it does not necessarily mean that it certainly causes the disease [138]. If so, antioxidants would not be the appropriate target for the treatment of neurodegenerative diseases. In addition, oxidative stress may not be the only cause of the disease. It should be noted that other deleterious processes such as inflammation and excitotoxicity also could be involved in the pathogenesis of neurodegenerative disease [139]. Therefore, it would be necessary to apply the combination of antioxidants with other drugs or multifunctional agents in treating neurodegenerative diseases.

Third, it is plausible that one single antioxidant may not be sufficient to resist the oxidative damage since the oxidative stress is modulated by a complex system of endogenous and exogenous antioxidants. In this regard, the combinatory approach of antioxidants would be necessary to be studied in the treatment of neurodegenerative diseases [120, 138].

Fourth, the timing of antioxidant therapy might not be optimal in previous clinical trials. Most trials have assessed the efficacy of antioxidants in patients with advanced AD or PD. Given that antioxidants may exert a prophylactic role in neurodegenerative disease, the earlier application of antioxidants, even before the onset of symptoms, may be effective [120, 139]. The optimization of time and duration of antioxidant therapy would be necessary to be elucidated.

Fifth, inter-individual differences in the levels of endogenous antioxidants may affect responses to antioxidant therapy [139]. Before starting antioxidant treatment, it might be helpful to

Table 1. A summary of antioxidants in clinical studies for neurodegenerative diseases

Authors	Study design	Participants	Intervention/ Duration	Outcome measures	Results
Alzheimer's disease (AD)					
Morris et al.[121]	Prospective longitudinal study	633 elderly with normal cognition at baseline	<ul style="list-style-type: none"> ▪ 4.3 years of follow-up ▪ Intake of antioxidant nutrients from foods and supplements were computed based on food questionnaire 	Incidence of AD dementia	The higher-dose vitamin E and vitamin C supplements may lower the risk of AD
Morris et al.[122]	Prospective longitudinal study	815 elderly with normal cognition at baseline	<ul style="list-style-type: none"> ▪ 3.9 years of follow-up ▪ Intake of antioxidant nutrients from foods and supplements were computed based on food questionnaire 	Incidence of AD dementia	Vitamin E from food : reduced risk of AD , only in non-carriers of apolipoprotein ε4
Zandi et al, Cache County study [123]	Cross-sectional and prospective study	5092 elderly with normal cognition at baseline	<ul style="list-style-type: none"> ▪ 3 year of follow-up ▪ Participants were categorized according to their use of vitamin supplements (vitamin E or C and vitamin B) 	Prevalence and incidence of AD dementia	Reduced prevalence and incidence of AD
Sano et al, Alzheimer's Disease Cooperative Study [124]	DPRCT	341 patients with moderate stage of AD	<ul style="list-style-type: none"> ▪ 4 years of intervention ▪ Randomization <ol style="list-style-type: none"> 1) Selegiline (selective MAOI,10 mg /day) 2) α-tocopherol (Vitamin E, 2000 IU /day) 3) Both selegiline and α-tocopherol 4) Placebo 	Time to occurrence of any of following: death/ institutionalization/ loss of the ability to perform basic activities of daily living /severe dementia	Slowing of the disease progression
Petersen et al. [125]	DPRCT	69 patients with the amnesic type of mild cognitive impairment	<ul style="list-style-type: none"> ▪ 3 years of intervention ▪ Randomization <ol style="list-style-type: none"> 1) Vitamin E (2000 IU /day) 2) Donepezil (10 mg /day) 3) Placebo 	The incidence of progression to AD dementia	No benefit on risk of progression to AD
Dysken et al. [126]	DPRCT	613 patients with mild to moderate AD	<ul style="list-style-type: none"> ▪ 6 months to 4 years of intervention ▪ Randomization <ol style="list-style-type: none"> 1) α-tocopherol (2000 IU/day) 2) Memantine (20 mg/day) 3) Memantine + α-tocopherol 4) Placebo 	ADCS-ADL score	Slowing functional decline and decreasing caregiver burden
Galasko et al. [127]	DPRCT	78 patients with mild to moderate AD dementia	<ul style="list-style-type: none"> ▪ 16 weeks of intervention ▪ Randomization <ol style="list-style-type: none"> 1) Vitamin E (α-tocopherol, 800 IU/day) + vitamin C (500 mg/day) + α-lipoic acid (900 mg/day) 2) Coenzyme Q tid/day 3) Placebo 	CSF biomarkers related to AD and oxidative stress, MMSE, ADCS -ADL	No influence on CSF biomarkers related to amyloid or tau pathology.
Adair et al.[128]	DPRCT	47 patients with probable AD dementia	<ul style="list-style-type: none"> ▪ 24 week of intervention ▪ Randomization <ol style="list-style-type: none"> 1) N-acetyl cysteine (50 mg/kg/day) 2) Placebo 	MMSE, Activities of Daily Living, cognitive battery	Improvement in some, but not all cognitive testing
Bergamasco et al. [129]; Weyer et al.[130]; Gutzmann and Hadler. [131]	DPRCT	300 patients with AD dementia with mild to moderate stage	<ul style="list-style-type: none"> ▪ 2 years of intervention ▪ Randomization <ol style="list-style-type: none"> 1) Idebenone (coenzyme Q, 30 mg tid) 2) Idebenone 90 mg tid 3) Placebo 	ADAS-Total	Dose-related beneficial effects of idebenone on cognition

Table 1. Continued.

Authors	Study design	Participants	Intervention/ Duration	Outcome measures	Results
Thal et al, Alzheimer's disease Cooperative Study.[132]	DPRCT	536 patients with probable AD dementia	<ul style="list-style-type: none"> ▪ 1 year of intervention ▪ Randomization <ol style="list-style-type: none"> 1) Idebenone 120 mg tid 2) Idebenone 240 mg tid 3) Idebenone 360 mg tid 4) Placebo 	ADAS-Cog and CGIC	No evidence of clinical benefit
Parkinson's disease (PD)					
Zhang et al. [133]	Prospective longitudinal study	From two large cohorts <ul style="list-style-type: none"> ▪ the Nurses' Health Study (76,890 women) ▪ the Health Professionals Follow-up Study (47,331 men) 	<ul style="list-style-type: none"> ▪ Nurses' Health Study : for 14 years ▪ Health Professionals Follow up Study : for 12 years ▪ Intakes of vitamins E and C, carotenoids, vitamin supplements were assessed by food questionnaire 	The incidence of PD	Higher dietary intake of vitamin E reduces risk of PD
Parkinson study group, DATATOP study. [134, 135]	DPRCT	800 early PD patients who received a diagnosis of PD within 5 years	<ul style="list-style-type: none"> ▪ 2 year of intervention ▪ Randomization <ol style="list-style-type: none"> 1) Deprenyl (MAOI, 10 mg/day) 2) α-tocopherol (2000 IU/ day) 3) Deprenyl plus α-tocopherol, 4) Placebo 	The onset of disability prompting the clinical decision to begin administering levodopa	No evidence of clinical benefit
Shults et al. [136]	DPRCT	80 patients with early PD who did not require treatment for their disability	<ul style="list-style-type: none"> ▪ 16 months of intervention ▪ Randomization <ol style="list-style-type: none"> 1) Coenzyme Q10 300 mg/day 2) Coenzyme Q10 600 mg/day 3) Coenzyme Q10 1200 mg/day 4) Placebo 	UPDRS score	Coenzyme Q10 appeared to slow the progressive deterioration of function in PD
The Parkinson Study Group. [137]	DPRCT	600 patients with early PD who received a diagnosis of PD within 5 years	<ul style="list-style-type: none"> ▪ 16 months of intervention ▪ Randomization <ol style="list-style-type: none"> 1) Coenzyme Q 10 (1200 mg/day) 2) Coenzyme Q 10 (2400 mg/day) 3) Placebo ▪ All of each group combined with Vitamin E (1200 IU/day) for synergistic antioxidant effects 	UPDRS score	Coenzyme Q10 showed no evidence of clinical benefit

DPRCT: double-blind, placebo-controlled, randomized clinical trial, MAOI: monoamine oxidase inhibitor, ADCS-ADL: alzheimer's disease cooperative study-activities of daily living, MMSE: mini-mental status examination, ADAS-total: alzheimer's disease assessment scale-total score, ADAS-Cog: alzheimer's disease assessment scale-cognitive subcomponent, CGIC: clinical global impression of change, UPDRS: unified parkinson disease rating scale.

select participants who could be the potential responders such as those with low levels of endogenous antioxidants, rather than the probable non-responders with high or normal levels of endogenous antioxidants [140].

CONCLUSIONS

The role of oxidative stress in the pathogenesis of neurodegenerative diseases has been well demonstrated in many preclinical and clinical studies. However, the benefit of antioxidant therapy for neurodegenerative diseases is still controversial in human, although the pre-clinical studies have shown promising

results. One of reasons for such discrepancy would be that there was no effective measurement of oxidative stress in the brain. Unfortunately, peripheral biomarkers may not necessarily represent oxidative stress in the brain and changes in neuronal function. Therefore, proper central biomarkers for oxidative stress should be identified to detect objective benefits to the brain and find exact therapeutic targets in treating neurodegenerative diseases.

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