Chapter

Neurodegenerative Diseases: Potential Effect of Glutathione

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Abstract

Neurodegenerative diseases are characterized by the progressive deterioration of neuronal function in the central or peripheral nervous system and ultimately the death of nerve cells. There is a big evidence that oxidative stress is an essential mediator implicated in neurodegenerative processes and may be a key event triggering various forms of cell death. Here, we review the hypothesis that neuronal loss resulted from oxidative stress may be initiated by a drastic decrease in the antioxidant molecule glutathione (GSH). The impairment of physiological glutathione's levels and the alterations in the activities of its related enzymes in neuronal cells are increasingly suggested to be implicated in the initiation and progression of neurodegenerative diseases. GSH plays a vital role in cellular redox homeostasis in the nervous system and protects neurons against a variety of oxidative insults. GSH depletion can enhance oxidative stress and may increase protein aggregation leading to initiate cell death in distinct neuronal populations. Evidence demonstrates a grand impact of oxidative stress and loss neuronal GSH in Parkinson's disease and Alzheimer's disease.

Keywords: mitochondria, oxidative stress, protein aggregation, cysteine, redox signaling

1. Introduction

During the past thirty to forty years, and along with the global rise in life expectancy, neurodegenerative diseases of the brain that affect the elderly in particular have become a burden on society more and more. The European Union (EU) Joint Programme-Neurodegenerative Disease Research (JPND) states that by the year 2030, a quarter (25%) of the European population will be over age 65, a significant increase over the current 16% [1]. Thus, the scientists place a special focus on age-related neurodegenerative diseases (ADD) research. These ADD such as Alzheimer's Disease (AD) and Parkinson's Disease (PD) have become more common and have drawn a lot of attention due to their irreversibility and lack of effective treatment [2]. Neurodegenerative diseases (DD) are known by gradual damage in neural cells and neuronal loss, which conduct to impaired motor or cognitive function. Largely, treatment is accomplished by reducing symptoms more than researching disease physiology and heading to the mechanisms that limit disease progression [3]. The mediating pathogeneses in neurodegenerative diseases are still not fully illustrated; however, great evidence demonstrates that reactive oxygen species (ROS) could be a key event as an elevated level of oxidative stress (OS) has been observed in the brain of DD patients [4]. In recent decades, a broad

range of studies has shown that the development of age-dependent neurodegeneration is due to a decrease in the antioxidant efficacy and an increase in oxidative damage. Cumulative ROS can cause damage to biomolecules such as lipids, proteins and DNA, in addition to mitochondrial dysfunction [5]. The increasing prevalence of DD and their profound hindrance to the quality of patient's life make it necessitous to come up with effective and novel treatment approaches, such as enhancing glutathione level in neurons. Glutathione (GSH) is a major antioxidant whose levels are found to decrease in aging as well as in DD. Scientists are currently heading to fully understand the role of GSH depletion in these diseases in addition to exploit that to develop GSH-based treatment. Glutathione is an essential cellular component, as it is primarily responsible for protecting and defending cells against any risk caused by exposure to ROS, and this role is evident, especially in the brain. Thus, GSH homeostasis disturbance and GSH-dependent enzymes inactivation lead to the breakdown of the main protective barrier against ROS and as a result, the cell becomes more vulnerable to the damage caused by OS [6].

In this chapter, we highlighted the OS in terms of its stimulating effect on the initiation and development of common ADD. We also demonstrated the great importance of glutathione in preserving nerve cells from the damage that OS may cause and the intracellular changes resulting from its depletion that may exacerbate the disease.

2. Oxidative stress and neurodegenerative disorders

2.1 Reactive oxygen species in brain

Although the human brain makes up only 2% of the body's weight, its oxygen requirements are estimated at 20% of the body's oxygen consumption. The brain is classified among the most generating organs for the reactive oxygen species (ROS), where 4% of the oxygen consumed by the mitochondria is converted into super oxide superoxide ion (O2•–), which possesses exceptionally high reactivity, particularly as a powerful oxidizing agent and an initiator of radical reactions. There are three radical reactions mainly initiated by superoxide "**Figure 1**" (i) under the influence of superoxide dismutase (SOD), the superoxide is converted into hydrogen peroxide (H_2O_2), which is subsequently converted to water and molecular oxygen by GSH peroxidase (GPx) or catalase. (ii) H_2O_2 can also react with iron, found in high concentration in brain, via the Fenton reaction to form hydroxyl radicals (OH.), which trigger lipid peroxidation. (iii) Superoxide also interacts with nitric oxide, which is formed in large quantities in the brain by an enzyme neuronal NO synthase (nNOS). This reaction is a million times faster than Fenton and produces the toxic oxidant peroxynitrite (ONOO−), which can spread 10,000 times farther than hydroxyl radicals. Harmful effects of ONOO− are varied including oxidation of macromolecules (DNA, proteins, lipids), nitration of amino acids and inactivating mitochondrial enzymes leading to mitochondrial dysfunction. It is now possible to detect specific markers that are byproducts of the oxidized endogenous macromolecules. For instance, 4-hydroxyl 2, 3-nenonal (HNE) is a marker of unsaturated lipid oxidation, which it has many cellular toxic effects such as irreversible formation of protein adduct and inhibition of GPx activity, and thus contributes in elevated levels of H_2O_2 [7].

2.2 Underlying role of oxidative stress in neurodegeneration

A large body of evidence demonstrates a particular susceptibility of neurons to ROS because of their distinctive characters: High energy demands, high oxygen

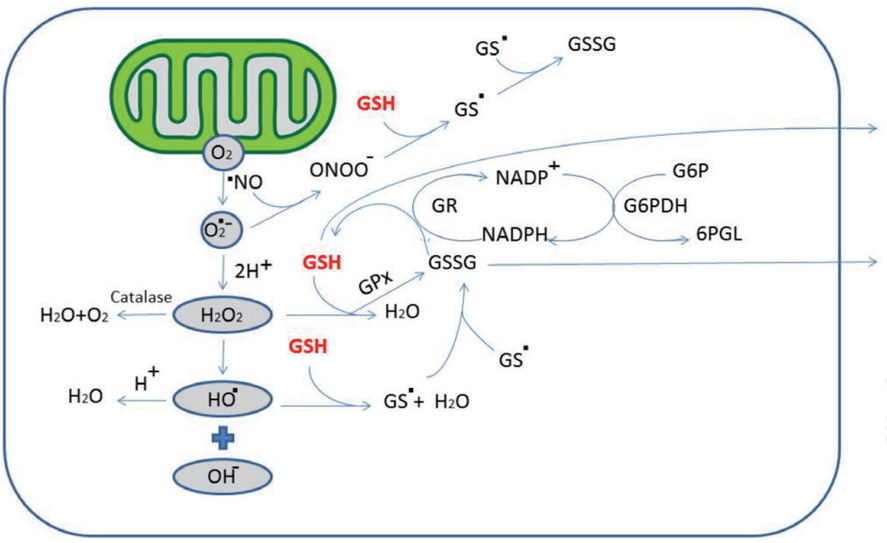

Figure 1.
Generation of ROS and Implication of glutathione in ROS/RNS elimination. As a result of mitochondrial respiration, the superoxide (O2-) is generated from O2. This latter can be converted into hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD). A number of other ROS such as hydroxyl radicals (·OH) and hydroxyl anions (OH-) can be produced from H_2O_2. Hydroxyl radical and nitric oxide or peroxynitrite may interact directly with GSH forming GSSG. GSH serves as an electron donor for the reduction of H_2O_2 or other peroxides, catalyzed by GPx, and as result, it is converted to GSSG.

consumption, high levels of iron, polyunsaturated fatty acids and, in particular, low anti-oxidative protection [8]. The defense enzymatic system in neuronal cells is weak where the SOD, catalase, and GPx activities are low compared to other organs. In addition, glutathione, an essential anti-oxidant component, is present in the brain at low concentrations. These findings suggest the involvement of ROS in neurodegenerative diseases [4, 8, 9].

The OS was one of the important axes of research conducted to understand the pathogenesis of neurodegeneration. A number of research confirmed a strong involvement of oxidative stress in the pathophysiology of neurodegenerative diseases through a variety of mechanisms including induction of oxidation of macromolecules such as nucleic acids, proteins, lipids, mitochondrial dysfunction, glial cell activation, amyloid β deposition, apoptosis, and proteasome dysfunction [3, 5, 10, 11]. A systemic review showed that these mechanisms of neurodegeneration are involved in many harmful cellular pathways. It has been observed that the interference in these pathways in complex ways has the greatest impact on disease development [12], apoptosis, cytokine production and inflammatory responses, and proteasome dysfunction. Currently, there is an increasing focus on the effects of OS on the pathogenesis of neurodegenerative diseases (DD) and the effectiveness of antioxidants as a promising treatment for DD.

3. Glutathione homeostasis and neurodegenerative diseases

Glutathione (GSH) is a thiol-containing tripeptide of major significance in normal brain function. GSH is formed from glutamate, cysteine, and glycine. The γ-carboxyl group of glutamate links the N-terminal glutamate and cysteine residues, unusual peptide bond. This specific peptide bond protects GSH against cleavage by intracellular peptidases preventing its hydrolysis and making GSH moderately stable in the cell. In addition, the presence of the C-terminal glycine residue in GSH structure prevents its cleavage by intracellular γ-glutamyl cyclotransferase. The cysteine residue is an effective functional component of GSH as

it provides thiol group, a principle responsible for the GSH activity. Moreover, cysteine residues form the intermolecular dipeptide bond in the oxidized form of GSH. Glutathione disulfide (GSSG), the major oxidized form, involves two residues of GSH that have been oxidized and connected by an intermolecular disulfide bond.

3.1 GSH content and synthesis

GSH is present in brain at elevated concentration (2–3 mM) compared to blood (15 μM) and cerebrospinal fluid (CSF) (5 μM) [13, 14]. Some evidence has been demonstrated that GSH is very poorly transported intact across the blood-brain barrier (BBB). However, it is probable that the blood is not the major source of cerebral GSH. This indicates that there is an avid brain system assures its synthesis in situ [15].

Generally, for maintaining GSH homeostasis in brain, there are at least two possible mechanisms: (i) glutathione constituents (cysteine moieties) may be recovered and recycled during the turnover of GSH in the brain, and (ii) precursors for brain glutathione synthesis (cysteine, cysteine-containing molecules) might be transported across the blood-brain barrier [13]. Cysteine is the rate-limiting substrate for neuronal GSH synthesis [15, 16]. In contrast, the availability of glutamine or glycine does not limit neuronal glutathione synthesis [13]. Therefore, cysteine alone is the crucial amino acid for neuronal GSH synthesis [17]. The neuronal uptake of cysteine is mediated by sodium-dependent systems, mainly the excitatory amino acid transporters (EAATs) [18]. EAATs have a significant function in removing extracellular glutamate in the CNS [19]. EAAT can transport not only excitatory amino acids, for example, glutamate and aspartate, but also cysteine, in particular, EAAT3, also known as EAAC1 that can transport cysteine at a rate comparable to that of glutamate [19].

Cystine, an oxidized form of two cysteines with a disulfide [20] linkage, is other source of free cysteine and employed as a substrate for GSH synthesis in some types of brain cells. Cystine moieties are transported into brain as (i) γ-glutamylcystine or as (ii) cystinylbisglycine which are possible origins of GSH in brain [21]. Cystine is especially important in maintaining glutathione levels in astrocytes [22], while it has no significance in the synthesis of neuronal GSH due to the inability of neurons to uptake it. Therefore, Content of cysteine or cysteine precursors determines the glutathione level in neurons since neurons are not able to use the cystine but rather rely on the availability of cysteine for their glutathione synthesis [20]. In addition to cysteine, neurons can utilize the cysteine donors such as CysGly, γGluCys, and N-acetylcystein (NAC) as precursors for glutathione. The presence of methionine does not increase neuronal glutathione levels [23]. Methionine is the main precursor of cysteine in liver, which supplies 50% of the cysteine needed for GSH synthesis. However, its role in producing cysteine in the brain is negligible and thus the neuronal GSH synthesis is not related to supply of methionine [16]. Among the exogenous precursors of glutathione, the dipeptide CysGly may be the most important. CysGly is efficiently utilized by neurons in micromolar concentrations [24].

Astrocytes store and synthesize high levels of GSH compared to neurons [13, 25, 26]. This is explained by the inability of neurons to directly uptake GSH. As well as, neurons utilize cysteine, not cystine, for GSH synthesis, whereas astrocytes utilize both [27, 28]. According to the above, neurons rely mainly on astrocytes to supply the necessary cysteine to neuronal GSH synthesis. GSH, released by astrocytes, undergoes a cleavage process by γ-glutamyl transpeptidase (γGT) [29] producing a γ-glutamyl moiety and a dipeptide CysGly which is an essential precursor of neuronal glutathione. The dipeptide CysGly could be uptake into neurons via a peptide transporter as has been described for astrocytes [30]. The dipeptide CysGly is hydrolyzed, upon entry into the neuron, by a neuronal ectopeptidase, providing cysteine and glycine [20] which subsequently are taken up as precursors for

glutathione synthesis. Glutathione is synthesized by two successive enzymatic steps dependant on ATP [13, 20]. the first step include γ-glutamylcysteine synthetase (GCL) which mediates the first reaction between glutamate and cysteine to form a dipeptide, γ-glutamylcysteine (γGluCys) which in turn combines with glycine to produce GSH. When a sufficient amount of glutathione is synthesized, a feedback occurs where GCL is inhibited [31]. Conversely, GSH depletion causes in the short term an increase in GCL activity and consequently an increase in GSH synthesis.

3.2 GSH activity against OS

The adult mammalian brain has a great demand for energy, and it almost relies entirely on metabolism of glucose. Most of the glucose is completely oxidized to carbon dioxide to meet energy requirements. This very high ability to oxidize glucose indicates that the brain may produce ROS at a remarkable rate. This increase in ROS production combines with low levels of defense mechanisms such as catalase and a high lipid content in brain. All of these indicate that the brain may be particularly vulnerable to OS.

GSH plays a leading role in reducing high levels of ROS and minimizing oxidative damage in brain (**Figure 1**). This importance has been established by several studies demonstrating that OS was aggravated by the GSH depletion, while increased intracellular GSH improved this damage [32]. GSH is a great component that provides protection against OS in brain by a direct interact with superoxide [33], NO [34], hydroxyl radical [35], and ONOO− [36]. The GSH capacity to scavenge superoxide is higher than NAC or cysteine [37]. Moreover, GSH is a principle hydroxyl radical scavenger because of unavailability of enzymatic defense against these radicals. On the other hand, GSH participates in enzyme-catalyzed redox cycling. The most important enzyme in glutathione redox reaction is glutathione peroxidase (GPx) due to its leading role in the reduction of toxic H_2O_2 (or lipid peroxide, ROOH) to H_2O (or ROH). GSH serves as an electron donor for the reduction of H_2O_2 or other peroxides, catalyzed by GPx, and as result, it is converted to GSSG [21]. The glutathione redox cycle, is completed by glutathione reductase (GR). This GSH redox cycle takes place in the cytosol and mitochondria, whereas GSH is compartmentalized in mitochondria [38], the major intracellular source of ROS [39], after its synthesis in cytosol. Catalase also reduces H_2O_2 to H_2O but it is unable to detoxify lipid peroxides and is not exist in mitochondria of most tissues. For these reasons, GPx is especially significant in protecting of mitochondria against H_2O_2,that are constantly generated during cell respiration [40, 41]. Mitochondria contain 5–15% of total cellular GSH [42]. The maintenance of this mitochondrial GSH pool occurs through the action of a high-affinity GSH uptake system [43] which is a main determinant of neuronal susceptibility to OS [44]. Depletion of this pool in brain mitochondria makes them more vulnerable to toxic effects of H_2O_2 leading to irreversible damages [45] and death. If mitochondria are not protected against OS insult, the organelles become irreversibly damaged through a process culminating with induction of a mitochondrial permeability transition (mPT) which is associated with the collapse of mitochondrial membrane ($\Delta\Psi$) and colloid osmotic swelling of the matrix [46]. As well, GSH detoxifies many agents that can induce the mPT in brain mitochondria including 4-hydroxyhexenal (a lipid peroxide) [39]. These findings indicate that GSH has a high significance in maintaining mitochondrial integrity in brain and other organs. Moreover, GSH is a substrate for glutathione S-transferase (GST) that catalyze GSH-dependent reduction of lipid peroxides. In addition to the above, there is a potential synergistic relationship between reduced glutathione and vitamin E, another line of defense. This vitamin is well recognized as antioxidant incorporating into cellular membranes to inhibit lipid peroxidation [47]. Lipids are protected

against ROS by α-tocopherol (vitamin E), which quenches ROS and by that, converts to α-tocopheroxyl radical. This latter can re-reduced non-enzymatically to α-tocopherol by GSH [48]. This reaction and those that are catalyzed by GPx and GST possess peroxidase activity and form a protective barrier of the brain against damaging effects of H_2O_2 on polyunsaturated fatty acids in biomembranes (lipid peroxidation) [49].

Many of studies have been demonstrated the specific toxicity of Hydrogen peroxide to brain [42, 43, 50]. This peroxide induces apoptosis in neuronal cells which are particularly sensitive to its toxic effects [51]. Nevertheless, neurons can detoxify H_2O_2, but apparently this capacity is more greater in astrocytes for which they play a putative role in the modulation of the neurotoxic effects of H_2O_2 [45, 46, 52]. The neuronal defense system against H_2O_2 is mainly based on glutathione redox cycle. This role of GSH is clearly illustrated by a rapid oxidation of GSH when H_2O_2 is applicated to neurons [53]. Intracellular GSH depletion enforces mitochondrial damage and causes cell death. Apoptosis has been hypothesized to be mediated through the induction of free radicals via oxidative pathways. Thus, a direct cause/effect relationship between GSH depletion and apoptosis was evidenced in neuronal cell [54]. In addition, GSH depletion is an early hallmark in the progression of cell death [55].

3.3 Implication of glutathione in neurodegenerative disorders

It has been previously emphasized that the breakdown of the balance between ROS and antioxidant defense systems is the main manipulator triggering the initiation or progression of a number of common neurodegenerative diseases such as Parkinson's (PD) and Alzheimer's (AD) diseases. Each of these diseases depends on a number of factors including mainly OS. However, the causative link between OS and neurodegeneration is not in the scope of this part as it focuses on the dysregulation of the GSH-based antioxidant network in the context of common neurodegenerative diseases: Parkinson's disease, Alzheimer's disease [6].

3.3.1 Parkinson's disease (PD)

The primary pathologic hallmarks of PD are loss of dopaminergic neurons located in an area of the brain called the substantia nigra pars compacta, and the presence of Lewy bodies, intracellular aggregates of misfolded α-synuclein, in dopaminergic neurons and likely contribute to the death of these neurons. Neurons in the substantia nigra pars compacta produce dopamine, a neurotransmitter (chemical messenger) that transmits signals from the substantia nigra to other parts of the brain. These other parts of the brain are collectively called the "basal ganglia". Communication among neurons of the substantia nigra pars compacta and the basal ganglia produce smooth, purposeful movement. When the neurons in the substantia nigra are damaged in large numbers, the loss of dopamine prevents normal function in basal ganglia and causes the motor symptoms of PD: tremor, rigidity, impaired balance, and loss of spontaneous movement [56].

Dopaminergic SN cells are usually pigmented with black neuromelanin, produced from of the autoxidation [57] or enzyme-mediated oxidation [58] of the cytoplasmic dopamine (DA) to DA-o-quinone, which then Polymerizes. Usually, this process is accompanied with production of H_2O_2 rendering dopaminergic SN cells are particularly sensitive to OS probably. It has been reported that dopaminergic SN neurons having high basal levels of DA oxidation, heavily pigmented, is particularly vulnerable to degeneration in PD [59].

A massive loss of nigral GSH is the most notable distinctive changes that occur in the earliest stage [60] in the parkinsonian SN. This GSH loss is uncorrelated to

altered activities of biosynthetic enzyme and not accompanied by an increase in GSSG levels [61]. It has been indicated that the drastic drop in GSH is attributed to raise in activity of γ-GT, causing an increased removal of both GSH and GSSG from cells [61]. It is interesting that GSH depletion is characteristic to the parkinsonian SA and is not observed in other neurodegenerative disorders of the basal ganglia [62].

GSH depletion cause indirectly formation of endotoxins in the cytoplasm of pigmented SN cells that contribute to the degeneration of these neurons in PD "**Figure 2**." As previously mentioned the activity of γ-GT is up-regulated significantly in the parkinsonian SN [61]. This enzyme is involved in translocation of free cysteine into dopaminergic SN neurons and expulsion of GSH out of these cells. Thus, the profound loss of nigral GSH, main storage form of cysteine, makes the free cysteine, which is increasingly transported into the cell, more likely to bind to oxidizing dopamine and formation DHBTs (dihydrobenzothiazines) by a series of consecutive reactions [63]. These compounds are lethal and evoke profound neurobehavioral responses, especially DHBT-1 which cause irreversible inhibition of mitochondrial complex I [64].

The presence of Lewy bodies, aggregated misfolded α-synuclein, in SN is also characteristic hallmarks to PD which mainly participate in neurodegeneration [65]. The plurality of intracellular proteins is degraded by the ubiquitin (Ub)–proteasome pathway (UPP). In this pathway, the protein Ub, belongs to a family of heat shock proteins (HSPs), is covalently bound by thiol groups to misfolded or damaged proteins and contributes in their breakdown by transferring them to the protease 26S complex. There are three enzymes contributing to UPP: E1 (Ub-activating enzyme) and E2s (Ub-carrier) prepare Ub for conjugation, but the main enzyme in the process is the E3 (Ub-protein ligase) which transfers the activated ubiquitin to the protein substrate to be degraded [66]. Any defect in the components of UPP or a lack of their activity result in accumulation of α-synuclein protein and subsequent aggregation leading in turn to the formation of Lewy bodies. The depletion in GSH in dopaminergic SN neurons leads to decreased E1 activity and subsequent UPP

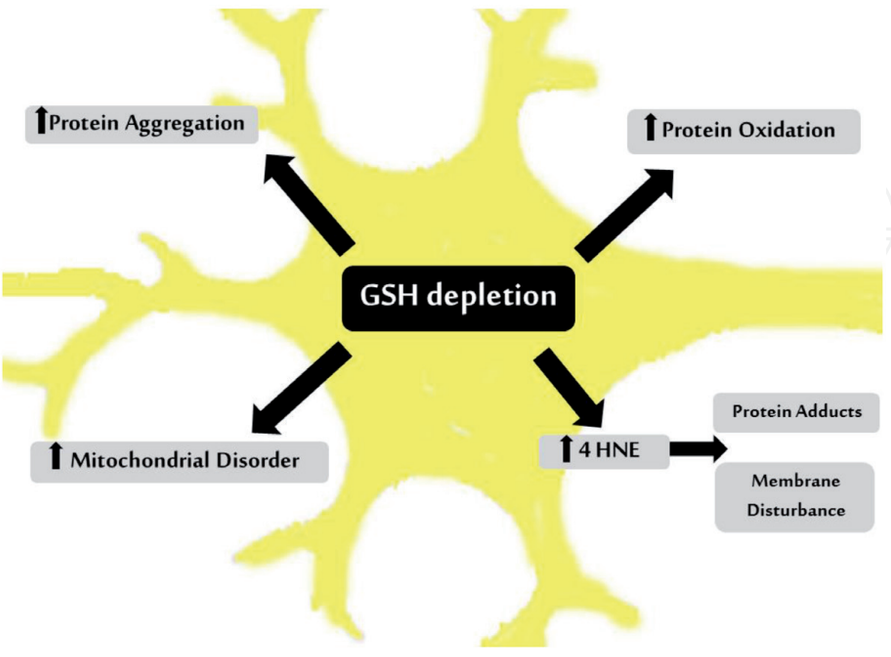

Figure 2.
Consequences of GSH depletion in SN dopaminergic neuronal cells in PD. Drastic loss of GSH is associated with protein aggregation which form Lewy bodies, mitochondrial dysfunction resulted from inhibition of complex I activity and oxidative damage including protein oxidation and the deleterious effects of the lipid peroxidation by-product 4HNE.

disturbance [67]. This finding indicates that GSH protects the active sites of these enzymes from being oxidized during oxidative stress, and thus keeps them performing in the Ub-proteasome pathway.

Additionally, the early GSH loss in parkinsonian SN accompanied by increased OS leads to raise in oxidized proteins. In the early stage of PD, HSP proteins are expressed at high levels to prevent the deleterious effects resulting from accumulation and aggregation of damaged proteins in dopaminergic neurons. As the disease progresses, these defenses become unable to control the build-up of protein aggregates [68].

OS also target the mitochondria and interfere with all of their functions. Mitochondrial disorders occupy a crucial place in the mechanisms that mediate neurodegeneration associating with the pathology of PD [69]. Since glutathione is the main component in detoxification of hydroperoxides in mitochondria, its depletion in the brain is believed to promote mitochondrial insult most likely via increased ROS. The mitochondria are known by their vulnerability to OS that might interfere with all of their functions. By serving as the main component in detoxification of hydroperoxides in mitochondria, GSH may reduce the oxidative insults that affect mitochondria. GSH depletion in the brain therefore is believed to promote mitochondrial insult most likely via increased ROS [70].

In synaptic mitochondria, the major role in control over oxidative phosphorylation is attributed to complex I that at 25% inhibition, energy metabolism is disturbed and ATP synthesis is drastically affected. To manifest similar effects [71], complex III and IV inhibition up to 80% is necessitated. The reduced complex I activity in the SN is known as a considerable biochemical characteristic in Parkinsonian brain [72]. Evidence suggest that Early depletions in nigral GSH levels may be directly lead to mitochondrial complex I activity inhibition and subsequent mitochondrial dysfunction which ultimately induces dopaminergic cell death related to PD. The complex I is the most severely influenced mitochondrial enzymes during OS [73]. It is believed that OS, due to decreased GSH availability in the brain, is the major responsible of mitochondrial complex I activity inhibition. This susceptibility of complex I to OS might be explicated by the oxidation of thiol (SH) groups of protein and the existence of accessible oxidation sensitive iron-sulfur centers in this complex [74].

It is recognized that GSH controls the activity of thiol-dependent proteins by keeping the SH groups of protein in a reduced state and preventing them from oxidation [75]. GSH conjugates with oxidized thiol groups to form protein-SS-G and subsequently can be re-reduced to protein and GSH by GR, thioredoxin or protein disulfide isomerase. In addition, GSH, present in dopaminergic cells, can bind to quinones resulted from dopamine oxidation preventing their reaction with SH groups in protein [76].

Lastly, during oxidative insult, aldehydes are formed as a byproduct; the most common of these types is 4HNE. This latter is able to incorporating into the membranes causing changes in their fluidity [77]. In addition, 4HNE can form adducts with important proteins like Na/K ATPas making them inactive. GSH may help reduce the levels of 4HNE by conjugating it via GST. In the PD brains, the loss in GSH in the SN results in high levels of 4HNE adducts [78].

3.3.2 Alzheimer's disease (AD)

AD, the most common age-related neurodegenerative disease, is known by progressive dementia affecting older populations. This disease is pathologically characterized by depositions of amyloid β (Aβ) plaques and neurofibrillary tangles (NFTs) [14]. The presence of amyloid plaques, which are mainly composed of Aβ peptide, in the extracellular space of AD brain is a main hallmark of disease. The excess of

Aβ levels, especially Aβ 42, the most neurotoxic peptide, causes the emergence of familial forms of Alzheimer's disease. This increase in Aβ 42 leads to the formation of soluble oligomers, causing permanent changes in synaptic function. In parallel, Aβ 42 is aggregated forming mostly β-sheet rich fibrils that enhance local inflammatory responses (microgliosis and astrocytosis). Synaptic spine loss and neurotic dystrophy are also observed. Over time, these events result in a biochemical changes including oxidative stress, altered ionic (e.g.; calcium) homeostasis [79]. Amyloid plaques are the determining factor in triggering a signaling pathway leading to AD progression. Recent evidence suggests that Aβ plaques induce neuronal apoptosis in the brain and in primary neuronal cultures, and this Aβ-induced neuronal death may be responsible in part for the cognitive decline found in AD patients. In addition, aggregated Amyloid-β activates the p38 mitogen activated protein kinase (MAPK) in cell leading to hyperphosphorylation of protein Tau and formation of neurofibrillary tangles (NFTs) inside neurons, making the microtubules unstable and causing the loss of neuron functionality [80].

Evidence demonstrates that Soluble Aβ oligomers are able to block the EAAC1-mediated cysteine uptake leading to a GSH loss in cultured human neuronal cells [81]. This is supported by autopsy brain of AD patients, which exhibit aberrant EAAC1 accumulation in pyramidal neurons of the hippocampus [82] and decreased GSH/GSSG ratios with the progress of disease [83].

Based on the above, it is possible to emphasize the notion of EAAC1 dysfunction in Alzheimer's disease.

Oxidative stress is considered a major pathogenic factor in AD. Since GSH depletion is of immense implication in oxidative stress, it is expected to have a role in the emergence and development of the disease. A recent clinical study using NMR spectroscopy showed that GSH level is depleted in AD patients as compared to healthy subjects [84]. This finding may have a profound clinical significance. In addition, the analysis of the blood samples of AD patients showed a decrease of GSH concentration in red blood cells compared to age- and gender-matched controls [85]. This is also observed in mild cognitive impairment (MCI) which is a preclinical stage of AD. MCI patients showed a decrease in GSH/GSSG ratios and GST activity in the hippocampus compared to healthy age-matched controls [86]. According to these results, it is suggested that disturbances in GSH metabolism precede the onset of AD. Genetic polymorphisms in the GPx-1 and GST genes were identified as positive risk factors for AD [87]. This can be the reason of decreases in GPx and GST activities in AD [88].

As was previously mentioned, ROS formation is induced by Aβ aggregating and cause a number of oxidative damages and metabolic insults including generation of HNE in hippocampal neurons, which could in turn mediate the toxicities of such insults [89]. Several studies have been shown an increase in lipid peroxidation in the brain of AD patients compared with age-matched controls [90]. As a result of lipid peroxidation, HNE, secondary bioactive aldehyde, is produced at a high levels in several brain regions of late-stage AD subjects [89]. The significant role of HNE in the progression of AD is supported by many findings. Accordingly, an increased level protein-bound HNE in brain of MCI patients was observed [91]. Many proteins were found to be significantly HNE-modified in AD such as ATP synthase, glutamine synthase, DRP-2, and MnSOD. These proteins have a great implication in the regulation of structural functions of brain cell in addition to a number of cellular functions including cellular signaling, energy metabolism and detoxification. Evidence showed that GSH could prevent oxidative damage induced by Aβ and HNE in cultured neuronal cells. This finding suggests that GSH depletion exacerbates oxidative insults stimulated by Aβ and HNE and therefore accelerates the development of the disease.

4. Conclusion

GSH is an interesting subject studied intensively in the brain for the past several decades. The purpose of such research is not only to understanding the potential role of intracellular GSH in preventing DD progression but also to provide the mechanistic insights contributing to the cellular dysfunctions associated with these diseases. GSH depletion is a common feature of DD triggered by a wide variety of cause including disturbance in GSH homeostasis and modification of the GSH related enzymes. Multiple cellular problems attributed to dysregulation of GSH and GSH-dependent enzymes contributes to impairment in the function of mitochondria, elevation in oxidative damage, disruption of intracellular signal transduction pathways, protein aggregation, and eventually cell death.

It is important to note that further research is necessary to determine more accurately the involvement of disruption of the network of glutathione-dependent reactions in the neurodegenerative events and find new ways to prevent or limit these events. As well to suggest more effective approaches therapy for DD patients.

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Acronyms and abbreviations

EU	European union
JPND	joint programme-neurodegenerative disease research
GSSG	glutathione disulfide
GPx	glutathione peroxidase
GST	glutathione S-transferase
GS	glutathione synthetase
GR	glutathione reductase
PD	Parkinson's disease
AD	Alzheimer's Disease
DD	neurodegenerative diseases
RNS	reactive nitrogen species
ROS	reactive oxygen species
GSH	reduced glutathione
OS	oxidative stress
4HNE	4-hydroxyl 2, 3-nenonal
EAAC1	excitatory amino acid transporter C1
EAAT	excitatory amino acid transporter
GCS	γ-glutamylcysteine synthetase
NFTs	neurofibrillary tangles

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