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Glutathione, iron and Parkinson's disease

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Abstract

Parkinson's disease (PD) is a progressive neurodegenerative disease involving neurodegeneration of dopaminergic neurons of the substantia nigra (SN), a part of the midbrain. Oxidative stress has been implicated to play a major role in the neuronal cell death associated with PD. Importantly, there is a drastic depletion in cytoplasmic levels of the thiol tripeptide glutathione within the SN of PD patients. Glutathione (GSH) exhibits several functions in the brain chiefly acting as an antioxidant and a redox regulator. GSH depletion has been shown to affect mitochondrial function probably *via* selective inhibition of mitochondrial complex I activity. An important biochemical feature of neurodegeneration during PD is the presence of abnormal protein aggregates present as intracytoplasmic inclusions called Lewy bodies. Oxidative damage *via* GSH depletion might also accelerate the build-up of defective proteins leading to cell death of SN dopaminergic neurons by impairing the ubiquitin–proteasome pathway of protein degradation. Replenishment of normal glutathione levels within the brain may hold an important key to therapeutics for PD. Several reports have suggested that iron accumulation in the SN patients might also contribute to oxidative stress during PD.

Keywords: Parkinson's disease; Oxidative stress; Mitochondrial dysfunction; Lewy bodies; Glutathione; Iron

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease affecting approximately 1% of the human population aged 65 and above [1,2]. PD is a slowly progressive neurodegenerative disorder and is clinically manifested by defective motor function, a decline in cognitive function and depression [3]. Physiologically, PD is characterized by dopamine deficiency owing to the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) region of the midbrain [4]. Its pathological features also include the presence of intracytoplasmic inclusions known as Lewy bodies [5]. Research in the recent years has accumulated substantial evidence supporting the hypothesis that oxidative stress triggers a cascade of events leading to the death of neuronal cells during PD [6].

During different processes of cellular aerobic metabolism such as mitochondrial oxidative phosphorylation, reactive oxygen species (ROS) like superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$) and peroxynitrite (ONOO–) are generated. Excess production of these molecules can potentially damage different macromolecules such as proteins, nucleic acids and lipids thereby leading to cellular degeneration [7]. To counter this, the cells maintain a battery of detoxifying enzymes and small molecule antioxidants a list of which is provided in Table 1. When there is either an increased production of ROS or a decrease in the levels of antioxidant defenses or both, the toxic effects of such a scenario can be summed as oxidative stress [4,8].

Although the body in general has evolved several defense mechanisms to counteract oxidative stress, the brain appears to be more susceptible to this damage than other organs. Dopaminergic neuronal cells of the SNpc are particularly vulnerable to such insult due to the ROS generated during dopamine metabolism. Levels of both

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Abbreviations: PD, Parkinson's disease; SNpc, substantia nigra pars compacta; ROS, reactive oxygen species; MAO, monoamine oxidase; GSH, glutathione; GSSG, oxidized form of glutathione; γ-GCS, gamma-glutamyl cysteine synthetase; GPx, glutathione peroxidase; GR, glutathione reductase; γ-GT, gamma-glutamyl transpeptidase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydro pyridine; 4HNE, 4-hydroxynonenal; MPP+, 1-methyl-4-phenyl pyridium; Ub, ubiquitin; LIP, labile iron pool.

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Table 1
A brief list of the various antioxidant molecules present in the cell [8]

Antioxidant molecules
Glutathione
Ascorbic acid
Lipoic acid
α-Tocopherol
Ubiquinol
Carotenoids
Uric acid
Flavanoids
Antioxidant enzymes
Catalases
Superoxide dismutase
Glutathione peroxidase
Glutathione-*S*-transferase
GSSG reductase
Repair enzymes like DNAses, RNAses, lipases and proteases
Thioredoxin reductase

lipid peroxidation and the DNA oxidation by-product 8-hydroxy-deoxyguanosine (8-OHDG) have, for example, been demonstrated to be elevated in the SN of PD patients compared with age-matched controls [9–12]. The nonenzymatic oxidation of dopamine leads not only to the generation of quinone and semiquinone molecules but also ROS such as H_2O_2. Enzymatically, both the synthesis of dopamine by tyrosine hydroxylase and catabolism by monoamine oxidase (MAO) can also lead to H_2O_2 production [13,14]. Nigral neurons contain neuromelanin that can bind iron and initiate its reaction with H_2O_2 *via* the ''Fenton reaction'' to form the highly reactive $^{\bullet}OH$. Hydroxyl radicals can in turn extract methylene hydrogens from polyunsaturated fats in neural membrane phospholipids, initiating lipid peroxidation and cell death. Increases in iron levels have been implicated in inducing cytotoxicity during PD by promoting excessive accumulation of $^{\bullet}OH$ [15]. The sequence of oxidative events within the nigrostriatal system becomes interlinked because neurodegeneration due to oxidative stress in turn increases dopamine turnover leading to increased H_2O_2 production. An important player in protecting dopaminergic SN cells against oxidative stress is the antioxidant molecule glutathione (GSH).

2. GSH, the universal antioxidant

The tripeptide GSH (γ-L-Glu-L-Cys-Gly) is the most abundant intracellular nonprotein thiol compound in mammalian cells [16]. GSH is also present as glutathione disulphide (GSSG), the oxidized form of GSH and as GSSR representing GSH-cysteine disulphides on proteins. Most importantly, GSH functions as an antioxidant with a crucial role as a scavenger of toxic free radicals and detoxification of xenobiotics. Other functions include maintenance of thiol redox potential in cells by reducing the thiol groups of proteins, transport and storage of cysteine and as a cofactor in certain isomerization reactions [17,18]. Recent research data suggest GSH may also have a role in signal transduction, cell proliferation, regulation of gene expression and apoptosis [19–21]. The nitroso form of GSH (GSNO) is considered to be a storage and transfer form of nitric oxide [16,22,23]. Furthermore, GSH also appears to play a role in various cellular processes such as DNA metabolism, protein synthesis, activation of certain enzymes and enhancement of immune function [24,25].

3. Regulation of GSH levels

De novo GSH synthesis in all cell types *in vivo* occurs from the constituent amino acids in two consecutive steps catalyzed by γ-glutamyl cysteine synthase (GCS) and GSH synthase. GCS catalyzes the formation of γ-glutamyl cysteine, the rate-limiting step in GSH synthesis, and the synthase completes the tripeptide by adding glycine. Both the reactions occur in the cytosol and require ATP (see Fig. 1 for the scheme of GSH metabolism). Mammalian γ-GCS is a heterodimer consisting of a catalytic heavy subunit (γ-GCS_H; 73 kD) and a regulatory light subunit (γ-GCS_L; 31 kD). Mammalian GSH synthetase is a homodimer (monomer MW 52 kD) [26]. The synthesis of GSH also includes GSSG reductase-mediated regeneration. GSSG reductase is a flavoenzyme, which catalyzes the transfer of the reduction equivalent from NADPH onto GSSG [27]. On the other hand, cellular levels of GSH are diminished when it is either consumed during the formation of GSH-*S*-conjugates by GSH-*S*-transferases (GST) [28], by conversion to GSSG by GSH peroxidase (GPx) [27] or by the release of GSH from cells [29,30]. Approximately 10% of total cellular GSH is transported to the mitochondria through an energy dependent-mechanism. Recently, Shi *et al*. [31] have shown that knockout mice harboring a null mutation in the heavy chain of γ-GCS were embryonic lethal and that the animals failed to gastrulate demonstrating that GSH synthesis is indispensable during the early stages of mammalian development. In cell culture, permanent cell lines derived from the mutant blastocysts could be rescued by the addition of GSH to the medium. These cells also remained healthy and proliferated indefinitely if grown in GSH-free medium supplemented with *N*-acetyl cysteine (NAC). These results show that GSH or an antioxidant equivalent, NAC, can rescue cells suggesting that GSH-like molecules are essential for cell growth. This is likely *via* GSHs crucial role as part of the primary cellular defense against oxidative stress. GSH reacts with toxic free radicals both nonenzymatically and also acts as an electron donor in the reduction of peroxides catalyzed by GPx. The resultant GSSG is acted upon by GSH reductase thus recycling the GSH. GPx plays a major role in the recycling of GSH as suggested by the data that GPx knockout mice challenged with toxins like

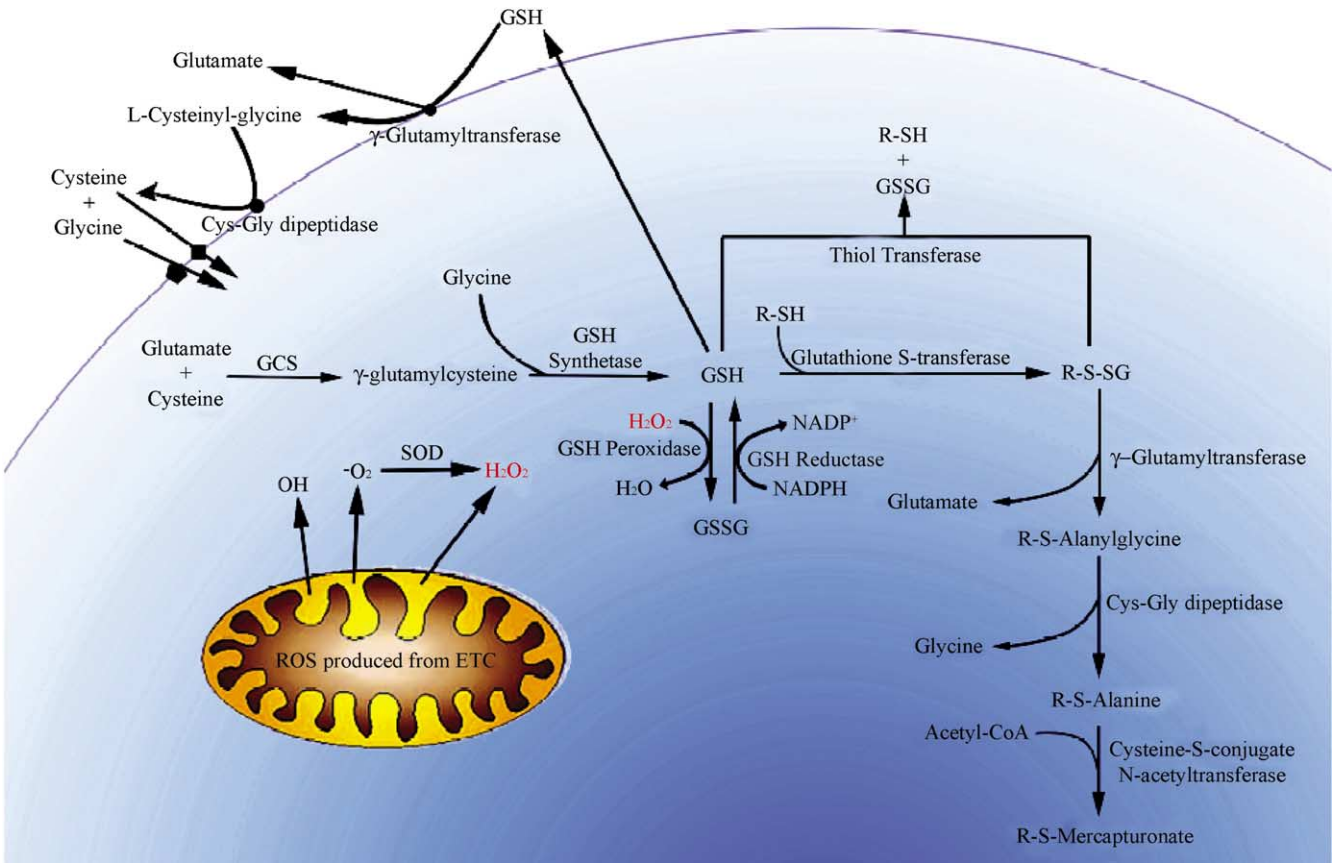

Fig. 1. A schematic representation of the different pathways involved in GSH metabolism. GSH is synthesized *de novo* in the cytoplasm from its constituent amino acids in a two-step reaction. The cellular GSH pool is also contributed to by conversion from GSSG, the oxidized form of GSH. H_2O_2 produced in the mitochondria is detoxified by the conversion of GSSG to GSH. GSH also participates in the formation of mixed disulfides with thiol-proteins (RSH), which can further become metabolized. GSH that is secreted from the cell is broken down into its constituent amino acids which can be taken up by the cells to be used for *de novo* GSH synthesis. For more details, see references [16,17,27].

1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) exhibited greater depletion of dopamine compared to age matched control mice [32].

A steady-state balance between the synthesis and depletion of GSH maintains its cellular levels. The synthesis of GSH depends, among other things, on the expression levels and posttranslational modification status of γ-GCS, availability of the substrates and feedback inhibition by GSH on the enzyme [26]. GSH is a nonallosteric feedback inhibitor of the γ-GCS enzyme. In the presence of thiols like GSH, the disulfide bridge connecting the heavy and light subunits of GCS heterodimer is reduced inducing a conformational change within the substrate binding site of GCS_H. The relaxed substrate-binding site can accommodate tripeptides like GSH with greater affinity thereby inhibiting substrate binding leading to decreased synthesis of GSH. Studies suggest that phosphorylation of serine/threonine residues on the γ-GCS_H molecule can lead to decreased activity of the enzyme thus lowering the synthesis of GSH [26].

The pathways resulting in loss of GSH mainly involve the conversion to GSSG by GPx, conjugation reaction with proteins [33] and 4-hydroxynonenal (4HNE) [34,35] and transport of GSH/GSSG across the plasma membrane to the outside of the cell. Once outside the cell, γ-glutamyl transpeptidase (γ-GT), a membrane bound enzyme initiates the degradation of extracellular GSH by catalyzing the transfer of the γ-glutamyl moiety from GSH/GSSG or a GSH conjugate onto an acceptor molecule. The product of this reversible reaction is further hydrolyzed into the constituent amino acids cysteine and glycine, which are taken up by cells *via* a peptide transporter and utilized for synthesis of GSH [36]. Hence an increase in γ-GT activity should theoretically lead to a decrease in the cellular pool of GSH. In fact Sian *et al.* [37] have proposed that the decrease in GSH observed in the PD SN is due to an increase in γ-GT activity.

4. GSH in the brain

Compared to other organs in the body, the brain is more susceptible to oxidative damage due to several factors which include high oxygen utilization [38], high iron content [39], presence of excess unsaturated fatty acids which are targets for lipid peroxidation [40], and decreased

activities of detoxifying enzymes like superoxide dismutase (SOD), catalase and GR [18]. Mitochondria, nitric oxide synthase, arachidonic acid metabolism, xanthine oxidase, MAO and P450 enzymes are all sources of ROS in the brain. GPx is the major enzyme for the detoxification of H_2O_2 in the brain since the brain has reduced catalase activity.

GSH is present in the brain in millimolar concentrations [18]. Although there are some reports favoring the transport of GSH across the blood brain barrier and their uptake into brain cells, it still needs to be established whether these play a role in GSH homeostasis in the brain [41,42]. Nevertheless, the constituent amino acids of GSH may cross the blood brain barrier and be utilized for GSH synthesis in the brain following the same pathway as described earlier [27]. Several reports employing different techniques have shown the presence of GSH both in neurons and glial cells [27,43,44]. The concentration of GSH appears to be higher in brain astrocytes compared to neurons [45]. Although there are reports which suggest that GSH is released from brain cells, very limited information is available about the exact mechanism [46–48]. Apart from the antioxidant functions of GSH in the brain, extracellular GSH has been hypothesized to have additional functions as a neurotransmitter [49], neurohormone, in the detoxification of glutamate and in leukotriene metabolism [27]. According to recent evidence accumulated based on coculture experiments, brain astrocytes and neurons appear to interact metabolically with one another in terms of GSH metabolism [18].

It has been observed that there is an age-dependent depletion in intracellular GSH of many organisms including humans [50]. In humans, there appears to be a decline in the GSH levels in the cerebrospinal fluid during aging [51]. Studies have shown that aged mice have a 30% decrease in levels of GSH compared with younger animals [52,53]. Since the brain requires extensive ROS detoxification, it is evident that a decrease in GSH content could increase oxidative damage making the brain more susceptible to neurological disorders.

5. GSH and PD

GSH plays an important role in the adult brain by removing H_2O_2 formed during normal cellular metabolism. In general, SN has lower levels of GSH compared to other regions in the brain. Previous experiments from our laboratory [54] have demonstrated that the relative variations in levels of GSH in different brain regions are cortex > cerebellum > hippocampus > striatum > SN which is consistent with previous reports [55,56]. However, the GSH profiles of all regions are the same through the lifespan, namely, high values during growth dropping to a maturation plateau and then decreasing 30% during aging [56]. It has been observed that during PD, there is a further reduction in GSH levels within the SNpc [57,58]. In fact, GSH depletion is the first indicator of oxidative stress during PD progression suggesting a concomitant increase in ROS. Although GSH is not the only antioxidant depleted during PD, the magnitude of GSH depletion appears to parallel the severity of the disease and occurs prior to other hallmarks of the disease including decreased activity of mitochondrial complex I (described later) [59,60]. GSH levels are not altered in areas of the brain other than SN or in other diseases affecting dopaminergic neurons. In a related work from our laboratory [61], systemic administration of buthionine sulfoximine (BSO), a GCS inhibitor resulted in neurodegenerative effects on the dopaminergic SN neurons that were not generalized to other neuronal cell populations in the brain. BSO treatment has been shown to potentiate the effects of both MPTP and 6-hydroxydopamine (6-OHDA), toxins used to model PD in the SN and striatum [62,63]. These data suggest that these neurons may be especially susceptible to the effects of disturbance in cellular redox *via* GSH depletion. Early depletion in nigral GSH levels observed in Parkinsonian brain is not explainable by increased oxidation of GSH to GSSG as levels of both are found to be decreased. Furthermore, there is no failure of GSH synthesis because the activity of γ-GCS in the SN is normal in PD [37]. GSH losses have rather been suggested to be due to increased activity of the enzyme γ-GT resulting in increased removal of both GSH and GSSG from cells [37]. The increased activity of γ-GT may be the initial step in the pathogenesis of PD.

Mitochondria are responsible for generating 90% of the ATP required for all cellular functions, for detoxifying ROS produced *via* mitochondrial respiration, for controlling the cellular redox state, and for regulating cytoplasmic calcium levels by acting as the major intracellular sink for this ion. Oxidative damage to the mitochondria might interfere with all of these functions. Mitochondrial dysfunction appears to play a major role in the neurodegeneration associated with the pathology of PD [64]. Decreased GSH availability in the brain is believed to promote mitochondrial damage most likely *via* increases in levels of oxidative stress to this organelle. Depletion of brain GSH has been shown to result in decreases in mitochondrial enzyme activities in preweaning rats as well as losses in ATP production in the aging murine brain [65,66]. Previous studies have shown that complex I in bovine heart submitochondrial particles is particularly affected by oxidative stress [67,68]. Reduction in the activity of mitochondrial complex I in the SN have been reported as a major biochemical feature in the pathogenesis of PD [60,69,70]. Interestingly, complex I has been found to be one of the most severely affected mitochondrial enzymes during oxidative stress [71]. In synaptic mitochondria, complex I exerts a major control over oxidative phosphorylation such that a decrease in its activity by 25% drastically affected ATP synthesis and the overall energy metabolism within the cell. On the other hand, inhibition of

complex III and IV up to 80% was necessary to show similar effect [72]. To understand the consequences of GSH depletion on mitochondria in dopaminergic neurons of the SN during PD, we constructed a dopaminergic PC12 cell line model system wherein the levels of the γ-GCS enzyme were inducibly decreased resulting in a reduction in GSH synthesis [73]. A decrease in the mitochondrial GSH in these genetically engineered cells resulted in increased oxidative stress and impaired mitochondrial function as reflected by decreased pyruvate-mediated mitochondrial respiration and ATP synthesis. Although there is no reported decrease in the activity of the γ-GCS enzyme in PD brains, the ultimate effect of the genetic manipulation mimics that which is seen in the Parkinsonian brain, that is a decrease in the GSH levels with no corresponding increase in GSSG levels. An interesting feature in our experiments was that GSH depletion in these cells led to selective inhibition of mitochondrial complex I activity. These results suggest that the early observed GSH losses in the SN may be directly responsible for selective inhibition of mitochondrial complex I activity and the subsequent mitochondrial dysfunction which ultimately leads to dopaminergic cell death associated with PD. One of the possible reasons for the susceptibility of complex I to oxidative damage is thiol oxidation and the presence of accessible oxidation sensitive iron-sulfur centers within this enzyme complex [74]. GSH is the chief molecular player in maintaining the SH groups of protein in reduced state thus controlling the activity of thiol dependent proteins [75]. GSH is known to protect proteins from oxidation by conjugating with oxidized thiol groups to form protein-SS-G mixed disulfides which can then be re-reduced to protein and GSH by GR, thioredoxin or protein disulfide isomerase [76]. In dopaminergic cells *in vivo*, GSH can also bind to quinones formed during oxidation of dopamine and prevent these compounds from reacting with protein sulfhydryl groups [77,78]. Sriram *et al*. [79] have demonstrated that thiol oxidation and loss of mitochondrial complex I activity precede excitatory amino acid mediated neurodegeneration. Both could be prevented by treatment with antioxidant thiol agents. GSH has been suggested to be involved in the repair of oxidized iron-sulfur centers of mitochondrial complex I [80]. In a recent study, when GSH was administered to PD patients by i.v. injections daily for up to a month, a significant improvement in disease related disability was observed [81]. Whether such treatment is effective in actually altering GSH levels in the brain and can have a prolonged effect in retarding the progression of the disease is unclear, however it can be implied that maintenance of thiol homeostasis is critical for the protection of dopaminergic SN neurons against neurodegeneration.

Selective inhibition of complex I *via* systemic administration of either MPTP or rotenone give similar patterns of morphological damage as that observed in the Parkinsonian brain. MPTP is a protoxin which causes selective destruction of dopaminergic neurons in SNpc [82,83]. The active form of MPTP is 1-methyl-4-phenyl pyridium (MPP+) which is formed within glia catalyzed by MAO B (MAOB). MPP+ diffuses out of glia and is actively taken up by SN dopaminergic neurons through a receptor-mediated mechanism where it exerts its toxic effects at least in part by accumulation and direct inhibition of mitochondrial complex I activity resulting in reduction in ATP synthesis thus contributing to mitochondrial dysfunction. Experiments involving SOD transgenic and GPx knockout mice indicate that this agent mediates its deleterious effects at least in part through induction of oxidative damage [32,84]. MPP+ has been shown to produce superoxide, H_2O_2 and $^{\bullet}OH$ [85–87]. Apart from oxidative damage *via* ROS production, MPTP also contributes to oxidative stress by depleting the levels of GSH [88,89]. This amplifies the damage caused because depletion of GSH causes further increase in ROS levels thereby contributing synergistically to mitochondrial dysfunction perhaps *via* direct inhibition of complex I activity.

PD is also characterized by the presence of proteinaceous neuronal inclusions in the midbrain known as Lewy bodies, which may also contribute to subsequent neurodegeneration [5,90]. Rare familial forms of PD exist which involve genetic mutations in various components of the ubiquitin (Ub)-proteasome degradation pathway which could contribute to the accumulation of proteins leading to the formation of Lewy bodies [91–94]. Ub belongs to a family of heat shock proteins (HSPs) involved in various stress response pathways. The protein Ub is covalently attached through thiol groups to misfolded or damaged proteins and aids in their degradation by transporting them to the 26S proteasome complex. The first step in this process is the activation of Ub by an activating enzyme called E1 through formation of a thiol ester bond. Ub is then transferred onto a thiol group of one of several specific Ub carrying enzymes (E2s). From the E2 enzyme, the Ub molecule is next transferred *via* one of several specific Ub ligases (E3) onto a lysine residue on the protein substrate to be degraded [95]. In familial forms of PD, the protein α-synuclein accumulates in the SN forming aggregates and is found to be a major component of Lewy body deposits [96,97]. The gene *Parkin*, which is mutated in some rare early onset PD cases, was found to contain sequences with striking homology to Ub. Recent evidence suggests that *Parkin* is an E3 ligase which acts to ubiquitinate α-synuclein. Furthermore, *Parkin* is also one of the components of the proteinaceous deposits which make up the Lewy bodies [98]. In a work recently published from our laboratory, we have demonstrated that GSH depletion in dopaminergic cells results in decreased E1 activity and subsequent disruption of the Ub pathway [99]. These data suggest that GSH is essential for preventing cysteine residues at the active sites of these enzymes from being oxidized leading to decreased proteolysis *via* the Ub-proteasome pathway. Taking all these data into consideration, it can be implied

that the early loss of GSH in sporadic PD might play an important role in the subsequent accumulation of protein aggregates in the SN in sporadic PD. During different kinds of stresses including oxidative stress, Ub and other related HSPs are expressed to help in the removal of defective proteins thus preventing formation of aggregates [95]. Results suggest that administration of MPTP might induce several HSPs *in vivo* [100]. It might be possible that in the early stages of PD, due to depletion of GSH in the SN, there is an elevated level of oxidative stress leading to the activation of expression of HSP and HSP-related chaperone molecules to protect the neuronal cells against the accumulation of oxidized proteins and aggregation. But during progression of the disease, there is a further increase in oxidative stress with a concomitant decrease in the levels of GSH thus leading to a scenario where the existing defenses against accumulation of defective proteins cannot control the build-up of protein aggregates. The depletion in GSH may also interfere with the ability of the Ub–proteasome system to degrade proteins leading to proteinaceous build-up and deposits [99].

During oxidative damage of lipids, aldehydes are formed as by-products, the most prevalent of these being 4HNE. 4HNE has been proposed to integrate into membranes affecting *in vivo* membrane fluidity. It has been found that GSH forms conjugate with 4HNE in a reaction catalyzed by GST preventing it from incorporating into the membranes [34,35]. Additionally, 4HNE may also form adducts with important proteins like Na^+/K^+ ATPase rendering them inactive. GSH has also been observed to prevent 4HNE from conjugating with proteins [33]. In the PD brains, the levels of 4HNE adducts have been reported to be elevated which might be reflective of the loss in GSH in the SN [11].

Lastly, by serving as the major storage form for cysteine in the cell, GSH may help reduce the levels of free cysteine which can bind to dopamine quinones to form adducts which have been proposed to inhibit complex I activity [101]. Therefore, changes in GSH levels may affect the ability of the cell to protect against toxic levels of dopamine quinones and cysteine which can form various deleterious adducts by conjugating to one other or to thiol groups on proteins. Fig. 2 highlights the functions of GSH in counteracting against oxidative stress during PD.

6. Role of iron in oxidative stress and PD

Besides decreased levels of GSH and impaired mitochondrial complex I, a third component supporting the role of oxidative stress in PD is iron. Sofic *et al.* [102] have demonstrated that total iron levels in the substantia nigra

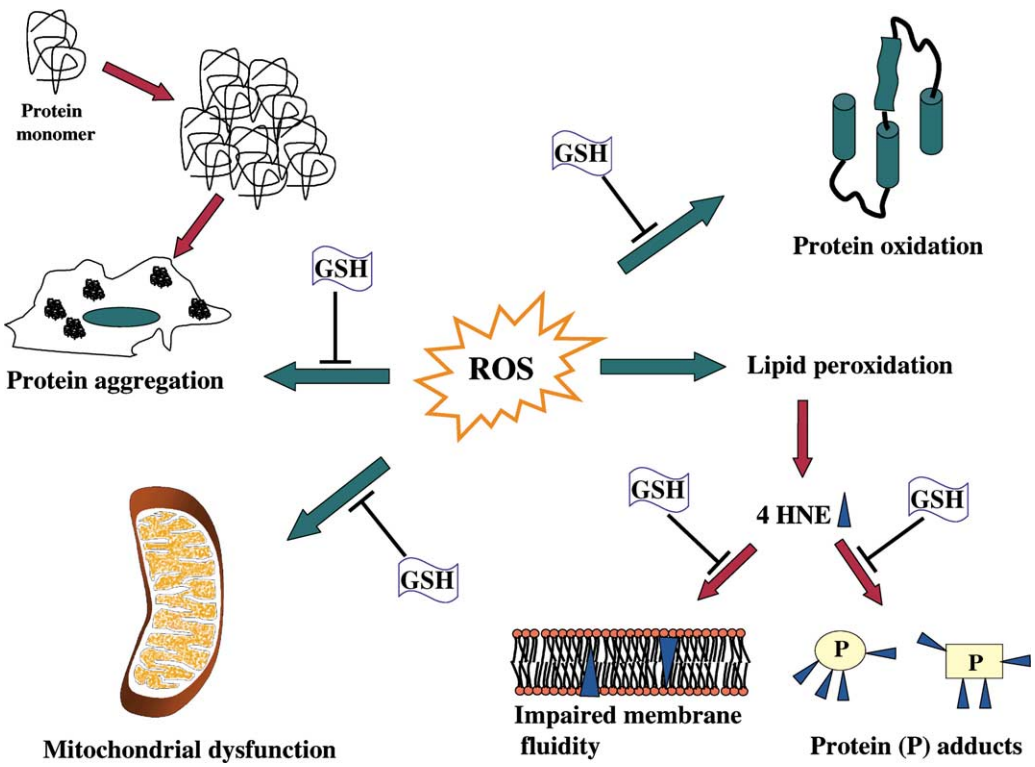

Fig. 2. The different roles of GSH: a schematic representation of the antioxidant properties of GSH as relevant to SN dopaminergic neuronal cells in PD. Apart from the detoxification of ROS themselves [16,17], GSH may protect neurons against the build-up of protein aggregates which form Lewy bodies within the cell [99]; mitochoindrial dysfunction due to inhibition of complex I activity [73]; the deleterious effects of the lipid peroxidation by-product 4HNE [33–35]; and protein oxidation [75,76].

(SN) of PD patients are higher than age matched controls [103]. Similar findings utilizing various methods have been reported in literature [104–111]. Additionally, what gives credence to the role of iron in pathogenesis of the disease is its ability to generate •OH *via* the Fenton reaction. Intracellular iron levels are stringently regulated as a labile iron pool (LIP), which provides optimum iron levels for vital biochemical reactions and limits the availability of free iron for generation of ROS. Ferritin is the major iron storage protein in the body which maintains iron in a nonreactive form in the cell. It is uncertain whether the excess iron observed in the PD brains is in a free form or bound to ferritin [106,112–115]. Griffiths *et al*. [105] demonstrated that in PD patients, ferritin is heavily loaded with iron implying that even if there is an increase in ferritin levels to counter excess iron levels, the ferritin molecules are saturated with iron. In the event of a superoxide or catechol-mediated release of iron from loaded ferritin pool, there could be an increase in LIP causing an increase in reactive iron available for generation of ROS [116]. Furthermore, iron promotes autooxidation of dopamine in SN neurons, releasing additional H_2O_2 [117]. Iron also catalyses the conversion of excess dopamine to neuromelanin, an insoluble black-brown pigment that accumulates in all dopaminergic neurons with age in humans [118]. Neuromelanin in general is neuroprotective and sequesters redox active ions in the cell with a high affinity for Fe^{3+} ions. However, when bound to excess Fe^{3+}, neuromelanin tends to become a prooxidant and reduces Fe^{3+} to Fe^{2+}, which then gets released from neuromelanin owing to weak affinity [119]. This increases the neuronal LIP and also the fraction of iron capable of reacting with H_2O_2. Under normal conditions, GSH constantly clears H_2O_2, thus preventing the production of •OH radicals. GSH also conjugates with quinones formed during dopamine oxidation and prevent them from facilitating release of iron from ferritin [120]. However under conditions of GSH depletion as in PD, this protection is attenuated leading to oxidative stress. It has been suggested that liberation of iron from ferritin or neuromelanin alters the homeostasis of mitochondrial Ca^{2+} with subsequent depletion of tissue GSH, resulting in oxidative stress [121]. A typical neuron of the SN in a PD patient containing dopamine, neuromelanin, high levels of iron and depleted GSH levels thus has a very conducive environment for generation of ROS. Hence oxidative stress produced during PD is likely the consequence of H_2O_2 production due to a combination of dopamine oxidation, GSH depletion and Fe^{3+} generated by neuromelanin or released from ferritin, thus allowing the Fenton reaction to proceed at a considerable rate resulting in neuronal death. This hypothesis gains support from the observation that pigmented neurons are preferentially lost during the course of PD [122–126]. It remains to be elucidated whether iron accumulation precedes injury of pigmented neurons or occurs as a consequence of neuronal degeneration. Intranigral iron injection in rats produces a selective lesioning of dopaminergic neurons, resulting in behavioral and biochemical Parkinsonism [127]. The biochemical changes due to oxidative stress resulting from tissue overload of iron (referred to as siderosis) are similar to those identified in the SN of PD brains [128].

It has been proposed that the catecholaminergic neurotoxin 6-OHDA can cause Parkinsonian symptoms in animal models and that oxidative stress, enhanced by iron, may play a key role in its toxicity. Neurotoxicity *via* 6-OHDA has also been linked to the release of iron from its binding sites in ferritin [130]. During dopamine metabolism, many toxic products such as hydrogen peroxide, oxygen-derived radicals, semiquinones, and quinones are generated which can exert neurotoxic effects. Pretreatment of rats with desferrioxamine, an iron chelator, attenuates 6-OHDA lesioning of nigrostriatal dopamine neurons [131]. Ben-Shachar *et al*. [132] have demonstrated that dopamine administration in rats pretreated with the MAO inhibitor pargyline caused mortality in a dose-dependent manner. Desferrioxamine proved to also be neuroprotective against this dopamine-induced neurodegeneration. It has been proposed that catecholamines can exert neurotoxic effects not only by inducing oxidative stress but also by affecting the mitochondrial electron transport chain. Glinka *et al*. [129] have demonstrated that 6-OHDA but not its oxidative products could reversibly inhibit complex I in isolated brain mitochondria.

Although these observations support the role of iron as a neurotoxin, it remains to be established whether accumulation of iron in PD is primary or secondary to other known events such as GSH depletion and complex I inhibition. It has been found that in patients with incidental Lewy body disease (ILBD) exhibiting preclinical PD symptoms, GSH depletion in SNpc are similar to that seen in PD patients. Since these patients exhibit physiological iron levels and normal mitochondrial complex I activity, it has been suggested that GSH depletion might be an upstream biochemical event in nigral neurodegeneration [133].

It is also important to take into account the lack of specificity of iron accumulation and its association with neuronal degeneration in various pathological conditions. Alterations in iron levels have been described in many neurodegenerative diseases such as multiple system atrophy, progressive supranuclear palsy, Huntington's disease, Alzheimer's disease, multiple sclerosis and spastic paraplegia [134]. Furthermore, iron levels are increased in the SN of 6-OHDA lesioned rats [135] and MPTP treated monkeys [136,137] suggesting that iron accumulation can occur as a secondary event during neuronal degeneration, irrespective of the causative agent. These nevertheless do not undermine the role of iron in disease progression. Recent findings that iron-related oxidative stress might promote α-synuclein aggregation strengthens the putative role of iron as an important link between the biochemical lesions and Lewy bodies during PD progression [138–141].

7. GSH as a therapy for PD

Based on all of the information described above, we can conclude that one of the most important events during pathogenesis of PD is depletion of GSH within the SN dopaminergic neurons. Hence it is plausible that one of the ways to counteract this problem is to replenish the GSH pool either by increasing the synthesis of the tripeptide or by slowing its degradation. There are several studies where administration of precursors of GSH metabolism such as γ-glutamyl cysteine have been used to increase the levels of GSH in rat brains [142]. Similarly, precursors of cysteine synthesis have been administered in various animal models with the view to accelerate cysteine production in the brain thus increasing GSH levels [142]. These efforts have been hampered by the problem that elevating levels of cysteine in the brain itself may be toxic to cells. GSH replacement can also be achieved by administering thiol reagents such as GSH itself or GSH analogs. GSH cannot easily penetrate the blood brain barrier due to the presence of the cysteine SH group and is not efficiently absorbed into neuronal cells in the brain [27,41,42]. Hence rather than GSH, modified forms of GSH such as GSH analogs (e.g. GSH esters) have generally been used *in vivo*. Yamamoto *et al*. [143] have shown that YM 737, a GSH analog, has protective properties against cerebral ischemia in rats. It would be interesting to test such compounds in animal models of PD to see if they are effective.

Gene expression analyses in animal models of PD using cDNA microarray approaches have suggested that neurodegeneration in PD is a complex process [144,145]. Although the exact molecular events leading to neurodegeneration have not been elucidated yet, the results of microarray analyses indicate the role of genes related to oxidative stress, glutaminergic excitatory, neurotrophic factors, nitric oxide-mediated and inflammatory processes. Since each of these events is complex and involves several biochemical mechanisms, it could be surmised that a single drug may not be completely effective against PD. Hence, drugs to counteract oxidative stress such as thiol reagents (GSH esters/GSH analogs) might be more effective if administered as a part of multi-drug therapy involving a cocktail of drugs [146].

8. Conclusions

In the last decade, there has been significant progress towards establishing that PD neuropathology may be at least in part attributed to depletion of the cellular GSH pool within SN dopaminergic neurons. This has been shown to affect various cellular processes including mitochondrial function which has been suggested to be the main source of neurodegeneration in the disease. GSH depletion might also affect protein degradation thus contributing to the build-up of defective proteins such as proteinaceous deposits which form Lewy bodies. Accumulation of iron in the SN has also been demonstrated to contribute to oxidative stress during PD. There have been several efforts in the recent years involving the use of GSH or related molecules in GSH metabolism as therapeutics in the treatment of PD to elicit an increase in brain GSH levels.

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