



Review Article

Thiol redox homeostasis in neurodegenerative disease

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ABSTRACT

This review provides an overview of the biochemistry of thiol redox couples and the significance of thiol redox homeostasis in neurodegenerative disease. The discussion is centred on cysteine/cystine redox balance, the significance of the x_c^- cystine–glutamate exchanger and the association between protein thiol redox balance and neurodegeneration, with particular reference to Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis and glaucoma. The role of thiol disulphide oxidoreductases in providing neuroprotection is also discussed.

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Abbreviations: ALS, amyotrophic lateral sclerosis; AD, Alzheimer's disease; AP1, activator protein 1; BOAA, β -N-oxalylamino-L-alanine; DAXX, death-associated receptor; GCL, ganglion cell layer; Grx, glutaredoxin; GSH, glutathione (reduced); GSR, glutathione reductase; GSSG, glutathione disulphide (oxidised glutathione); GST, glutathione-S-transferase; L-DOPA, L-3,4-dihydroxyphenylalanine; mGluR, metabotropic glutamate receptor; MMP, mitochondrial membrane potential; MPP⁺, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NF- κ B, nuclear factor- κ B; NGF, nerve growth factor; NMDA, N-methyl-D-aspartate; NRF2, nuclear factor (erythroid-derived 2)-like 2; NTG, normal tension glaucoma; POAG, primary open angle glaucoma; PD, Parkinson's disease; Prx, peroxiredoxin; SOD1, superoxide dismutase 1; Trx, thioredoxin; TrxR, thioredoxin reductase; TxNIP, thioredoxin inhibitory protein; xCT, functional subunit of the x_c^- cystine–glutamate exchanger

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Introduction

Regulation of thiol redox balance is critically important for multiple metabolic, signalling and transcriptional processes in mammalian cells. Thiol groups, whether in proteins or small molecules, are highly reactive and susceptible to oxidation that may cause significant loss of biological activity. In proteins, oxidation of free thiol groups produces modifications that, depending on their location, may impact on the structure, catalytic activity or ability to engage in protein–protein interactions. A critical function of cell-based thiol redox buffering systems is to protect thiol groups from oxidation and to repair those that may have become oxidised as a result of normal or aberrant cellular metabolism. The key components of the thiol redox buffering system are the cysteine/cystine and glutathione (GSH)/glutathione disulphide (GSSG) redox pairs, and the thiol disulphide oxidoreductases that include thioredoxin (Trx), glutaredoxins (Grx) and peroxiredoxins (Prx).

In this review, we describe the biochemistry of cellular redox couples and present recent findings on the association between thiol redox stability and neurodegenerative disease. A wealth of studies has implicated GSH redox balance in brain disorders that are the subject of several recent reviews [1–3]. Here, we focus primarily on the GSH precursor, cysteine, and the association between protein thiol redox balance and neurodegeneration, using Parkinson's disease (PD), Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS) as examples. A section on thiol redox homeostasis in glaucoma is included that illustrates common disease mechanisms between this and other neurodegenerative diseases.

Cellular redox couples

Physiologically, sulphur exists in many different oxidation states ranging from +6 to –2 in an oxidative environment [4]. In cysteine, the thiol group is mildly acidic with pKa values ranging from ~4 to 9 depending on the structure of the protein and the local environment [4,5]. Its reactivity is further increased in the deprotonated thiolate anion (RS⁻) form. Therefore, the thiol side chain may be readily oxidised to give a variety of different post-translational modifications such as sulphenic acids and disulphides which are reversible, or higher oxidation products such as sulphinic and sulphonic acids [6]. Thiols act as depots for nitric oxide through reversible formation of nitrosothiols. Due to its high reactivity, the thiol group of cysteine plays a major role in many biological activities like catalysis, metal binding and in acting as a 'molecular switch' activating or deactivating protein activity and function [6]. Early studies of thiol reactivity were conducted in isolated chemical systems and these may be far removed from the actual cellular and organismal redox potential and it is therefore important to consider the feasibility of chemical reactions within local cellular conditions and the thermodynamic feasibility of redox reactions within biological systems.

GSH is the major cellular thiol antioxidant. It operates within an important biological network of redox couples comprising NAD⁺/NADH, NADP⁺/NADPH and GSH/GSSG that work in concert with GSH/ glutathione reductase (GSR), Grx/GSH, Trx/oxidised Trx and thioredoxin reductase (TrxR) and Prx to maintain redox homeostasis (see Fig. 1). In neurons, oxidation of glucose via the pentose phosphate pathway provides the NADPH needed by GSR to regenerate GSH from GSSG [7]. Moreover, neurons preferentially oxidise glucose for antioxidant defence rather than energy production, due to low activity of the key activator of glycolysis, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 [8]. The abundance of GSH in cells and the ready conversion of sulphenic

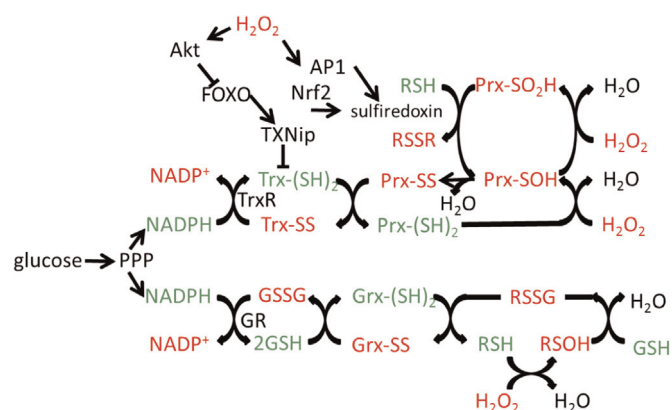


Fig. 1. Cellular redox couples in biological systems that show the link between reducing nucleotides (NADPH) derived from the pentose phosphate pathway (PPP) and maintenance of cellular redox state through reduction of hydrogen peroxide and oxidised proteins. GSH, glutathione; GSSG, glutathione disulphide; GR, glutathione reductase; Grx, glutaredoxin; Prx, peroxiredoxin; PrxSOH, peroxiredoxin sulphenate; PrxSOOH, peroxiredoxin sulphinate; PrxSS, oxidised peroxiredoxin; RSH, protein; RSSG, glutathionylated protein; RSOH, protein sulphenate; RSSR, protein disulphide; trx, thioredoxin; TrxR, thioredoxin reductase.

acids and S-nitroso derivatives to S-glutathione mixed disulphides suggests that reversible S-glutathionylation may be a common feature of redox signal transduction and regulation of the activities of redox sensitive thiol-proteins [9].

Cysteine is the precursor for GSH, hydrogen sulphide and taurine, each of which has significant antioxidant, neuroprotective or neuromodulatory properties. Free cysteine readily oxidises to the corresponding disulphide, cystine. In normal cells, cysteine is the dominant form, due to electron transfer from other cellular thiol/disulphide systems, particularly GSH/GSSG. The cytosolic cysteine/cystine redox potential (Eh) is typically –140 to –160 mV, whereas extracellularly, the oxidised form prevails and the cysteine/cystine ratio is close to 1:5, with an Eh of –80 mV. The GSH/GSSG redox potential is more reduced than that of cysteine/cystine and has an intracellular mean value of –230 mV, compared to –140 mV extracellularly. The steady-state redox potential for reduced/oxidised Trx in cells is the most reduced at –280 mV. The intracellular concentration of cysteine is typically in the low micromolar range and is an order of magnitude lower than the concentration of GSH. Extracellularly, the concentration of cystine (40–50 μM) is greater than either GSH (2.8 μM) or GSSG (0.14 μM).

The extracellular cysteine/cystine ratio shifts towards a more oxidised value during ageing in humans and is viewed as a significant risk factor for disease [10]. For example, a more oxidised cysteine/cystine redox potential significantly increases metabotropic glutamate receptor 5 (mGluR5)-mediated phosphorylation of extracellular signal-regulated kinase (ERK) in astrocytes, leading to increased expression of the transcription factor, nuclear factor-κB (NF-κB), and inducible nitric oxide synthase, release of reactive oxygen and nitrogen species and increased neurotoxicity [11]. It is believed that the extracellular portion of mGluR5, which contains a cysteine-rich domain incorporating many disulphide bridges, alters to facilitate interaction between glutamate and the receptor at the more oxidised potential. Based on studies using the 6-hydroxydopamine rodent model of PD, it has been proposed that nutritional strategies aimed at manipulating the extracellular cysteine/cystine redox potential may prove beneficial in slowing the rate of neurodegeneration in this and other age-related neurodegenerative diseases [12].

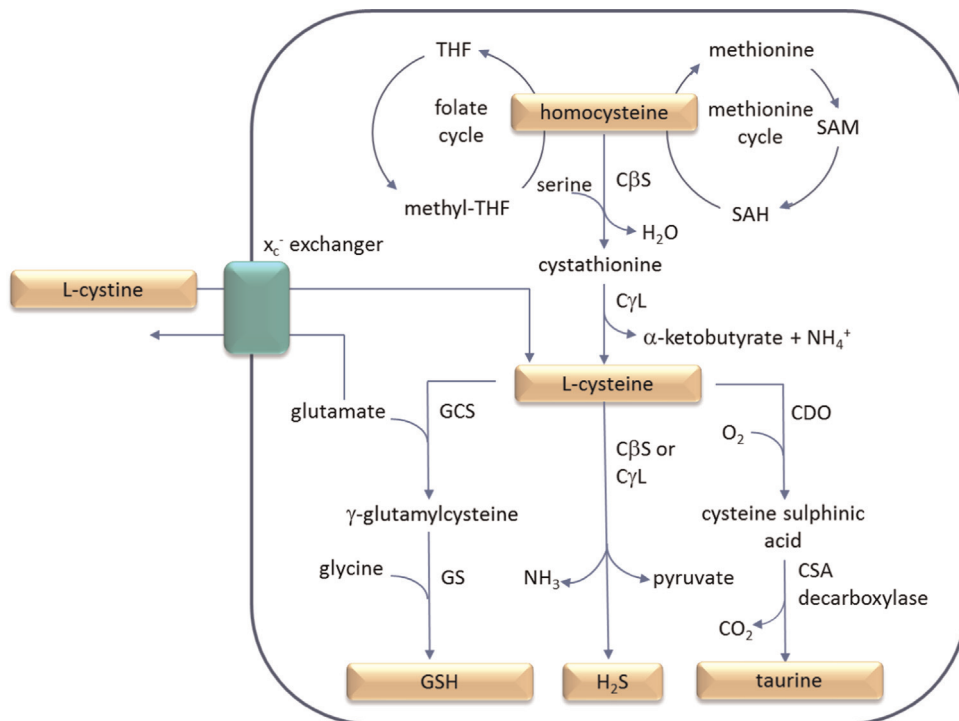


Fig. 2. Pathways of cysteine metabolism; cysteine is derived from extracellular cystine via the x_c^- cystine glutamate exchanger and from methionine, via the transsulphuration pathway. Cysteine is the precursor for GSH, hydrogen sulphide (H_2S) and taurine. $C\beta S$, cystathionine- β -synthase; CDO, cysteine dioxygenase; $C\gamma L$, cystathionine- γ -lyase; CSA, cysteine sulphinic acid; GCS, γ -glutamylcystine synthase; GS, glutathione synthase; GSH, glutathione; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; THF, tetrahydrofolate.

Cysteine

In the brain, cysteine must be supplied either from the extracellular medium, or in situ synthesis from methionine (see Fig. 2). Astrocytes and microglial cells are the main sites of GSH production in the brain and import cystine, which is immediately reduced to cysteine on entering the cytosol. In astrocytes, uptake of cystine provides approximately two thirds of the cysteine required for GSH synthesis. The remaining one third is derived from methionine via the transsulphuration pathway, but this contribution increases during oxidative stress [13,14]. Uptake of cystine is mediated by the plasma-membrane x_c^- cystine-glutamate exchanger (SLC7A11 carrier) that releases glutamate in a 1:1 ratio with cystine uptake (Fig. 2). Immature neurons readily take up cystine, but this facility is lost in mature cells. Instead, neurons take up cysteine, which originates from astrocytic GSH that is exported and degraded extracellularly [15,16]. Therefore, de novo synthesis of GSH in astrocytes must meet the antioxidant requirements of both astrocytes and neurons.

It is increasingly recognised that the x_c^- exchanger participates in an intracellular-extracellular cysteine/cystine redox cycle that serves to regulate the extracellular redox potential and is not directly associated with GSH synthesis [17–19]. Key features of the cycle are cystine uptake, intracellular reduction to cysteine and secretion of cysteine that is surplus to intracellular requirements to the extracellular space. The net effect is a reduction in the extracellular cysteine/cystine redox potential [19]. Additional support for the cycle comes from the observation that x_c^- -deficient mice do not display depletion of GSH [20].

The x_c^- exchanger has a significant role in regulating the extracellular concentration of glutamate and, increasingly, attention is drawn to the physiological and pathological implications of exchanger-mediated glutamate release [21]. For example, x_c^- -derived glutamate stimulates group 2/3 metabotropic glutamate receptors (mGluR2/3) in tissue slices of the nucleus accumbens or

prefrontal cortex, leading to a reduction in excitatory tone [22]. Moreover, withdrawal from cocaine reduces extracellular glutamate levels in the nucleus accumbens by decreasing cystine-glutamate exchange [22].

The x_c^- exchanger in neurodegenerative disease

Increased expression of the x_c^- exchanger and consequent elevation of glutamate release has been recorded in a number of neurodegenerative disorders, including brain tumours [18,23], HIV infection [24], multiple sclerosis [25] and hereditary haemochromatosis, a neurodegenerative disorder of the visual system [26,27]. Direct sampling of peritumoural extracellular fluid in Grade IV glioblastoma multiforme (GBM) patients by microdialysis has revealed concentrations of glutamate in excess of 100 μM at the tumour margin [28], leading to widespread glutamate-mediated excitotoxicity and neurodegeneration. siRNA-mediated silencing of the xCT subunit of the exchanger protects against neurodegeneration and reduces glioma-associated oedema [29]. Equally, blockade of x_c^- exchanger with sulphasalazine or the cyclic glutamate analogue 4-carboxyphenylglycine (4-CPG) has an anti-proliferative and cytotoxic effect on glioma cell lines [30–32]. While additional research is needed, the results cited here illustrate the therapeutic potential of treatments targeting the x_c^- exchanger in glioma therapy.

Aberrant activity of the cystine-glutamate exchanger is associated with neurodegenerative changes in AD and ALS. In AD, activated microglia display increased expression and functional activity of the x_c^- exchanger and elevated glutamate release [33]. The toxicity of amyloid beta peptide 1–40 to cultured neurons was increased on exposure of the cells to activated microglia due to glutamate release via the x_c^- exchanger. Inhibition of N-methyl-D-aspartate (NMDA) receptors or system x_c^- prevented the microglia-enhanced toxicity of the peptide [33]. More recently, it has been hypothesised that excessive glutamate release from activated

microglia may be a significant contributor to motor neuron degeneration in the symptomatic stages of ALS. In support of this theory, expression of xCT is elevated in spinal cord and isolated microglia from mutant superoxide dismutase 1 (SOD1) ALS mice and in the spinal cord of ALS patients [34]. Moreover, xCT expression correlates with increased inflammation and enhanced release of glutamate. xCT is absent from motor neurons [34]. Deletion of xCT in ALS mice leads to earlier symptom onset, but ultimately, increases the number of surviving motor neurons. Taken together, these observations raise the possibility that targeting the xCT exchanger could provide a mechanism to slow disease progression after onset of clinical symptoms.

Thiol redox homeostasis in glaucoma

Glaucoma is the leading cause of irreversible blindness worldwide [35] and is characterised by progressive damage or degeneration to the optic nerve [36]. Since the retina and optic nerve are part of the central nervous system, glaucoma shares mechanistic and epidemiological similarities with other CNS neurodegenerations, including PD, ALS, AD and Huntington's disease [35].

Several hypotheses exist on the mechanism of ganglion cell death in glaucoma, including high intraocular pressure (IOP) and hypoxia [37], oxidative or nitrate stress [38,39], glutamate toxicity [40], loss of neurotrophic factors [41] and autoimmune reactions assisting degeneration of retinal ganglion cells [42]. The retina is one of the most vascularised tissues in the body and is particularly vulnerable to oxidative stress due to its high consumption of oxygen and exposure to light [43]. It has one of the highest oxidative metabolic rates per tissue weight [44]. Retinal cells require the maintenance of normal GSH levels, turnover rates and oxidation state [45]. GSH deficiency is manifested largely through an increased susceptibility to oxidative stress, and the resulting damage is thought to be a key step in the onset and progression of glaucoma [46]. Enzymes involved in GSH synthesis and metabolism in different types of retinal cells are shown in Fig. 3.

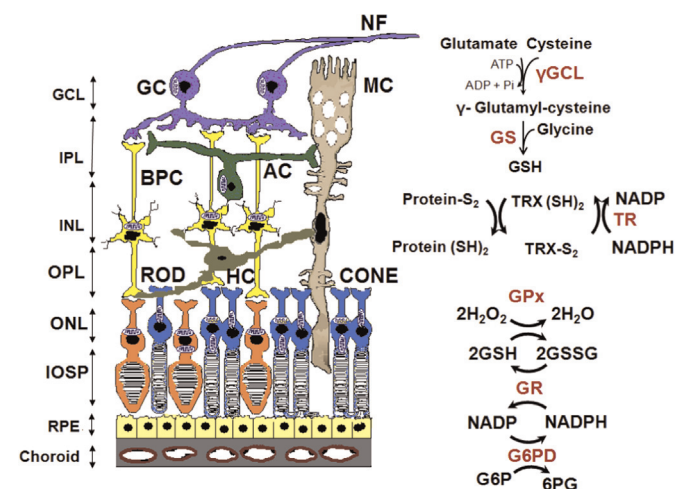


Fig. 3. Scheme of enzymes involved in glutathione synthesis and metabolism in the retina. AC, amacrine cell; BPC, bipolar cell; GC, ganglion cell; GCL, ganglion cell layer; γ GCL, γ glutamate cysteine ligase; G6PD, glucose-6-phosphate dehydrogenase; 6PG, 6-phosphogluconate; GPx, glutathione peroxidase; GR, glutathione reductase; GS, glutathione synthase; GSH, glutathione; HC, horizontal cell; INL, inner nuclear layer; IPL, inner plexiform layer; IOSP, inner and outer segments of photoreceptors; NF, nerve fibre; ONL, Outer nuclear layer; OPL, Outer plexiform layer; protein-S₂, protein-S-S; protein (SH)₂, protein-SH. RPE, retinal pigment epithelium; TR, thioredoxin reductase; TRX (SH)₂, thioredoxin; TRX-S₂, oxidised thioredoxin.

GSH levels decline in human eyes with age [47] and decreased GSH levels have been associated with glaucoma [48]. Moreno et al. analysed retinal GSH levels in a rat glaucoma model induced by chronic injection of hyaluronic acid in the eye anterior chamber [48]. Reduced GSH levels were assessed in the retinas from hypertensive eyes at 6 weeks of ocular hypertension compared to controls, whereas, at 3 or 10 weeks of treatment, levels of GSH did not change compared to eyes injected with vehicle. In accordance with animal studies, plasma glutathione levels measured in 21 patients with newly diagnosed primary open-angle glaucoma and 34 age- and gender-matched control subjects revealed that glaucoma patients exhibited significantly lower levels of reduced and total glutathione than did control subjects [49].

In a recent study performed on humans, levels of circulating total GSH (GSH and GSSG) were analysed in 34 primary open angle glaucoma (POAG) patients, 30 normal tension glaucoma (NTG) patients and 53 controls [50]. Total blood GSH levels were also determined in this study by the glutathione reductase-5,5 dithio-bis-2-nitrobenzoic acid (DTNB) recycling procedure. Independent of age, POAG and NTG patients demonstrated significantly lower GSH and total GSH levels than age-matched controls. Additionally, a lower redox index, defined as the GSH/GSSG ratio, was found only in POAG patients, in comparison to both NTG and control groups. GSSG levels were similar between all study groups. This study demonstrated that both POAG and NTG patients exhibit lower GSH and total GSH levels than age-matched controls, indicating a compromise of the antioxidant defence systems in glaucoma.

Glutathione S-transferase (GST) catalyses the conjugation of GSH to electrophilic centres on a variety of substrates including toxic species [51]. In a recent study, the aqueous humour proteome was analysed by antibody microarray in samples obtained from 10 clinically uncontrolled POAG patients and 10 cataract patients immediately before trabeculectomy and cataract surgery, respectively. Aqueous humour GST levels were significantly lower in POAG patients than in controls reflecting a weakening of GST-mediated mechanism of protection against harmful electrophiles produced during oxidative damage [52]. Serum antibodies against GST have also been reported to be higher in patients with POAG than in controls [53].

Glutamate toxicity is an important mechanism of ganglion cell death in glaucoma [54]. Increased levels of glutamate have been reported in the vitreous of glaucomatous patients and a prolonged elevation of extracellular glutamate is possibly caused by chronic ocular hypertension [55]. The cystine–glutamate exchanger is upregulated by oxidative stress in retinal ganglion cells [56] and, given that glutamate is toxic to these cells via over-stimulation of the NMDA receptor [40], upregulation of the exchanger under oxidative stress conditions in glaucoma would have injurious consequences.

Protein redox state in neurodegeneration

Alterations in protein redox state have been implicated in the pathology of neurodegenerative diseases [57–59]. Early important observations of cell death under redox stress have been reviewed and linked to more recent, elegant experiments describing mechanisms of redox-regulated cell death in AD, PD and ALS. Key pathways that are affected by redox state and predispose to death are the apoptosis signalling kinase 1 (ASK1)/JNK and ASK1/p38MAPkinase cascades [9,60]. Mitochondrial dysfunction is considered pivotal to cell death as a source of increases in thiol-oxidising reactive oxygen species and during intrinsic apoptotic signalling. Consequently, a number of studies have investigated Grx, Trx and Prx activity and mitochondrial function in neurodegeneration models. While the majority of articles focus on

mitochondria as a source of oxidants that contribute to cytotoxicity, mitochondria have also been reported to play an important role in removal of hydrogen peroxide, e.g. generated by redox cycling of chemicals such as paraquat [61,62]. Nicotinamide nucleotide transhydrogenase capitalises on the mitochondrial proton gradient to generate NADPH from NADH and NADP⁺, so providing a link between energetics and removal of hydrogen peroxide via the Trx2/TrxR and Prx systems [63].

Glutaredoxin as a neuronal survival factor

Glutathionylation/deglutathionylation reactions are regulated by the expression and activity of Grx and the availability of GSH substrate which is required for chemical reduction of Grx. Grx exists in at least 3 forms in humans, however, most studies focus on Grx1 found in the cytoplasm and Grx2 found in the nucleus and mitochondria. In 2004, Kenchappa et al. described that down-regulation of Grx using anti-sense oligonucleotides prevented recovery of mitochondrial complex I activity in the striatum after 1-methyl, 4-phenyl, 1,2,3,6-tetrahydropyridine (MPTP) treatment [64]. This study suggested a critical role for Grx in recovery of mitochondrial function in brain. A follow up study investigated the mechanism of toxicity of the excitotoxin β -N-oxalyl amino-L-alanine (BOAA) [65]. While male mice were susceptible to mitochondrial complex I loss and GSH depletion only ovariectomised female mice showed mitochondrial-dependent neuronal loss. Grx expression, central to neuroprotection via maintaining complex I activity, is regulated by oestrogen; treatment of SH-SY5Y cells with oestrogen upregulated Grx1 and protected from BOAA mediated mitochondrial membrane potential (MMP) loss. Later, Lee et al. demonstrated that Grx2 is involved in glutathionylation of protein cysteine sulphhydryl residues in the mitochondria and is required for iron sulphur cluster biogenesis and complex I activity. Following inhibition of Grx2 in vitro, complex I activity declines in a manner consistent with the pathological events associated with PD [66]. The concomitant rise in intracellular iron further predisposes neurones to oxidative stress. Consistent with these findings, Karunakaran elegantly demonstrated that downregulation of Grx2 using antisense oligonucleotides in the mouse brain in vivo resulted in partial loss of complex I activity supporting the concept that Grx2 may help maintain complex I function in the mitochondria. These findings were reinforced in vitro by over-expression of Grx2 in neuroblastoma cells which abolished 1-methyl-4-phenylpyridinium (MPP⁺)-mediated toxicity [67].

Despite its localisation to the cytoplasm, downregulation of Grx1 by shRNA resulted in loss of MMP that is associated with oxidation of thiols on voltage dependent anion channels [68]. Loss of MMP was preventable by the antioxidant, α -lipoic acid, or by cyclosporine A, an inhibitor of mitochondrial permeability transition suggesting that either there is direct interaction between Grx1 and proteins in the mitochondrial membrane or that an intermediate redox active protein transduces the reducing signal.

Most commonly, familial ALS is associated with the aggregation of mutant SOD1, but whether SOD1 aggregates represent a cause, a correlate or a consequence of processes leading to cell death is unclear. As well as being localised in the cytoplasm, SOD1 is found in the intermembrane space and in ALS mitochondria, fission and dysfunction has been attributed to mutant SOD aggregation. Aggregation can be prevented by Grx2 overexpression, preserving mitochondrial function and preventing apoptosis [69]. Related to this, biochemical studies by Bouldin et al. have described the propensity for the intramolecular disulphide bond between two SOD monomers to be reduced by Grx1/GSH; they showed that ALS mutant SOD was more susceptible to reduction to monomers and inactivation than WT [70]. This is significant because it is the apo- and reduced forms of ALS mutant hSOD1 that are especially

vulnerable to destabilisation and readily aggregate under mild oxidative stress conditions. In ageing yeast, glutathionylation was shown to be essential for human SOD1 activation [71]. Further work is needed in this area to rationalise the role of Grx in mutant SOD aggregation.

Aberrant regulation of protein glutathionylation can disrupt both apoptotic and survival signalling pathways. More recent studies have focussed on potential signalling pathways by which Grx may support neuronal survival. DAXX, the death associated protein downstream from ASK1, has surfaced as an important transducer of death signals from the nucleus in PD. Based on studies of DJ-1, a putative gene recessively linked to early onset PD, reduced DAXX is normally retained in the nucleus. DJ-1 normally functions as an antioxidant, transcriptional co-activator, and molecular chaperone that is maintained active in a reduced state by Grx1. However, down-regulation of Grx 1 caused loss of DJ-1 protein, translocation of DAXX from nucleus, and cell death. This effect was eliminated in the DJ-1 cys mutant which was not susceptible to oxidation or degradation so maintained DAXX in the nucleus [72]. More recent studies using the brains from DJ-1 (–/–) mouse model of PD have suggested that the animals have adapted to loss of DJ-1, increasing consumption of hydrogen peroxide and through upregulation of the Trx/TrxR /Prx system [73] and in fact that the ability to adapt to stress may be the key factor in overt disease. In a model of AD, using SH-SY5Y exposed to amyloid beta, both Grx1 and Trx1 were oxidised, associated with nuclear export of DAXX. Moreover, amyloid beta toxicity was inhibited by insulin-like growth factor-I and by overexpressing Grx1 or Trx1 [74] highlighting the importance of maintaining redox state for neuronal survival.

Another hydrogen peroxide generating drug relevant to PD is L-3,4-dihydroxyphenylalanine (L-DOPA). While it is used as a therapeutic, L-DOPA causes oxidative inactivation of Trx and Grx, with concomitant activation of ASK1 [59,75]. A potential mechanism for this has been investigated by Sabens et al. [75]; in biochemical studies, Grx underwent irreversible adduction with dopaquinone to its nucleophilic active-site Cys-22. This caused enzymatic inactivation but did not cause protein degradation, consistent with observations after L-DOPA treatment. Whether this mechanism may also contribute to cognitive decline observed in PD patients remains to be explored.

Thioredoxin and neuronal survival

Trx exists in multiple forms and acts as a reducing agent within the cell and extracellularly. It restores the activity of oxidised Prx and is in turn recycled at the expense of NADPH by TrxR. In addition to regulation of expression, TrxR activity is also regulated post-translationally by thioredoxin inhibitory protein (TxNIP).

Trx has been recognised as a neurotrophic factor for over 10 years; the earliest studies showed it to support nerve growth factor (NGF) signalling and to play a critical role in NGF-mediated neurite outgrowth in PC12 cells [76]. It exists in at least two isoforms, Trx1 in the cytosol and Trx2 in the mitochondria. Trx1 is a small, 12-kDa, conserved and ubiquitous multifunctional protein with several redox-active cysteine residues. It reduces disulphide bonds and sulphenic acids and exhibits transnitrosylation activity [77–80]. Trx1 has many interaction partners depending on its cellular localisation. Through its reductase activity it may regulate apoptosis, cell growth, differentiation and development [81,82]. In the nucleus, Trx1 binds directly to a number of different transcription factors including p53, NF- κ B, and activator protein-1 (AP1) and thereby modulates their DNA-binding activity [83,84]. With respect to apoptosis inhibition, at least three binding partners have been identified in the cytoplasm; ASK-1, TxNIP and actin, where actin protects Trx1 from degradation and preserves

its anti-apoptotic function [81,85]. The cytoprotective protein DJ-1 which is frequently mutated in PD, binds to ASK1 in a Cys-106 redox-sensitive manner and can be reduced by Trx1 [86,87]. It has been suggested that DJ-1 is an atypical peroxiredoxin-like peroxidase that scavenges hydrogen peroxide through oxidation of Cys-106. In WT mice, an increase of Cys-106 oxidised DJ-1 was observed after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment [88]. DJ-1 has also been suggested to act as an upstream activator of the transcription factor, nuclear factor (erythroid-derived)2-like 2 (NRF2). NRF2 regulates Trx, and its overexpression inhibits ASK1/JNK and ASK1/p38 pathways that are frequently activated in neurodegenerative disease [89]. While overexpression of DJ-1 resulted in increased NRF2 protein levels, nuclear translocation and binding to the ARE site in the Trx1 promoter, NRF2 knockdown abolished DJ-1-mediated Trx1 induction and cytoprotection against hydrogen peroxide [11]. In PD patients with mutant DJ-1, alternative NRF2 activators may prove to be a useful strategy for upregulating Trx1 expression.

Neurotoxins associated with risk for PD frequently associate with oxidised Trx and activate unique pathways. For example, paraquat oxidised Trx1 impairing its ASK1 inhibitor activity and leading to JNK and caspase 3 activation whereas 1-methyl-4-phenylpyridinium (MPP⁺) and rotenone oxidised Trx2 without activating the JNK pathway [90,91]. Injection of MPP⁺ into mouse brain decreased levels of TrxR 1 mRNA, protein and activity [92]. Others have shown that MPP⁺ toxicity is due to ER stress which can be inhibited by Trx-1 overexpression; Trx-1 played a neuroprotective role in MPP⁺-mediated neurone loss by suppressing ER stress [93]. Interestingly, Trx acts not only as a reducing agent but also as a chaperone; when Trx mutants were studied in the Pael-R drosophila model of PD, it was the chaperone rather than redox activity of Trx that proved to be more significant for enhancing cell survival [94].

Trx1 also associates with the plasma membrane and is trafficked with a limited number of cytosolic proteins via the leaderless secretory pathway [95]. We have shown previously that under oxidative stress, Trx1 secretion is altered [96]. Trx1 and Grx1 are also released to the cerebrospinal fluid. In 120 patients the early stages of AD and with mild cognitive impairment Trx1 and Grx1 levels correlated with the established AD biomarkers tau and phospho-tau suggesting their potential involvement in the pathogenesis of disease [97].

The neuroprotective effect of Trx1 and Trx2 were investigated in a rat glaucoma model [98]. Expression of Trx1 and Trx2 was observed in the retinal ganglion cell layer (GCL), nerve fibre (NF) layer and inner nuclear layer (INL). Trx1 level decreased 2 weeks after glaucoma induction and more notably after 5 weeks. No change in Trx2 levels was reported. The effects of Trx1 and Trx2 overexpression on retinal ganglion cell survival were evaluated 5 weeks after glaucoma induction. Trx1 and Trx2 preserved 45 and 37% of cells, respectively that were destined to die in glaucomatous retinas.

Peroxioredoxin and neuronal survival

Basal expression of the six different Prx isozymes shows a distinctive distribution profile within brain regions and different cell types. On the one hand, Prx1 and 6 are expressed in glial cells but not in neurons; conversely, Prx2, 3, 4, and 5 are expressed in neurons [99]. Of these enzymes, it is Prx3 that is found in mitochondria. Prx 6 differs from the other Prx enzymes and is a 1-Cys Prx that lacks an internal resolving cysteine residue. The other mammalian Prx enzymes are 2-Cys Prx where the peroxidatic cysteine is first oxidised to a sulphenic acid and then it oxidises a second resolving cysteine to form a di-sulphide bond. The latter is reduced via Trx and TrxR.

Prx2 is highly expressed in many tissues and along with Prx 6 is the focus of most research in AD. In a transgenic Alzheimer's mouse diseased brain, Prx2 levels were significantly elevated. This was suggested to be an adaptive response that protected the mice against amyloid beta toxicity [100]. In a histochemical study of brains from AD patients, nitrated Prx2 was identified. Randall et al. investigated the functional consequences of Prx2 tyrosine nitration, and demonstrated that nitration was on a non-catalytic residue that resulted in increases in peroxidase activity and resistance to over-oxidation [101]. Prx2 is also S-nitrosylated forming SNO-Prx2 by reaction with nitric oxide at two critical cysteine residues (C51 and C172). In contrast to the effect of nitration, this prevents its reaction with peroxides [102].

The role of Prx6 is less clear; on the one hand, apoptosis was inhibited after amyloid beta treatment in PC12 cells overexpressing wild-type Prx6, but not in cells that overexpressed the C47S catalytic mutant. This indicates that the peroxidase activity of Prx6 protects PC12 cells from amyloid-induced neurotoxicity [103]. However, in a mouse model after amyloid beta infusion, memory impairment in Prx6 transgenic mice was worse than C57BL/6 mice. In addition, the astrocytes and microglia cells of amyloid-infused Prx6 transgenic mice were more activated, lipid peroxidation and protein carbonyl levels were increased and glutathione levels were lower, suggesting that Prx6 is promoting rather than preventing oxidative stress [104].

In the PD field, interest has been in Prx1, 2 and 3. In MN9D cells, overexpression of Prx1 protected against 6 hydroxydopamine toxicity, prevented p38 MAPK activation and subsequent activation of caspase-3. In contrast, apoptotic death signals were enhanced by RNA interference-targeted reduction of Prx1 [105]. Hu et al. explored the role of Prx2 and showed it inhibited 6 hydroxydopamine -induced ASK1 activation by modulating the redox state of Trx1 so preventing its dissociation from ASK1 [106]. In cells expressing a common p.G2019S LRRK2 gene mutation (rs34637584:A > G) that is responsible for up to 30–40% of PD cases in some ethnic populations, the phosphorylation of Prx3 is increased. LRRK2 interacts with Prx3 and mutations in the LRRK2 kinase domain significantly increased phosphorylation but decreased peroxidase activity and increased death in neuronal cells [107]. These findings point towards a common downstream pathway of redox imbalance in PD that may be mediated through different upstream effectors and by either environmental or genetic factors.

Evidence for redox disturbance in neurodegeneration

One of the more compelling arguments for thiol regulatory networks in neurodegenerative disease is evidence of altered expression in post-mortem tissue or in circulating cells and plasma. To this end, there is limited analysis of Grx1 in human neurodegenerative disease. However, of note, a recent study has examined post-mortem midbrain samples from PD patients, in which it was observed that Grx1 content is decreased in PD, specifically within the dopaminergic neurons [108]. This further supports a role for redox in PD. Although whether it is causal is unclear. Increased SNO-Prx2 has been described in human PD brains, and S-nitrosylation of Prx2 inhibits both its enzymatic activity and protective function from oxidative stress [102].

In post-mortem brain tissue of patients with AD, many blood vessels exhibited Prx6 staining that appeared to be due to the astrocytic foot processes [109]. Furthermore, an increase in Grx1 and a decrease in neuronal Trx1 have been described in AD brains by one group, while a second group reported from 120 patients that the Trx1, Trx2, Grx1, and Grx2 expression pattern was altered in hippocampal tissue sections from AD patients compared to

controls. In addition, oxidative modifications to Prx-2 and Prx-6 were increased in AD plasma [110].

Conclusion

Much progress has been made in understanding the significance of thiol redox imbalance as a contributing factor to neurodegenerative disease. The biological reactivity of the sulphur atom in cysteine, whether as the free amino acid or incorporated into proteins and peptides, is a significant factor in determining susceptibility to oxidative damage, excitotoxicity and neurodegeneration. It is clear that restoration of redox balance may offer a useful approach to minimise neuronal loss during neurodegeneration.

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