PLOS PATHOGENS

PEARLS

Redox mechanisms and their pathological role in prion diseases: The road to ruin

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Abstract

Prion diseases, also known as transmissible spongiform encephalopathies, are rare, progressive, and fatal neurodegenerative disorders, which are caused by the accumulation of the misfolded cellular prion protein (PrP^{C}). The resulting cytotoxic prion species, referred to as the scrapie prion isoform (PrP^{Sc}), assemble in aggregates and interfere with neuronal pathways, ultimately rendering neurons dysfunctional. As the prion protein physiologically interacts with redox-active metals, an altered redox balance within the cell can impact these interactions, which may lead to and facilitate further misfolding and aggregation. The initiation of misfolding and the aggregation processes will, in turn, induce microglial activation and neuroinflammation, which leads to an imbalance in cellular redox homeostasis and enhanced redox stress. Potential approaches for therapeutics target redox signalling, and this review illustrates the pathways involved in the above processes.

Citation: Spiers JG, Chen H-JC, Steinert JR (2023) Redox mechanisms and their pathological role in prion diseases: The road to ruin. PLoS Pathog 19(4): e1011309. https://doi.org/10.1371/journal.ppat.1011309

Editor: Cathryn L. Haigh, National Institutes of Health, UNITED STATES

Published: April 27, 2023

Funding: JGS was supported by a Royce Simmons Foundation Mid-Career Research Fellowship through the Dementia Australia Research Foundation. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Introduction

Prion disease or transmissible spongiform encephalopathies are a type of rare, progressive, and fatal neurodegenerative disorders caused by misfolding of the cellular prion protein (PrP^{C}) and accumulation of its disease-associated scrapie prion isoform (PrP^{Sc}). Symptoms of prion disease include rapidly developed dementia, difficulty thinking and speaking, impaired coordination, and behavioural changes. There is no known cure for prion disease, with current treatment focused primarily on symptom management. The exact molecular mechanisms of prion disease are yet to be fully characterised, but hallmark histopathological features can be observed including the development of widespread spongiform lesions, neuronal loss, and gliosis. Recently, there has been a great deal of interest in the role of redox imbalance/oxidative stress in contributing to those aforementioned clinical features of prion disease. This heavily coincides with disruption of metal homeostasis in the brain, particularly redox-active metals such as iron and copper, which may further contribute to the associated oxidative stress by

acting as an important neurotoxicity trigger. Here, we summarise the advances in understanding the functional link between redox stress and prion disease.

Prion and redox imbalance/oxidative stress

Neuroinflammatory redox stress is a common observation in the pathophysiological development and progression of prion diseases in both animal models and human forms of the disease [1–3]. This neurodegenerative neuroinflammation generally involves microglial cells, with the polarisation of these towards an "activated" M1 phenotype resulting in the release of pro-inflammatory cytokines and an up-regulation of oxidative/nitrosative stress machinery to combat potential pathogens [4]. While microglial-derived neuroinflammation is generally viewed as a mechanism leading to neuronal death, particularly where excessive oxidative stress occurs, in prion disease, this mechanism becomes less clear-cut. Microglia become classically activated at the site of spongiform lesions and display this phenotype in the early stages of infection prior to neuronal death, suggesting they play a role in prion-induced neurodegeneration [5]. Activated microglia express high levels of the superoxide radical-generating NADPH oxidase enzyme capable of inducing oxidative stress in neighbouring cells, which may contribute to the neurodegenerative phenotype seen in prion disease. Moreover, the antioxidant superoxide dismutase (SOD) enzymes also show reduced expression in the early development of prion disease, which likely exacerbates NADPH-oxidase-derived oxidative stress [6].

Importantly, microglial proliferation and activation also appears to be important in the effective clearance of prions, with pharmacological-mediated reductions in microglia resulting in enhanced prion deposition and neurotoxicity [7]. Moreover, the cytokine profile following prion infection is highly dependent on the prion strain and infected species, with some anti-inflammatory cytokines becoming more abundant in different rodent models and human Creutzfeldt–Jakob disease (CJD) [8,9]. Although up-regulated respiratory burst enzymes such as NADPH oxidase found in activated microglia play a critical role in prion-induced oxidative stress, mitochondrial dysfunction is also a key component of prion disease redox dysfunction. Mitochondrial oxidation is observed very early in prion disease pathogenesis and contributes to early oxidative stress [10–12]. A number of studies using different models of prion disease have shown the majority of differentially expressed proteins following infection mediate energy metabolism. This includes oxidative phosphorylation proteins such as ATP synthase, tricarboxylic acid cycle proteins including succinate dehydrogenase, aconitase 2, and malate dehydrogenase, and mitochondrial membrane proteins like mitoflin [13–16]. This broad degree of mitochondrial dysfunction contributes heavily to redox dysfunction and, together with pro-inflammatory microglial activation and reductions in primary antioxidant protection from SOD, likely forms the basis of oxidative stress in prion disease.

Prion and nitrosative stress

The elevation of nitric oxide (NO) within the central nervous system is known to be associated with the pathogenesis of several neurodegenerative diseases including prion infection [17–19]. Using lipopolysaccharide to generate an inflammatory up-regulation of inducible nitric oxide synthase (iNOS) or treating cells with an NO-donor (sodium nitroprusside) significantly elevates both mRNA and protein expression of the prion protein via the MEK and p38 MAPK signalling pathways [20]. Up-regulation of this inflammatory NO-producing enzyme, iNOS, has also been demonstrated in brains of scrapie-infected mice, which may contribute to the observed vacuolation and astrocytosis [21,22]. Interestingly, in addition to the immune-related iNOS, constitutive endothelial NOS was also markedly increased in the hippocampus of ME7 scrappie-infected mice, which showed a degree of specificity in reactive astroglial cells and

accumulated in the mitochondrial fraction [23]. The increase in NO and damaged mitochondria could, in turn, affect the function of the affected astrocytes and further contribute to the prion disease progression. In agreement with these studies, increased neuroinflammatory and altered nitric oxide signalling with an accompanied nitrergic stress have been observed in the hippocampus of hemizygous Tg37 mice that overexpress the cellular mouse prion protein using an alternative prion protein strain from Rocky Mountain Laboratory (RML) [24,25]. Suppression of NO production pharmacologically showed beneficial effects on hippocampal physiology and supressed prion protein misfolding highlighted the importance of nitrergic stress in the neuropathology of prion disease [24].

Prion and biometals

Normal PrP^{C} is a well-known divalent metal binding protein with a high binding preference for copper ions in the N-terminal octa-repeat domain of the protein [26]. Moreover, the reactive oxygen species produced by Fenton chemistry have been shown to actively participate with redox-active divalent metals like copper to facilitate β-cleavage into N2 and C2 prion protein fragments [27–29]. The binding of copper appears to be highly important for PrP^{C} physiological functionality, with studies showing normal PrP^{C} possesses SOD1-like antioxidant activity, stimulates PrP^{C} endocytosis and trafficking activity, and modulates N-methyl-D-aspartate (NMDA) receptor activity [30–32]. Specifically, it has been shown that PrP^{C} and copper cooperatively modulate NMDA receptor activity by mediating S-nitrosylation to prevent neurotoxicity [33]. The N-terminal copper binding site of PrP^{C} also appears to be essential for neurite outgrowth, acting as a neurotrophic factor [34]. It is highly likely that the combination of these effects contributes to the neuroprotective actions of cellular PrP^{C}. In prion-infected mice, it has been demonstrated that the reduction of NMDA receptor S-nitrosylation precedes the appearance of the clinical signs and neuropathological changes [35]. Therefore, dysregulation of NMDA receptor S-nitrosylation may act as a possible mechanism of neuronal death in prion pathology. During prion disease, the conversion of PrP^{C} to PrP^{Sc} is highly influenced by both the surrounding concentrations of available copper and the levels of copper bound to the PrP protein, with apo forms of the protein being more susceptible to conversion [36]. Moreover, the interaction of PrP^{C} with other redox-active divalent metals such as iron has enormous implications for the microenvironment surrounding PrP^{Sc} lesion sites. It has been shown previously that PrP^{C}-null mice have impaired copper metabolism and the oxidase activity of copper-dependent ceruloplasmin, which is known to regulate iron mobilisation and distribution [37]. Accumulation and dysregulated iron release, in particular, can be highly deleterious to surrounding protein, lipids, and nucleic acids due to the high levels of oxidative stress produced via Fenton chemistry.

In addition to this direct involvement of biometals on local oxidative stress conditions, alterations in divalent metal homeostasis can influence gene expression via metal response elements in promotor regions of several genes [38,39] or indirectly via iron–sulphur (Fe–S) cluster containing proteins [40]. Metal dyshomeostasis can alter gene expression very early in the pathogenesis of prion disease before functional neuronal changes can be detected. Importantly, this can alter metal transporters and binding proteins capable of mediating cellular metal ion pools required for structural and functional incorporation into enzymes and chaperones. For example, divalent metal transporters Slc11a2 and Slc39a14 show reduced expression prior to symptomatic onset in prion diseased mice [6]. This is observed alongside a >50% reduction in expression of Ccs, the copper chaperone to SOD, responsible for redox-active copper insertion and proper protein folding of SOD1. Importantly, this suggests that SOD expression is not only reduced, but antioxidant activity is also compromised very early in prion disease pathogenesis.

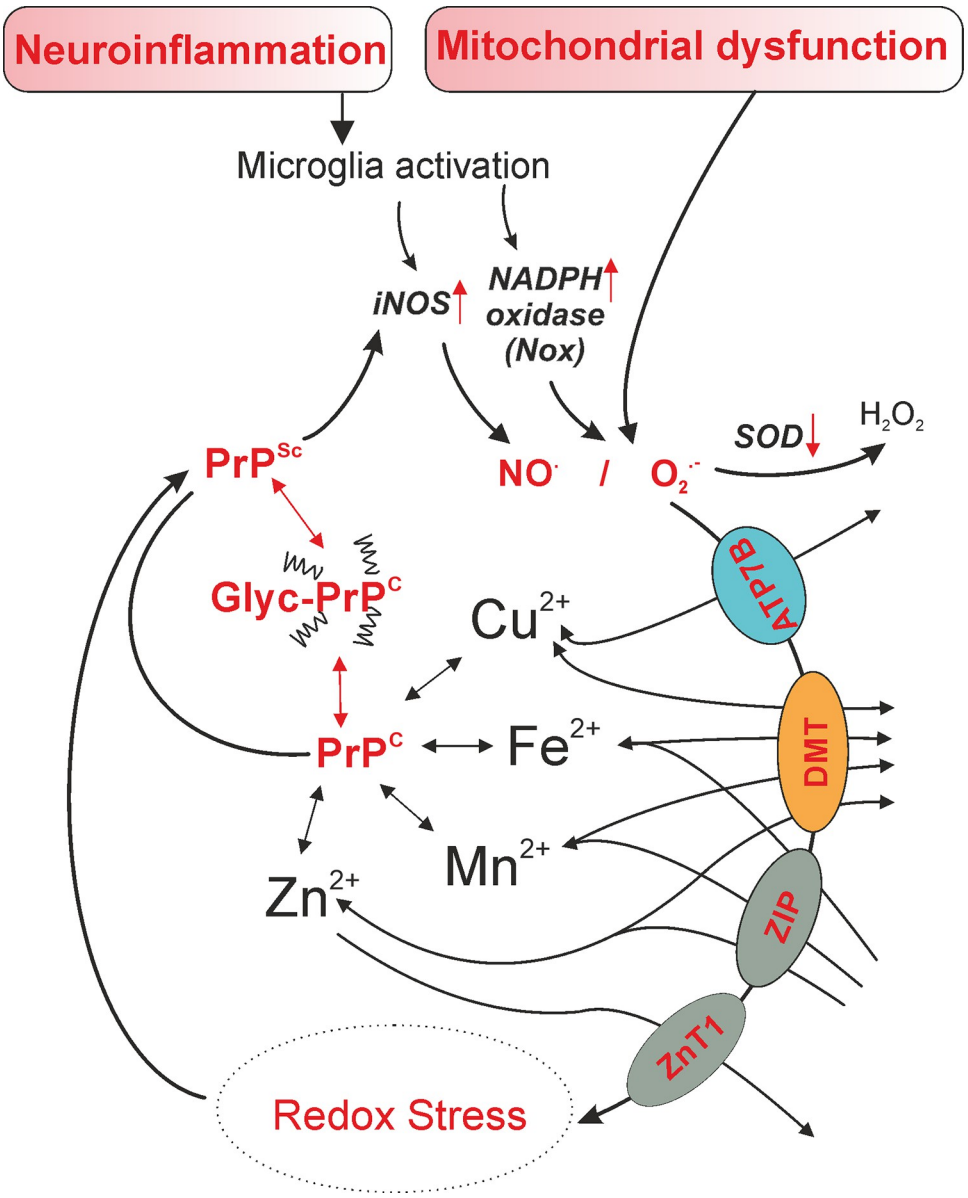

Fig 1. Hallmarks of prion disease pathogenesis include neuroinflammation and mitochondrial dysfunction, which facilitate production of reactive oxygen and nitrogen species via inflammatory-related enzymes including NADPH-oxidase (Nox) and inducible nitric oxide synthase (iNOS). These produce radical species such as superoxide ($O_2^{\cdot -}$) and nitric oxide ($NO^{\cdot}$), respectively. Under normal conditions, antioxidants such as superoxide dismutase (SOD) would neutralise these radicals to less reactive species or reduce them to water. However, in pathological conditions such as prion disease where oxidant production is favoured, antioxidants are overwhelmed, which results in cellular redox stress. This is further exacerbated by the mobilisation of redox-active and transition metals such as iron (Fe^{2+}), zinc (Zn^{2+}), copper (Cu^{2+}), and manganese (Mn^{2+}), which causes dyshomeostasis in cellular biometal distribution through various divalent metal transporters (ZnT1, ZIP, DMT, ATP7B) and promotes oxidative stress. Together, these conditions may favour glycosylation of the native prion protein (PrP^{C}), which facilitates conversion to the disease isoform (PrP^{Sc}), further promoting disease progression.

https://doi.org/10.1371/journal.ppat.1011309.g001

Outlook and treatment

Targeting biometals, such as copper and zinc, has been suggested as a potential therapeutic strategy for prion diseases. These biometals can bind to misfolded PrP^{Sc} proteins, which can

inhibit protein aggregation. Biometals can also modulate the activity of certain enzymes involved in disease processes, ultimately slowing disease progression. In addition, an interaction between PrP^{C} and manganese or zinc, but not copper, causes shedding of the N1 fragment of PrP^{C}, which, in turn, impacts prion N-glycosylation and its cellular functions, distribution, cellular trafficking, aggregation, and fibril formation [41,42]. In the unglycosylated mutants, PrP^{C} was localized on the cell surface and readily converted to PrP^{Sc}, indicating that glycans are not necessary for prion infection but rather a lack of glycation favours misfolding and PrP^{Sc} transmission [43]. Thus, targeting of redox metal homeostasis will strongly impact prion function and transmissibility (Fig 1).

However, a main question of how to minimise or slow down prion propagation during early infection stages remains to be addressed. Early detection methods in animal models involve the use of aptamers [44], single-stranded nucleic acids (DNA, RNA) that are usually 22 to 100 nucleobases long and have molecular recognition properties similar to antibodies. Aptamers have also been investigated as novel anti-prion compounds. It was shown that peptide aptamers binding to PrP^{C} prion protein can abrogate prion propagation [45]. Binding of the designed anti-prion aptamer with PrP^{C} prion protein increased α-cleavage, interfered with protein internalization, and inhibited prion replication. A recent study also explored the possibility of reducing pro-inflammatory signalling of prion-infection in cell culture. This study provides some novel therapeutic insights by highlighting the ability of mesenchymal stromal cells in regulating inflammation by secreting anti-inflammatory small molecules to promote angiogenesis and neurogenesis [46].

In summary, the detection of early biomarkers and biosensors [47], identifying early stage infection, prion seeding and propagation [44], targeting of inflammatory processes, and biometal dyshomeostasis are essential steps to develop potential therapeutic strategies for prion diseases. However, more research is needed to determine the translational potential from animal models to humans.

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