Original article

Folia Neuropathologica

Neurodegeneration and oxidative stress: prion disease results from loss of antioxidant defence

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Folia Neuropathol 2005; 43 (4): 229-243

Abstract

Prion diseases or transmissible spongiform encephalopathies (TSEs) are rare neurodegenerative disorders that can be acquired either by direct transmission, inherited through dominant mutations in the prion protein gene or via an unknown sporadic cause. This latter group constitutes the vast majority of cases. Like many neurodegenerative diseases the hallmarks of oxidative damage can be readily detected throughout the brain of the affected individual. However, unlike most other neurodegenerative diseases, prion diseases are connected with a dramatic loss of antioxidant defence. As abnormal protein accumulates in the diseased brain there is both an increase of oxidative substances and a loss of the defences that keep them in check. In particular the normal cellular prion protein has been shown to be an antioxidant. Conversion of this protein to the protease resistant isoform is accompanied by a loss of this antioxidant activity. This change creates a paradox as the loss of activity is not accompanied by a loss of protein expression. It is likely that this prevents other cellular defences from responding sufficiently to protect neurons from the heightened oxidative burden. Recent experiments with transgenic mice have shown that when prion protein expression is switched off during the course of prion disease, cell death is dramatically halted and the mouse recovers from the disease. This result clearly illustrates that the continued expression of non-function prion protein is essential for disease progression. This implies that the presence of this abnormal protein during prion disease causes a failure of cellular antioxidant defence. This failed defence is the fundamental cause of the massive neurodegeneration that results in the fatal nature of TSEs. The role of oxidative stress in TSEs and other neurodegenerative disorders are discussed in this review.

Key words: *prion, neurodegeneration, oxidative stress, Alzheimer's disease, Parkinson's diesease, ALS*

Introduction

A number of recent publications have reported on the relationship between metal ions such as iron (Fe), copper (Cu), manganese (Mn) and zinc (Zn) and neurological diseases (reviewed in [27,48]). In many disorders of this kind, an abnormal reaction occurs between a protein and a redox-active metal ion, for example Cu^{2+} or Fe^{3+}. This promotes the formation of reactive oxygen species (ROS), which can be detrimental to the nervous system [55,64,74]. Oxygen radicals may play an important role in the pathologies of a number of diseases of the central nervous system (CNS).

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The entire nervous system, including the brain, spinal cord, and peripheral nerves, is rich in metals [56]. The brain particularly is a specialized organ that concentrates metal ions. Although their importance in metabolism has frequently been marginalized, as illustrated by the use of terms such as "trace elements", the concentrations of metal ions such as Fe, Cu and Zn in the grey matter of the brain are quite significant, within the range 0.1-0.5 mM [67]. Under normal conditions, metal ions interacting with proteins can both generate and defend against ROSs. Experimental data of the past ten years has shown that Fe, Cu, Mn and Zn are key neurochemical factors. This chapter will discuss how their interaction with proteins can be of primary relevance to the neuropathophysiology of degenerative diseases like Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS) and Parkinson's disease (PD). A special emphasis will be laid on the rare but increasingly important group of diseases, the transmissible spongiform encephalopathies (TSEs).

Oxidative stress in CNS disease

The nervous system, is rich in both metal ions and unsaturated fats, which are prone to oxidation [55]. The high lipid content of the nervous tissue, coupled with its high metabolic (aerobic) activity, makes it particularly susceptible to damage by oxygen radicals [39]. There is now substantial evidence that oxidative stress is either a causative or an ancillary factor in the pathogenesis of major neurodegenerative diseases, including PD [42], AD [6,71] and ALS [54,75,90] as well as in cases of stroke, trauma, and seizures [37,44]. Important new data showing the role of free radical damage in transmissible spongiform encephalopathies (TSEs) has also come to light.

In PD patients, decreased levels of antioxidant enzyme activities have been found [46] and evidence of oxidative stress in the form of increased lipid peroxidation and oxidation of DNA bases is seen in the *substantia nigra*, the area of the brain affected by PD [64]. Similar increased lipid peroxidation and oxidation of DNA and proteins are seen in AD [83]. Also, increases in markers of oxidative stress (e.g., oxidation of proteins or of DNA) are observed in both familial ALS (FALS) and sporadic ALS (SALS) patients [47].

There are currently no real treatments for any neurodegenerative diseases. The symptoms of PD can be partially alleviated by using L-DOPA [61]. It has been suggested that the chances of developing AD may be reduced by following diets high in antioxidants [51]. A number of *in vitro* studies have shown that antioxidants, both endogenous and dietary, can protect the nervous tissue from damage by oxidative stress. Uric acid, an endogenous antioxidant, was found to prevent neuron damage in rats, both *in vitro* and *in vivo*, from the metabolic stresses of ischemia [107]. Tocopherol (vitamin E) was shown to prevent cell death (apoptosis) in rat neurons subjected to hypoxia followed by oxygen reperfusion and the same study showed that tocopherol prevented neuronal damage from reactive nitrogen species [96]. Both tocopherol and β-carotene shielded rat neurons from oxidative stress caused by exposure to ethanol [73]. In an experimental model of diabetes-caused neurovascular dysfunction, β-carotene protected cells most effectively, followed by tocopherol and ascorbic acid (vitamin C) [36].

Most *in vivo* and clinical studies of the effects of lipid-soluble antioxidant supplementation on neurological diseases have focused on tocopherol. A report in 1991 demonstrated that the rate at which PD progressed to the point when the patient required treatment with L-DOPA was slowed by 2.5 years in patients given large doses of ascorbic acid and synthetic tocopherol [45]. One study reported that high doses of tocopherol resulted in elevated plasma levels but failed to detect increased tocopherol levels in the cerebrospinal fluid (CSF) [77]. A later report, however, demonstrated that high doses of tocopherol did result in elevation of tocopherol levels in the CSF and possibly the brain [99]. Recently it was shown that the protein responsible for the uptake of tocopherol is in fact present in brain cells of patients suffering from tocopherol deficiency or diseases associated with oxidative stress [35]. In a Dutch study, it was found that the risk of PD was lower in subjects who had higher dietary intakes of antioxidants, particularly tocopherol [40]. The same group reported that a low dietary intake of β-carotene, the precurser to the atioxidant vitamin A, was associated with impaired cognitive function in a group of persons aged 55-95, though no such association was observed for either ascorbic acid or tocopherol [62]. Conversly, in an Austrian study, se-

rum concentrations of tocopherol were reported to be significantly associated with the cognitive function in adults aged 50-75 years measured by a standardized test [88]. In another study, it was found that patients suffering from PD had consumed less of the small-molecule antioxidants β-carotene and ascorbic acid than did non-sufferers of the disease, implying that dietary antioxidants do play a protective role in this disease [57]. About 20% of FALS cases are associated with a mutation in the gene for copper/zinc superoxide dismutase (Cu/Zn-SOD), an important antioxidant enzyme, and in vitro experiments demonstrated that expression of the mutant enzyme in neuronal cells caused cell death, which could be prevented by antioxidant small molecules such as glutathione and tocopherol [49].

Oxidative stress and TSEs

The possible role of oxidative stress in the pathology of prion disease is an intriguing new field of research. Prion diseases or transmissible spongiform encephalopathies (TSEs) are infectious, inherited or sporadic neurodegenerative disorders characterized by neuroamyloid formation and dementia (reviewed in [82]). Human TSEs include Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker (GSS) syndrome, kuru and fatal familial insomnia (FFI). Animal prion diseases include, most notably, scrapie and bovine spongiform encephalopathy (BSE). Spongiform degeneration in the brain, astrogliosis and neuronal loss are the classical neuropathological changes in TSEs. The underlying cause and mechanism of neuronal loss is not yet known. Several studies have suggested that the cellular prion protein (PrP^c), a molecule having a crucial role in these diseases, may be involved in cellular response and resistance to oxidative stress [12,15,32,105] and it was conclusively shown that cultured neurones and astrocytes from mice deficient in PrP^c were more sensitive to oxidative stress [13,14,23]. PrP^c is a protein expressed by most neurones and during the cause of a prion disease the protein is converted to an abnormal isoform often referred to as the scrapie isoform, PrP^{Sc}. In the inherited forms of human prion disease specific point mutations in the gene for PrP are known to result in the onset of the diseases (Figure 1). However, most forms of the disease are sporadic and the mechanism of conversion of the protein from a harmless or benevolent form to a disease specific form remains uncertain. There are two possible mechanisms of conversion which rely on introduction of preformed PrP^{Sc} into the system from an exterior source (i.e. infection). Alternatively, a rare change in the protein could cause spontaneous conversion of

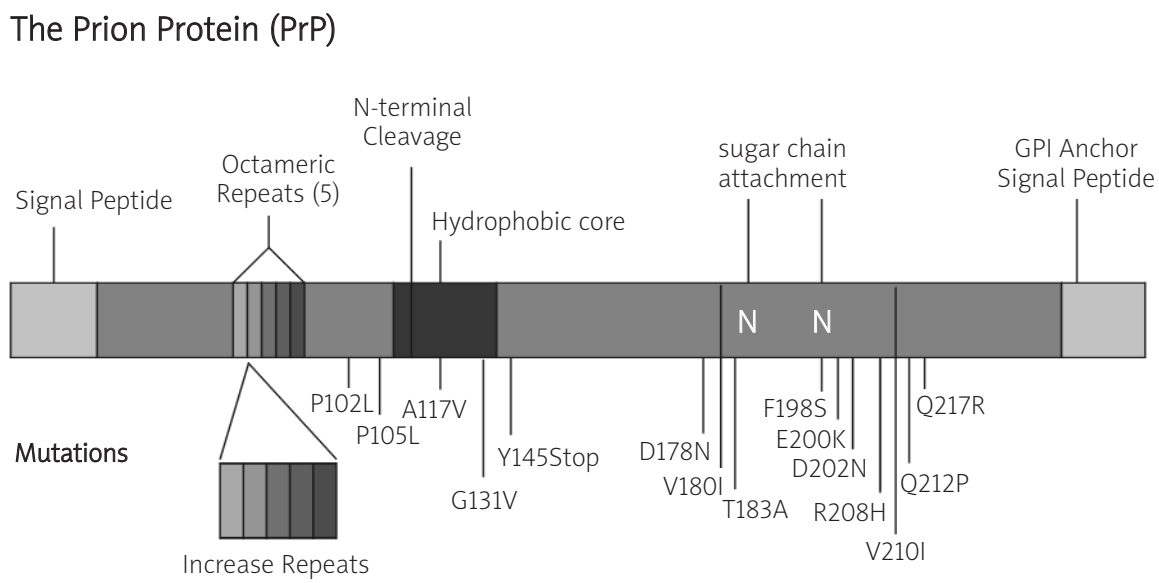

Fig. 1. The primary structure of the prion protein showing the locations of known mutations that cause the inherited forms of the disease in humans

the protein to an altered isoform able to catalyse conversion of PrP^{c} to PrP^{Sc} (Figure 2).

In vivo studies were made to see if free radical damage was involved in the neuronal degeneration following prion infection in an experimental TSE model. Immunohistochemical studies with prion infected mouse brain tissue were made using the following markers for oxidative stress: nitrotyrosine (NT), an indicator of peroxynitrite generation [92], heme oxygenase-1 (HO-1), an enzyme leading to the formation of antioxidant molecules [91] and also lipid oxidation markers [106]. A widespread increase in neuronal labelling of these markers was seen after infection, showing that peroxynitrite-mediated neuronal damage was present and confirming that oxidative stress was an important factor in the terminal stage of experimental TSEs. The immunocytochemical detection of NT and the changed immunohistological profile for HO-1 in the scrapie infected brains provided the first *in vivo* evidence that oxidative stress has a major role in neurodegeneration in TSEs. These results were in agreement with other work on neurodegenerative disease, in which free radical damage was shown as the major event [5,25,89,92,78]. In Alzheimer's disease, previous studies showed only vulnerable neurons as positive for NT [92]. Cells permanently infected with a TSE such as scrapie show decreased levels of antioxidants and are more susceptible to cell death induced by oxidative stress (Figure 3) Other *in vitro* experiments have investigated the effects of exposure of cells in culture to neurotoxic peptide derivatives of the prion protein. Exposure to the toxic peptide, PrP106-126 [16], induced oxidative stress in PC12 cell cultures, a cell line from rats [13] and HO-1 mRNA in cultured astrocytes [85].

In human and experimental TSEs, a number of investigators demonstrated the severe vulnerability of PV+ (parvalbumin immunoreactive) neurones to neurodegeneration [52,53]. Thus it would be expected that most of the damage by free radicals would be mainly in this neuronal subset. However, NT positive neurons were found to be widely distributed through the whole brain, even in regions without PV+ neurones [52]. This suggested that oxidative stress was a global event in TSEs, affecting almost all neurons. It is probable that PV+ cells, most vulnerable to free radical damage, degenerate first. In conclusion, damage by free radicals is a very likely stimulus of neurodegeneration in prion disease. It has also been proposed that antioxidants may be a potential therapy for these disorders [23].

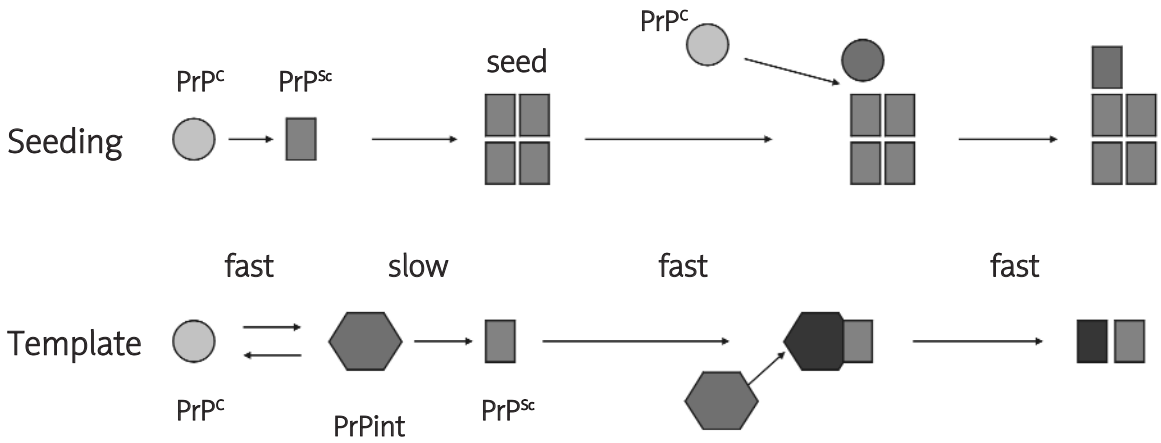

Fig. 2. The two main theories about the mechanism of conversion of the normal cellular prion protein (PrP^{c}) to the abnormal isoform (PrP^{Sc}) are illustrated in this figure. In the seeding (nucleation) theory a small aggregate must form first. The formation of this seed would be slow once the seed is formed it catalyses the reaction at a faster rate. In the template hypothesis conversion is dependent on a dimer or an intermediate which rarely forms PrP^{Sc} but can do so when interacting with PrP^{Sc}. The formation of the intermediate is reversible. However, the process is accelerated by the formation of more PrP^{Sc}. The seeding hypothesis is considered a more accurate measure of what occurs *in vivo*

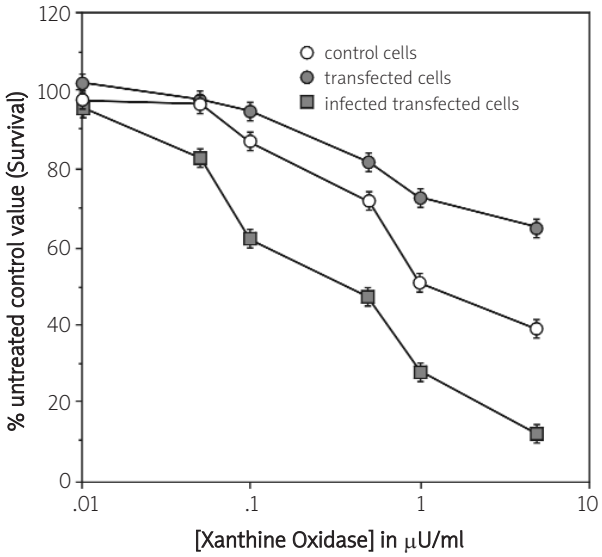

Fig. 3. Neuroblastoma cells were transfected with a plasmid construct to overexpress prion protein. These cells were also infected with PrP^{Sc} (ME7). The cells were exposed to increasing concentration of an enzyme that induces oxidative damage by the production of superoxide. The survival of the cells was measured. Increased PrP^{c} expression protects cells from oxidative damage but PrP^{Sc} production causes an increased susceptibility to oxidative damage

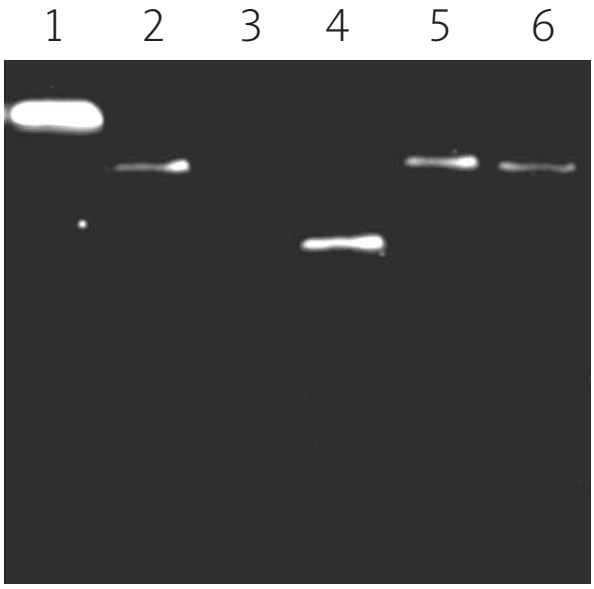

Fig. 4. In Gel Assay of PrP's SOD-like activity. An in gel assay was used to provide a visual demonstration of the SOD-like activity of wild-type PrP and some of the active mutants. 5 μg of protein was electrophoresed on a native polyacrylamide gel and stained for SOD-like activity. Shown are 1, wild type PrP protein; 2, PrPΔ35-45 3, PrPΔ112-136; 4, PrP23-171; 5 PrPΔ112-119 and 6, PrP45-231. This experiment shows that deleting the hydrophobic domain of PrP inactivates this activity. This domain is important to the active site of the protein

Another study from the lab of Kim [65] suggested that Fe induced oxidative stress might be the main mechanism of neuronal loss in scrapie. It is known that oxidative stress induced by free radicals is associated with iron accumulation; this association led to an examination of the levels of iron (total iron, Fe^{2+} and Fe^{3+}) in the brains of control and scrapie-infected mice by biochemical methods. In the scrapie-infected group, both the level of total iron and the Fe^{3+} level were significantly increased in the cerebral cortex, striatum, and brainstem as compared to the control group. A shift in the ratio of Fe^{2+}/Fe^{3+} was observed in the same regions of infected mice. Additionally, in this scrapie model, the presence of oxidative stress was confirmed, as evidenced by the increase of free malondialdehyde. These results suggested that iron metabolism is changed and that iron-induced oxidative stress partly contributes to neurodegeneration in scrapie infection [65].

Metals in TSEs

The central event in TSEs pathogenesis is believed to be the post-translational conversion of a normal cellular prion protein (PrP^{C}) into an abnormal isoform called scrapie PrP (PrP^{Sc}). The disease form of PrP is partially resistant to proteases and can be passed between individuals, producing symptoms of TSE [82]. An important and very current question in TSE research concerns the relationship between oxidative stress as a factor causing the symptoms and the role of the PrP protein as a possible cause of the disease. There has been considerable discussion about the normal function of the protein. It is likely that this function is dependent on the metal binding capacity of the protein. Although the *in vivo* function

of PrP^{C} remains to be confirmed, it has been demonstrated that both recombinant and brain-derived PrP^{C} have superoxide dismutase (SOD)-like activity when bound to Cu^{2+} [18,21] (Figure 4). The Cu^{2+} complexation in PrP is different, however, to that in cellular Cu/Zn-SOD [98]. It also appears that the protein transports Cu^{2+}, thereby increasing cellular resistance to Cu^{2+} toxicity [14-16]. Cu appears to be taken up in association with PrP^{C} into the cell [17,24,81]. However, PrP^{C} is not the main Cu-uptake protein expressed by cells. When PrP^{C} converts to PrP^{Sc}, this SOD-like function is lost [97]. It is therefore of considerable interest to consider this property in relation to oxidative stress as a cause of neuronal damage in TSEs. Several lines of investigation have been taken to see (*i*) if the metal binding of PrP^{C} is altered in TSEs, (*ii*) if metal imbalances also correlates with the loss of antioxidant function in PrP^{C} and (*iii*) whether these alterations correlate with the disease phenotype, such as PrP^{Sc} and also the PrP genotype at codon 129, which influences the manifestation of the disease.

NMR studies have shown that PrP^{C} consists of a structured C-terminal region, which is primarily α-helical, and an unstructured N-terminal region. PrP^{C} exhibits high affinity, cooperative Cu^{2+} binding through a histidine-containing octapeptide repeat domain in the unstructured N-terminal region [12,100]. It also binds Cu^{2+} along the more structured C-terminal domain of the protein [33,98]. Continuous wave electron paramagnetic resonance studies demonstrated that Cu^{2+} first binds and fills the C-terminal binding sites before occupying the octarepeats at the N-terminus [33]. Recombinant PrP^{C} was also found to have the capacity to bind other metal ions such as manganese (Mn^{2+}) [20] in both the octarepeats and the C-terminal sites [34]. In vitro metal ion occupancy experiments showed that when Mn^{2+} replaced the Cu^{2+} ion in the prion protein, PrP^{C} altered its structure and took on a more PrP^{Sc}-like conformation [20]. The prion protein also lost its SOD-like function [20].

Investigations on alterations in metal ion concentrations were carried out using mouse scrapie models [97] and in samples from sCJD cases [106]. Changes in the levels of Cu^{2+} and Mn^{2+} were detected in the brains of scrapie infected mice early in the disease, prior to the onset of clinical symptoms. In addition, a major increase in blood Mn^{2+} was also noted in the early stages of the disease. The analysis of purified PrP from the brains of scrapie infected mice also showed a reduction in Cu^{2+} binding to the protein and a proportional decrease in the antioxidant activity between 30-60 days post infection.

A striking elevation of Mn^{2+} and to a lesser extent Zn^{2+} accompanied by a significant reduction in Cu^{2+} binding to purified PrP were found in subtypes of sporadic Creutzfeldt-Jakob disease (sCJD), the most common type of human prion disease. Studies were made using brain tissues and affinity purified PrP preparations (i.e. PrP^{C}, PrP^{Sc} and possibly other abnormal PrP species) obtained from four major subtypes of sporadic CJD. These were identified according to the genotype at codon 129 of the PrP gene and the PrP^{Sc} type as established by [79]. Both Zn^{2+} and Mn^{2+} were undetectable in PrP^{C} preparations from control brain preparations. However, Cu^{2+} and Mn^{2+} changes were pronounced in sCJD subjects homozygous for methionine at codon-129 and carrying PrP^{Sc} type-1. It was also found that a decrease of up to 50% of Cu^{2+} and an approximately 10-fold increase in Mn^{2+} occurred in the brain tissues from sCJD subjects. Antioxidant activity of purified PrP was dramatically reduced by up to 85% in the sCJD variants, and correlated with an increase in oxidative stress markers in the sCJD brains. These results clearly point to the fact that metal-ion occupancy alterations in PrP play a pivotal role in the pathogenesis of prion diseases. Since the metal changes differed in each sCJD variant, they may contribute to the diversity of PrP^{Sc} and disease phenotypes in sCJD [106]. These fascinating and significant results could also have bearing on potential approaches to the diagnosis of CJD. The increase in brain Mn^{2+} associated with prion infection is potentially detectable by MRI, and the binding of Mn^{2+} by PrP in sCJD might represent a novel diagnostic marker.

Metals in other neurological diseases

Cells rely on several transition metals to regulate a wide range of metabolic and signaling functions. The diversity and efficiency of their physiological functions are derived from atomic

properties that are specific to transition metals, most notably an incomplete inner valence subshell. These properties enable the metals to fluctuate among a variety of positively charged ionic forms, and a chemical flexibility that allows them to impose conformational changes upon the proteins to which they bind. By this means, transition metals can serve as the catalytic centres of enzymes for redox reactions including molecular oxygen and endogenous peroxides [56]. In general, neurodegenerative diseases display two commonly recognised metal dependent reactions. Firstly, there are the metal-protein associations causing the abnormal aggregation of proteins. These can involve both redox-inert metal ions such as Zn^{2+} or redox-active ions such as Cu^{2+} or Fe^{3+}. Secondly, there are the metal-catalysed protein oxidations leading to protein damage and denaturation. These reactions involve only the redox-active metal ions such as Cu^{2+}, Fe^{3+} or Mn^{2+}. Both these reactions can lead to the functional demise of their target protein. It has been proposed that certain neurodegenerative diseases are caused by the abnormal interaction of metals enriched in the neural tissue, with specific target proteins that are susceptible to such interactions. These then cause aggregation and/or oxidation of the neural tissue mediated by the redox-active metal ion interacting with the target protein and finally, a loss of the function of the protein. Oxidative stress follows and leads to damage at the site of the abnormal metal-protein interaction.

An important mechanism of oxidative damage to proteins involves catalysis by redox active transition metals, which are bound to them. This process consists of the reduction of, for example, Fe^{3+} or Cu^{2+} by electron donors, such as O_2^-, catecholamines, L-ascorbate and mercaptanes, and generation of the OH radical through the reduction of H_2O_2 by the reduced metals. This highly reactive free radical attacks neighbouring amino acid residues, producing carbonyl-containing derivatives. In addition, carbonyls are introduced to protein as a consequence of oxidative cleavage of the peptide backbone. The carbonyls thus produced can serve as important markers of oxidative damage to proteins [7,29,94].

From the preceding discussion it can be seen that some of the symptoms of TSEs may be caused by an abnormality in an active Cu^{2+} site on the PrP protein. A similar phenomenon can also occur within other proteins, such as β-amyloid (Aβ) in AD or the Cu/Zn superoxide dismutase (Cu/Zn-SOD) in FALS (See below) and be a potential factor in other neurological diseases. Under normal conditions, Cu^{2+} binding to appropriate proteins is essential for oxidative stress homeostasis. Such Cu^{2+} active sites are very likely exposed to constitutively high concentrations of reactive oxygen species such as O_2^- and H_2O_2. H_2O_2 can react with Cu+, which is produced transiently at the active site of these proteins, and generate the highly reactive and detrimental ion, OH . In the normally folded proteins, (PrP^c in TSEs, APP in AD and SOD1 in FALS), the Cu^{2+} active site is probably shielded and therefore does not undergo this abnormal reaction. However, changes in the conformation of these proteins may expose the active site and make it more prone to react to produce the OH radical.

Alzheimer's disease (AD)

Alzheimer's disease (AD) appears to result when a specific protein in the brain, the Amyloid Precurser Protein (APP), is metabolised incorrectly to β-amyloid (Aβ), a 39-49 amino acid peptide. The disease is characterised by the deposition of Aβ as diffuse extracellular plaques or intracellular, neuritic plaques with dense cores. The deposition of Aβ is found predominantly in the hippocampus and temporal lobe cortex and is probably closely related to the primary pathogenesis of AD, with consequent neuronal death and increase in oxidative stress [3,30,93]. Many studies have confirmed that Aβ is neurotoxic in cell culture. There is an obvious similarity between AD and prion diseases, in that both are characterised by the deposition of a diseases causing form of a normal cellular protein. Unlike in prion diseases, the length of the Aβ species is considered to be the important factor in AD pathogenesis as Aβ is a proteolytically cleaved version of the normal APP.

The metallochemistry of Aβ has been investigated in some detail [27]. Aβ can be rapidly precipitated by Zn^{2+} ions at low physiological concentrations and it was recently reported that other metal ions like Cu^{2+}, Fe^{3+}, unlike Zn^{2+}, produced a greater aggregation of Aβ under weakly acidic conditions (pH 6.8-7.9) [2]. Such a mildly

acidic environment probably resembles conditions occurring in the brain. The significance of these *in vitro* studies with Aβ and metal ions is emphasised by other data showing that the homeostasis of Zn, Cu and Fe, are significantly altered in the AD brain [3]. Experiments utilizing microparticle-induced X-ray emission (PIXE) analysis of the cortical and accessory basal nuclei of the amygdala showed that these metals accumulated in the neuropil of the AD brain, and that their concentrations were increased 3-5 fold compared with age matched controls. The metals were found to be particularly high in the Aβ deposits [67]. Zn^{2+} in Aβ amyloid deposits was recently detected by histological fluorescent techniques in human brain [95]. It was also noted that the apolipoprotein E4 (apoE4) allele, which commonly appears in late onset AD cases, is associated with increased serum levels of Cu^{2+} and Zn^{2+} in AD. This suggests that the underlying perturbations in metal homeostasis associated with AD are systemic and not just confined to the brain [50].

Zn/Cu-selective chelators reportedly enhanced the resolubilization of pathological Aβ deposits from post-mortem AD brain samples, suggesting that Cu and Zn ions may play a role in assembling these deposits [31]. The metals could also play a more significant role other than purely facilitating fibril formation. *In vitro* work from Ashley Bush's lab reported that Aβ is a redox active protein, which reduces Cu(II) or Fe (III) and then produces H_2O_2 by electron transfer to O_2 [58,59]. Aβ cytotoxicity was shown to be mostly mediated by H_2O_2 produced directly by the Aβ variant, as the toxicity of the peptide was augmented by Cu^{2+} correlating with the degree of metal reduction by the same peptide. Aβ is very vulnerable to Cu(II)-mediated auto-oxidation, which leads to oxidative effects such as carbonyl adduct formation, histidine loss and dityrosine crosslinking and such modifications have been located on the Aβ deposits extracted from AD amyloid [1].

The metal mediated redox activity of Aβ may well play a significant role in the pathogenesis of AD in vivo, though this still remains to be shown. The affinities of the Zn^{2+} binding sites on Aβ1-40 were measured as 100 nM and 5 μM, indicating that they may be occupied under physiological conditions [28]. The highest affinity Cu^{2+} binding site on Aβ1-42 has a measured association constant (Ka) of 10-15 aM [4]. With such strong affinity for Cu^{2+}, Aβ species like Aβ1-42 are likely to bind Cu^{2+} in vivo. The increase in Cu^{2+} affinity of Aβ1-42 over the normal APP is related to APP proteolysis. APP is a membrane spanning protein and the Cu^{2+} binding site is probably hidden within the protein, becoming exposed in the proteolysed Aβ fragment. Also the peptide Aβ1-42 has a higher β-sheet content, and these structures are frequently found in the tertiary structure of Cu^{2+} catalytic sites.

Moreover, there is a further similarity between the Aβ in AD and the PrP^{Sc} in prion diseases. Studies made with the complex of Cu^{2+} with Aβ1-42 showed that it had a very strong reduction potential (+550 mM with respect to Ag/AgCl). This value is comparable with blue copper proteins such as SOD and is likely to denote a biological purpose for the metal binding [27]. Furthermore, Aβ binds both Cu^{2+} and Zn^{2+} and pulse radiolysis and cell culture experiments showed that Cu/Zn loaded Aβ possesses catalytic SOD-like activity. Thus the oxidative damage induced by Aβ might be mechanistically related to the oxidative stress induced by abnormal SOD activity. Here is another parallel with prion diseases.

In view of the relationship between Cu^{2+} binding and prion diseases, it is intriguing to note that APP, the precurser to Aβ, was also reported to mediate transport of Cu^{2+} out of cells [102,103]. APP has second Cu^{2+} binding and Zn^{2+} binding domains at its amino terminus and this activity therefore resembles that proposed for the prion protein, PrP^{c}. But in contrast to the PrP^{c} interaction with Cu^{2+}, the interaction of APP appears actually to promote oxidative damage in cultured neurons [102,103]. Another similarity has been reported in that the Cu^{2+} binding domain of PrP^{c} reduces Cu^{2+} and uses O_2 to produce H_2O_2, in a reaction sequence which is similar to that of Cu:Aβ.

Familial amyotrophic lateral sclerosis (FALS)

Familial amyotrophic lateral sclerosis (FALS) is a motorneurone degenerative disorder. A proportion of FALS are linked to missense mutations in the gene encoding Cu/Zn superoxide dismutase (Cu/Zn-

SOD1). The disease demonstrates a fascinating and slightly paradoxical principal in neuroscience when a normal antioxidant enzyme with redox-active metal catalytic sites suddenly becomes a pro-oxidant through upregulation. How this deleterious activity provokes neurological degeneration is a matter of some controversy. Some studies have proposed that the mutant Cu/Zn-SOD1 is able to generate highly toxic hydroxyl radicals that can damage essential cellular constituents [104]. The mutation causes a gain in the function in Cu/Zn-SOD1 that changes the activity of this ubiquitous antioxidant and the outcome is an aggressive, degenerative disorder. There is a wealth of literature on the oxidative insult caused by the FALS-linked SOD1 mutation [66,80] as well as the formation of SOD1 aggregates in affected motor neurons and glia [26]. The underlying biochemistry of this disorder and an understanding of how the change in SOD1 function causes it to become an aggressive oxidant might also be relevant to other more common disorders also characterized by oxidative stress.

As already discussed, Cu^{2+} is a versatile catalyst and, in an unbound state, possesses SOD activity with a rate constant the same as SOD1 itself [8]. Fortunately, free C^{u2+} is virtually undetectable. The purpose of the SOD1 protein is probably to harness the Cu^{2+} activity by binding to it and preventing it from undergoing other more destructive reactions. The latter would include, for example, the generation of OH formed by the reaction of reduced Cu^{+}, transiently made during the disproportionation of O_2^-, with the product H_2O_2. Hence the Cu^{2+} at the active site of SOD1 has the potential to be abnormally redox reactive, generating unwanted toxic species.

A recent paper described the likely mechanism for the pathogenicity of the mutant SOD1 [43]. It appears that the pathogenic SOD1 mutations do not cause a loss of function when the protein is fully loaded with Cu^{2+} or Zn^{2+}, but do cause a marked loss of affinity for Zn^{2+} [68]. An altered Cu^{2+} co-ordination makes Zn-deficient SOD (both wild type and mutant) a more efficient oxidant, able to oxidize ascorbate 3000 fold faster than Cu/Zn SOD. The altered reactivity of Zn-deficient SOD enables it to be reduced by cellular reductants (such as reduced glutathione GSH). SOD then donates an electron to O_2 to generate O_2^-, which then reacts with NO to form the strong pro-oxidant, peroxynitrite. Peroxynitrite is known to be very damaging to the nervous system. Thus, when SOD1 loses Zn^{2+}, its catalytic activity is diminished while it abnormally develops tyrosine nitration mediated by O_2^-, which is formed at the Cu^{2+} catalytic site [38].

In view of the above, it is intriguing to note that a loss of PrP activity was noted in a transgenic model of ALS [41]. The gene encoding the cellular prion protein PrP^C was specifically repressed in a transgenic model of ALS overexpressing the mutant Cu/Zn SOD. The analysis by Northern blot, semiquantitative RT-PCR and Western blot revealed that PrP^C down-regulation, which appeared early in the asymptomatic phase of the pathology, occurred preferentially in those tissues primarily affected by the disease (spinal cord, sciatic nerve and gastrocnemius muscle). The down regulation of PrP^C was found to be specifically linked to the overexpression of Cu/Zn SOD. As PrP^C has been shown to protect against oxidative stress [11,12] there is the intriguing possibility that its down regulation may contribute at least in part to ALS pathogenesis. It is interesting to note that overexpression of PrP^C results in an increased activity of Cu/Zn SOD possibly due to increased incorporation of Cu into the molecule [16]. Also PrP knockout mice show altered antioxidant expression including an increase in the extracellular SOD [23].

The SOD activity of PrP^C is also intriguing because of the neuropathological similarities between TSEs, AD and FALS. If PrP^C and Aβ act physiologically as SOD antioxidant enzymes, then TSEs, AD and FALS might share a common underlying cause, namely the break-down of an antioxidant protein containing a Cu^{2+} catalytic site.

Like mutant SOD1 in FALS, PrP^{Sc} might be a modified form of PrP^C, that induces a Cu^{2+} related gain in function, perhaps engendering abnormal free radical formation and consequently damage. Recent data suggests that this is a possible mechanism [69]. Cu^{2+} treatment of denatured PrP^{Sc} restored protease resistance and infectivity. In addition, it has been reported that the various conformations of different strains of PrP^{Sc} depend upon Cu^{2+} and Zn^{2+} interaction, and chelation of

these metals induces a conformational change in the protein, exposing novel proteolytic cleavage sites [101].

Parkinson's disease (PD)

The recent evidence implicating Cu^{2+} and Fe^{3+} in the overproduction of free radicals may also have a function in causing the death of the nigral cells in PD. Fe deposits selectively involving neurolmelanin in the *substantia nigra* neurons of PD patients have been found. Also the contents of the nigral Cu is decreased whereas its concentration in the cerebrospinal fluid (CSF) is raised. Increased oxidation markers and impaired mitochondrial electron transport mechanisms appear to be closely related to interactions between Fe and neuromelanin, and this results in the accumulation of Fe and a continuous production of cytotoxic species leading to neuronal death [63]. In familial PD, a mutation was identified in the alpha-synuclein gene. This is a component of Lewy bodies, which are typical of PD neuropathology. It is possible that the abnormal interaction of alpha-synuclein with Cu^{2+} in the formation of Lewy bodies may play a role in PD pathogenesis [76].

Failed antioxidant defence as a cause of TSEs

From the forgoing discussion it is clear that oxidative stress plays a major role in the damage to neurones observed in a wide variety of neurodegenerative disease. In many cases the damage has been linked to elevated levels of certain metals or to disturbances to the metabolism of specific metals. In TSEs there is an apparent elevation in Mn but no evidence that this causes oxidative damage. In ALS there is some evidence that a particular protein has gained a deleterious function and might initiate cellular destruction. In prion diseases an abnormal isoform of PrP is generated in access. It is unclear how this protein causes cell death although a multitude of in vitro experiments have suggested a direct toxic effect [22]. It is important to note that the presence of PrP^{Sc} alone is not sufficient to cause neuronal destruction [10]. In addition, co-expression of a different form of PrP that cannot be converted to PrP^{Sc} has a protective effect preventing neuronal damage [84]. A recent finding has confirmed that switching off expression of PrP during the course of a TSE results in a cessation of symptoms and an end to the disease progress [70]. These experiments involved mice expressing a conditional knockout of PrP expression. In this way the mice could express PrP and be infected with the disease and then during the disease progress the expression of PrP was switched off. What is unknown in this system is what other changes occur when PrP is switched off. Studies on traditional knockout mice have shown alterations in antioxidant defence [23] that would suggest alternative defence mechanisms are more active when PrP is not expressed. The implication of this is that the presents of non-functional PrP inhibits the activity of oxidative defence mechanisms (Figure 5). Cell lines can be generated that are conditionally infected with TSEs. These cell lines do not show significant cell death but they do show decreased levels of a number of antioxidants [72]. This supports the hypothesis that cell death in prion disease results from a failure in antioxidant defence rather than oxidative damage in excessive levels alone. This has an immediate implication relating to the function of PrP^{c}. This implies that the protein can act as a sensor for oxidative stress or could in itself be an antioxidant. As has already been mentioned, there is strong evidence that PrP^{c} has an activity like a superoxide dismutase [18]. This suggestion has largely been accepted. Adriano Aguzzi's group has hotly contested this finding, suggesting that the protein does not have this activity [60]. However, there evidence for this is based on studies with transgenic mice where mice lacking the expression of SOD-1 are compared to mice lacking the expression of both SOD-1 and PrP. In their system there is no difference in the level of SOD-like activity in the brains of the mice. The implication of this is that PrP does not have an SOD-like activity. Unfortunately, close examination of the experimental procedure used revealed that the experiments were carried out under conditions designed to eliminate any SOD activity other than that produced by SOD-1. The authors were repeatedly unable to detect SOD activity even from MnSOD which is highly expressed in the brain [87]. In addition, the methodology employed EDTA in high concentrations known to strip PrP^{c} of Cu [101]. Under these conditions PrP^{c} would not have any Cu related activity. Therefore, the findings of Aguzzi's group should be dismissed as misleading. Even, if PrP^{c} did not have an SOD-like activity it does not alter the fact that the majority of

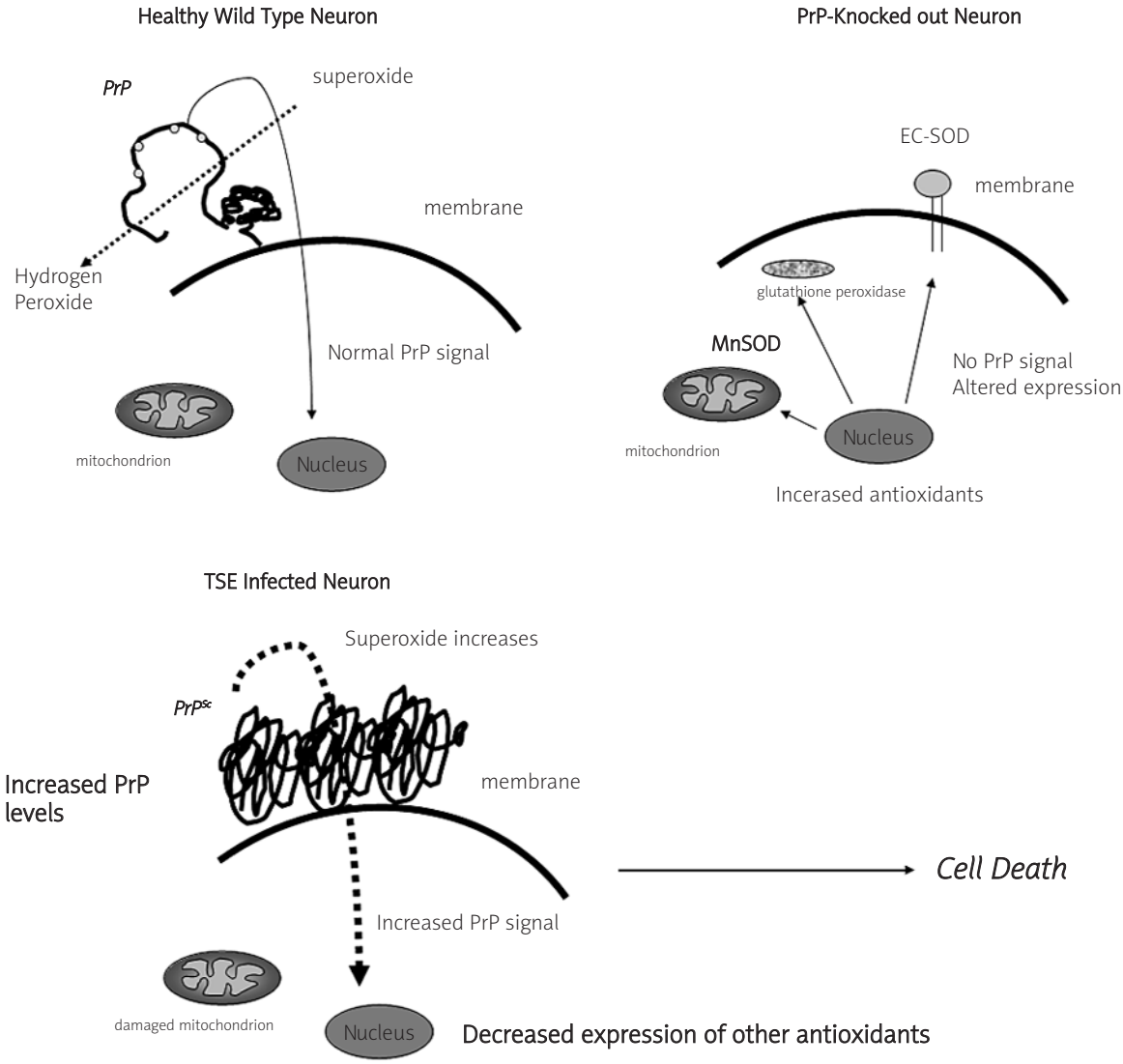

Fig. 5. The kind and level of PrP expression has a major influence on neuronal production of antioxidants. In a normal neuron expressing PrPc the cell has a normal level of expression of antioxidants and benefits from the superoxide dismutase activity of the protein. The expression of PrP^{c} the protein signals back to the nucleus maintaining the status quo. In the PrP-knockout mice there is no expression of normal or abnormal PrP and the signal from PrP to the nucleus is lost. This results in compensatory changes in protein expression, increasing levels of antioxidants such as glutathione peroxidase, MnSOD and EC-SOD. In the TSE infected neuron that expresses PrP which is converted to the scrapie isoform, PrP^{Sc}, the signal back to the nucleus is increased because PrP expressed by the cell is protease resistant and accumulates at high levels compared to the normal uninfected cell. This increased feedback decreases the levels of other antioxidants expressed by the neuron. In addition there is an increase in oxidative substances such as superoxide which initiate oxidative damage to the cell. This oxidative stress then results in programmed cell death

findings from many groups point to a role of PrP in antioxidant defence [86]. Therefore enhancing or augmenting antioxidant defence might prove protective against the neurodegeneration observed in TSEs. This finding is likely to lead to continued research in the future.

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