

## Prion diseases – current theories and potential therapies: a brief review

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### Abstract

*Prion diseases are a group of infectious diseases that cause lethal neurodegenerative disorders in both humans and animals. Affected patients usually die within one year from the appearance of the first clinical abnormalities. Unfortunately, no viable treatment options are available for prion diseases. The aim of this review is to describe the underlying prion disease pathology and discuss the therapeutic targets that have emerged from this.*

**Key words:** prion diseases, current theories, potential therapies.

### Introduction

Prion diseases are fatal neurodegenerative protein-misfolding disorders. Different prion species and strains are involved in the pathogenesis of these diseases as explained in detail by Weissmann [29]. They are also known as transmissible subacute spongiform encephalopathies (TSEs), a group that includes sheep scrapie, bovine spongiform encephalopathy (BSE) and Creutzfeldt-Jakob disease (CJD). Prion diseases gained widespread public interest and worry after an outbreak of BSE occurred in Europe, and evidence suggested that BSE is transmissible to humans [3,7]. The incidence of CJD in Iran remains rare, but this is thought to reflect underreporting of the condition [2]. Clinical symptoms such as dementia

and loss of coordination are suggestive of CJD but an accurate diagnosis requires inspection of post-mortem brain histology to detect the pathogenic prion.

It is commonly conceived that the agents responsible for TSEs are abnormal conformers of the prion protein (PrP<sup>C</sup>) [12]. The abnormal conformer, known as PrP<sup>Sc</sup>, is an insoluble form of PrP<sup>C</sup> that is resistant to protease digestion. PrP<sup>C</sup> is widely expressed, but the majority are found in the central nervous system as a glycoinositol phospholipid anchored cell surface protein. In contrast, the abnormal conformer PrP<sup>Sc</sup> accumulates intracellularly in cytoplasmic vesicles. The physiological function of PrP<sup>C</sup> remains uncertain but it may have a role in cellular signalling pathways such as calcium homeostasis. The conversion of PrP<sup>C</sup> to PrP<sup>Sc</sup> is believed to involve post-translational modification. The structure of PrP<sup>C</sup> is mainly  $\alpha$ -helical,

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whereas PrP<sup>Sc</sup> seems to be predominantly composed of  $\beta$ -sheet arrangement. The conversion from PrP<sup>C</sup> to PrP<sup>Sc</sup> can occur spontaneously, arising from genetic mutations in the PrP<sup>C</sup> gene, or be induced by infection with exogenous PrP<sup>Sc</sup>. PrP<sup>Sc</sup> is thought to induce PrP<sup>C</sup> conversion to PrP<sup>Sc</sup> [19].

Prions involve not only humans but also a wide variety of both domestic and wild animals and lead to transmissible and fatal diseases which are recognized by various names [8,11]. A summary of prion diseases are demonstrated in Table I.

### Potential therapeutic targets

Unfortunately, most of the CNS damage has already taken place when the clinical symptoms of prion disease manifest themselves. Since early diagnostic testing for prion disease is not yet available [8], there has been an interest in the development of drugs that could be beneficial following the onset of its clinical signs.

### PrP<sup>C</sup> conversion

Conversion of PrP<sup>C</sup> to PrP<sup>Sc</sup> is a central event in TSE pathology and, therefore, a potential therapeutic strategy has been an attempt to disrupt PrP<sup>Sc</sup> formation. Quinacrine (an antimalarial drug) and chlorpromazine (a widely used antipsychotic drug) were found to be efficacious inhibitors of PrP<sup>Sc</sup> formation *in vitro* [10]. It is thought that these compounds act by rearrangement of cholesterol into intracellular compartments resulting in the deterioration of the plasma membrane [17]. Animal studies examining these compounds have demonstrated limited effectiveness in the treatment of TSEs [28]. In fact, administration of quinacrine to CJD patients led to short-lived improvements of symptoms (lasting between 1 and 2 months) only and, ultimately, did not halt the disease [26].

As protein misfolding is a major event in prion disease pathology, there has been an investigation into the ability of pharmacological agents to stabilize the native folded state of PrP. These agents may allow for a reduction in the rate of their misfolding and hence prevent progression of disease. Recently, Nicoll et al. found that a cationic tetrapyrrole compound displays significant antiprion activity by binding to a folded domain of human PrP [24].

### Immunotherapy

Antibody-mediated therapy that targets the PrP conformers has been shown to inhibit PrP<sup>Sc</sup> propagation *in vitro* [13,22]. It is thought that these antibodies bind to PrP<sup>C</sup> and attenuate its availability for conversion into the abnormal prion conformers. This approach appears to be promising in animal models but issues that remain to be addressed include the delivery of the antibodies across the blood-brain barrier as well as the potential immunological pitfalls associated with tolerance to PrP, which is widely expressed in the immune system.

Another, more indirect, target may be the inflammatory mediators required for the development of follicular dendritic cells. These cells are known to be required for splenic PrP<sup>Sc</sup> accumulation. Neutralization of the lymphotoxin- $\beta$  receptor (LT $\beta$ -R) pathway by administration of a soluble LT $\beta$ -R-Ig fusion protein blocks follicular dendritic cell maturation and prevents scrapie neuroinvasion [14].

**Table I.** Human and animal diseases caused by prions

Affected species	Diseases
Cattle	Bovine Spongiform Encephalopathy (BSE) Mad cow disease
Goat, Sheep	Scrapie
Cat	Feline Spongiform Encephalopathy (FSE)
Mink	Transmissible Mink Encephalopathy (TME)
White-tailed Deer, Elk, Mule Deer, Moose	Chronic Wasting Disease (CWD)
Oryx, Nyala, Greater Kudu	Exotic Ungulate Encephalopathy (EUE)
Human	Creutzfeldt-Jakob Disease (CJD) Iatrogenic Creutzfeldt-Jakob Disease (iCJD) Variant Creutzfeldt-Jakob Disease (vCJD) Familial Creutzfeldt-Jakob Disease (fCJD) Sporadic Creutzfeldt-Jakob Disease (sCJD) Gerstmann-Sträussler-Scheinker syndrome (GSS) Fatal familial insomnia (FFI) Kuru

## RNAi

RNA interference (RNAi) exploits the cell's endogenous gene silencing machinery and is a well-recognized experimental tool, with the potential for targeted gene silencing in neurodegenerative diseases such as Alzheimer's. RNAi inhibits PrP<sup>C</sup> expression in neuroblastoma cells and prevents the build-up of the abnormal PrP<sup>Sc</sup> conformer in scrapie-infected cells [4, 30]. Recently, lentiviral vector-mediated RNA silencing of PrP<sup>C</sup> was shown to prolong survival of prion-infected mice [16,20]. Despite these promising results, this approach has not cured the disease. Although the onset of disease was delayed, all animals treated with RNAi against PrP<sup>C</sup> eventually died of the disease.

## Neurodegeneration

Apoptosis of neuronal cells is a central feature in prion diseases. The cause of neuronal cell death remains unclear but may be triggered by direct interaction of the prion-infected cells or, indirectly, by deficiency of the normal PrP<sup>C</sup> prion protein. Muller *et al.* noted that neuronal cells *in vitro* undergo apoptosis when incubated in the presence of PrP<sup>Sc</sup> and this can be mimicked by the PrP peptide fragment PrP106-126 [25].

PrP<sup>C</sup> has been shown to enhance intracellular calcium levels, which may contribute to neuronal apoptosis [18]. Other investigators have also shown that PrP<sup>Sc</sup> induces alterations in calcium homeostasis. For example, the activity of the calcium-dependent phosphatase, calcineurin, is enhanced as a result of PrP<sup>Sc</sup> formation [27]. Muckerjee *et al.* reported that the calcineurin inhibitor FK506 reduced severity of the clinical abnormalities and increased survival time compared to sham-treated controls. Another contribution from calcium mediated excitotoxicity is through effects on the N-methyl-D-aspartate (NMDA) receptor [25]. The NMDA receptor channel is widely expressed in the CNS and is highly permeable for the cations Ca<sup>2+</sup>, Na<sup>+</sup> and K<sup>+</sup>. Crucially, the neurotoxic activity of PrP<sup>Sc</sup> was blocked by the NMDA antagonists, memantine and MK-801, *in vitro* [25]. The finding that the PrP<sup>Sc</sup> induced neurotoxicity is prevented by antagonists of the NMDA receptor channel suggests that PrP<sup>Sc</sup> activates the NMDA receptor channel, resulting in a significant rise of [Ca<sup>2+</sup>]<sub>i</sub> level in neurons and eventually cell death. Flupirtine, a drug that is used clinically as a nonopi-

oid analgesic acts as an NMDA antagonist, but does not bind directly to NMDA receptors. Flupirtine increases the expression of the anti-apoptotic Bcl-2 protein in neuronal cells exposed to prion protein [5]. In view of its established pharmacokinetic actions, it is a potential drug for use in clinical trials for CJD.

There is research to suggest that there is serotonin (5-HT) dysregulation in prion diseases, particularly alteration in 5-HT levels and 5-HT receptor binding [1, 6]. It has been suggested that PrP<sup>C</sup> has a role to maintain 5-HT receptor coupling to G-proteins [6]. This work has yet to yield direct therapeutic targets but 5-HT drugs form a major group of pharmacological therapies, including those used to treat central nervous system disorders such as depression and anxiety.

Following neuronal damage and loss, any form of therapeutic intervention is limited. However, by analogy to other neurodegenerative diseases such as Alzheimer's disease, stem cells may yield benefits [23]. Embryonic stem cells and neural precursors are indeed known to migrate towards sites of brain damage and can differentiate into specific neuronal cell types [15, 21]. Although these therapies are still in the experimental phase of development, there is hope that stem cell based therapies may attenuate disease symptoms.

## Future perspectives and conclusion

Despite the varied approaches that have been undertaken to limit or reverse prion diseases, there has been frustratingly limited success to date. Despite this, our knowledge of the pathology of prion diseases and means of disease detection is continuously expanding and generating novel drug targets. Indeed, it may be that targeting more than one pathway or protein may provide synergistic benefits. It is hoped that prion diseases will remain rare, but if extensive human infection with BSE prions emerges in the next 10-20 years, there will be considerable pressure to combat this public health threat.

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