

Potential approaches for heterologous prion protein treatment of prion diseases

Davis M. Seelig^a, Patricia A. Goodman^b, and Pamela J. Skinner^b

^aVeterinary Clinical Sciences Department, University of Minnesota, Saint Paul, MN, USA;

^bVeterinary and Biomedical Sciences Department, University of Minnesota, Saint Paul, MN, USA

ABSTRACT. Prion diseases, or transmissible spongiform encephalopathies (TSEs) are progressive, fatal neurodegenerative diseases with no effective treatment. The pathology of these diseases involves the conversion of a protease sensitive form of the cellular prion protein (PrP^C) into a protease resistant infectious form (PrP^{res}). The efficiency of this conversion is predicated upon a number of factors, most notably a strong homology between cellular PrP^C and PrP^{res}. In our recently published study, we infected mice with the RML-Chandler strain of scrapie and treated them with heterologous hamster prion proteins. This treatment was seen to reduce clinical signs of prion disease, to delay the onset of clinical symptoms and to prolong survival. In this current article we discuss potential mechanisms of action of treatment with heterologous prion proteins. We also discuss potential extensions of these studies using a heterologous rabbit PrP-based treatment strategy or a peptide based strategy, and improvement of treatment delivery including a lentiviral-based system.

KEYWORDS. prion, treatment, PrP^{res}, PrP^C, heterologous prion proteins

The prion diseases, or transmissible spongiform encephalopathies (TSEs) are progressive, uniformly-fatal neurodegenerative diseases of infectious, genetic, or sporadic origin. TSEs include diseases that affect both humans (e.g. Creutzfeldt-Jakob disease [CJD], Gerstmann-Straussler-Scheinker syndrome [GSS], and fatal familial insomnia [FFI]) and animals (e.g.,

Correspondence to: Pamela J. Skinner; Email: skinn002@umn.edu

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bovine spongiform encephalopathy [BSE], chronic wasting disease [CWD] of cervids, and scrapie in sheep. Following a typically long incubation period, those affected by a TSE experience progressive, and ultimately fatal, neurocognitive decline. No effective therapy for prion diseases has been identified and nearly all patients with the most common form of prion disease, CJD, die within 14 months of their diagnosis.¹ While unique in their own right, the molecular and biochemical mechanisms, cellular infectivity, the progressive accumulation of misfolded protein mechanistically aligns the prion diseases with a larger group of protein misfolding neurodegenerative disease, including Alzheimer's, Parkinson's, and Huntington's diseases.

The hallmark event in the prion disorders is the misfolding of the normal cellular prion protein (denoted PrP^C) into a misfolded isoform (commonly denoted as PrP^{res} or PrP^{Sc}). It is widely accepted that the PrP^{res} formation is the key initiating event in prion disease pathogenesis and that its presence correlates with infectivity. As such, PrP^{res} is also the mostly widely used diagnostic surrogate marker for affected individuals and animals. Although the accumulation of PrP^{res} is the hallmark feature of prion diseases and the microscopic neuropathology is stereotypic (coincident gliosis, synaptic dysfunction, spongiosis, and neuronal loss), the mechanism(s) by which the misfolded prion protein exerts its toxic effects is unknown. However, it is likely that the consequences of prion infection result from a toxic gain of function of misfolded PrP, as depletion of PrP^C does not appear to be detrimental.²

The conversion of PrP^C to PrP^{res} is a highly specific process of templated conversion requiring direct interaction between the normal and abnormal forms of the protein.³ The efficiency of this conversion is predicated upon a number of specific reaction conditions, including the secondary structure of PrP^{res}, homology of the primary and secondary structures between PrP^C and PrP^{res}, and the architecture of the PrP^C-PrP^{res} complex.^{4,5} Increased contact between PrP^C-PrP^{res} at residue 129 and the relative rigidity of the β 2- α 2 loops in PrP^C are 2 important factors in mediating the efficiency

of PrP^{res} formation and TSE susceptibility.⁵⁻⁷ In addition to steric factors, the formation of PrP^{res} is favored by destabilization of PrP^C as a number of destabilizing pathogenic mutations in PrP are linked with increasing misfolding rates.⁸⁻¹⁰

In light of this work demonstrating the importance of a precise interaction in the PrP^C-PrP^{res} complex, it seems reasonable to probe the possibility that interference with this interface might be a potential therapeutic avenue. That is, to test the hypothesis proposed by Singh and Udgaonkar in their comprehensive review on PrP misfolding, namely that "...any ligand, whether small or large, that binds to the native conformation of the [PrP^C] protein would stabilize that state and can therefore be expected to decrease the native-state dynamics that drive misfolding."¹¹ Support for such an approach has been validated by antibody-based studies, which have stabilized the α 1 region of PrP^C and prevented prion disease in animals.^{12,13}

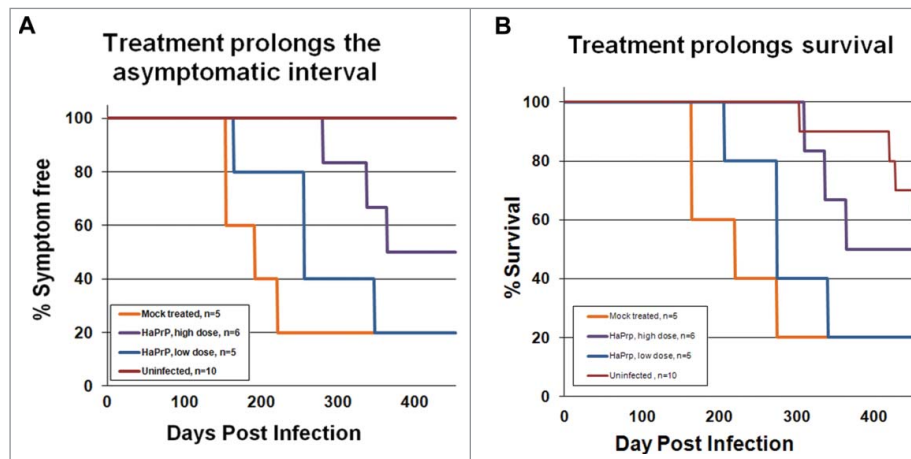
The concept of heterologous prion proteins (HetPrP) as potential therapeutics is not new. Horiuchi et al demonstrate that inclusion of a heterologous species PrP^C in a cell-free conversion system was capable of interfering with the formation of PrP^{res} between 2 homologous species.¹⁴ When they divide the process of PrP^{res} formation into 2 steps, namely initial binding between PrP^C and PrP^{res} followed by acquisition of protease resistance, the interfering effect of HetPrP appears to occur during the latter.¹⁴ Further *in vitro* studies demonstrate that expression of hamster prion protein (HaPrP) in persistently-infected mouse cells nearly completely eliminates the accumulation of PrP^{res},¹⁵ suggesting that heterologous HaPrP may either inhibit PrP^{res} production or enhance its clearance. Moreover, scrapie-infected mouse cells that are induced to express a rabbit prion gene produce substantially less PrP^{res} as compared to mouse cells that do not express rabbit prion proteins, suggesting that rabbit prion proteins may interfere with mouse PrP^{res} formation.¹⁶

In our work, we extended these *in vitro* observations into the mouse using the RML-Chandler strain of scrapie and HetPrP therapy

using bacterially expressed and purified recombinant HaPrP amino acids 23-231.¹⁷ In brief, mice were intracerebrally inoculated with a 0.01% RML-Chandler strain brain homogenate combined with 1 of 2 dosages of either HaPrP or vehicle control. Mice were then treated with HaPrP orally the following day. To assess the effect of the HaPrP dosage, 2 treatment groups were used, including a high dose of recombinant protein (0.7 mg/ml, high dose) and a low dose (0.35 mg/ml). Lastly, 2 control groups were included, namely a mock treatment group comprised of infected treated with vehicle only and an untreated / uninfected group. To assess the impact of treatment on clinical disease, mice were evaluated daily following infection, weekly during the first months and then daily in later months for signs of scrapie-related symptoms including ataxic gait, hind limb paresis, decreased motility, dull eyes, flattened stature, weight loss and kyphosis.

Clinically, treatment with high dose HaPrP effectively and significantly delayed the onset of clinical symptoms, including motor and non-motor deficits, and prolonged survival as compared to vehicle-treated animals (Fig. 1).¹⁷ Moreover, when the study was terminated at 452 d post-infection, half of the high dose treated animals were still free of scrapie symptoms. In addition to abrogating the clinical signs of prion disease, mice receiving the high-dose of HaPrP accumulated significantly less PrP^{res} in both brain and spleen as compared to mice treated with a low dose of HaPrP or with vehicle only. Additionally, HaPrP partially mitigated the neuropathologic consequences of prion infection as high dose treated animals showed a trend toward fewer activated astrocytes as revealed by immunohistochemistry for glial fibrillary acidic protein and less severe neuropil spongiosis.

FIGURE 1. Treatment with heterologous recombinant HaPrP delayed the onset of symptoms and prolonged survival. Kaplan-Meier plots showing the time at which mock-treated (orange, n = 5), low-dose-treated (blue, n = 5), high-dose-treated mice (purple, n = 6) and uninfected (red, n = 10) developed (A) detectable symptoms associated with scrapie infection, including ataxic gait, weight loss, and kyphosis, and (B) time of survival. We tested for differences between groups using a modified version of the Gehan-Wilcoxon test and found a statistically significant difference between the mock infected group and the high dose group ($p = 0.0348$). The low-dose group was not significantly different than the mock-treated control group. The uninfected control mice showed significantly longer survival times than the 3 groups of infected mice; uninfected versus mock ($p = 0.008$), uninfected vs. low dose ($p = 0.006$) and uninfected versus high dose ($p = 0.0201$). This figure is reprinted from our original article, Skinner et al., 2015, published in PLoS ONE.



Taken in concert, treatment with HetPrP^C inhibits both the formation of PrP^{res} and the clinical consequences of prion infection. However, the mechanism underlying this phenomenon is unknown. We believe that HetPrP binds to both PrP^{res} and PrP^C and blocks the production and elongation of PrP^{res} chains and amyloid formation (Fig. 2). The work of Horiuchi et al., offers 2 possible mechanistic models for this interference, based upon number and type of binding sites for PrP^C on PrP^{res}.¹⁴ In a “one binding system,” they posit that the binding of HetPrP to a growing PrP^{res} oligomer creates a functionally impotent aggregate that is then incapable of generating the steric interactions necessary for the continued production of PrP^{res}. Alternately, in a “2 binding system” they propose that the growing PrP^{res} oligomer contains 2 binding sites, namely a conversion-inducing site and a non-converting site. In such a 2 site system, HetPrP would interfere with the formation of PrP^{res} by binding and blockade of the conversion site without blocking the non-converting site. In addition to biochemical mechanisms, it is possible that the protective effect of HetPrP

in our study resulted from an evoked immune response that impacted PrP^{res} formations. However, Western blot analysis of serum from study mice did not reveal the presence of anti-hamster PrP antibodies at 1:500 or 1:1000 dilutions. Lastly, it is important to note that because mice were intracerebrally inoculated simultaneously with both scrapie prions and HaPrP, it is quite likely that the HaPrP in the inoculum served to inactivate the scrapie prion by binding to PrP^{res} and forming an inactive complex due to sequence incongruence.

Based upon *in vitro* and *in vivo* studies, it is increasingly apparent that HetPrP treatment safely inhibits the PrP^C to PrP^{res} conversion process. These results render feasible the prospect of treating prion diseases with HetPrP. While demonstrating efficacy, the treatment regime used in our study (intracerebral instillation of HetPrP at the time of infection followed by oral ingestion of heterologous PrP^C) is not ideal for treating patients with existing prion disease, and delivery via intracerebral injection is certainly not anticipated to allow HetPrP to make contact with and inactivate all PrP^{res}

FIGURE 2. Model of heterologous prion protein inhibition of protein misfolding, nucleation and formation of amyloid. Normal cellular prion proteins, PrP^C, bind with and take on the conformation of misfolded, protease resistant prion proteins PrP^{res}. This process continues with leads to seeds of oligomers of misfolded proteins, which elongate and form amyloid deposits. Heterologous prion proteins inhibit this process. We hypothesize that the mechanism mediating this inhibition is that heterologous prion proteins bind directly to PrP^C and PrP^{res} and do not conform to the shape of PrP^{res}, and block the progression of seed and amyloid formation.

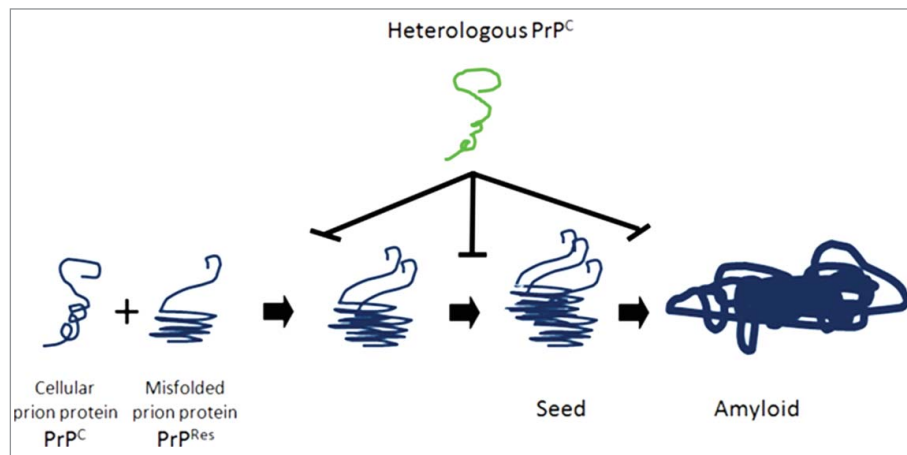


FIGURE 3. Comparison of human and rabbit prion protein amino acid sequences. The SIM - Local similarity program (Xiaoquin and Miller, 1991) was used to make the alignments.

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88.6% identity in 254 residues overlap; Score: 1300.0; Gap frequency: 0.4%

Rabbit      1 MAHLGYWMLLLFVATWSDVGLCKKRPKPGGGWNTGGSRYPGQSSPGGNRYPPQGGGGWGQ
Human      1 MANLGCWMLVLFVATWSDLGLCKKRPKPGG-WNTGGSRYPGQGSPGGNRYPPQGGGGWGQ
      * * * * *

Rabbit     61 PHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGGGTHNQNGKPSKPKTSMKHVAGAAAAG
Human     60 PHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGGGTHSQWNKPSKPKTNMKHMAGAAAAG
      * * * * *

Rabbit    121 AVVGGGLGGYMLGSAMSRPLIHFQNDYEDRYRENMYRYPNQVYRPFVDQYSNQNSFVHDC
Human    120 AVVGGGLGGYVLSAMSRPIIHFQSDYEDRYRENMHRYPNQVYRPMDEYSNQNNFVHDC
      * * * * *

Rabbit    181 VNITVKQHTVITTTKGENFTETDIKIMERVVEQMCITQYQQESQAAYQRAAGVLLFSSPP
Human    180 VNITIKQHTVITTTKGENFTETDVKMERVVEQMCITQYEREQAAYKRGSSMVLFFSSPP
      * * * * *

Rabbit    241 VILLISFLIFLIVG
Human    240 VILLISFLIFLIVG
      * * * * *

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in the system. As such, future studies should emphasize the development of more practical HetPrP delivery modalities and the evaluation of more effective HetPrP sequences.

While a wide range of mammal species are susceptible to prion infection, the efficiency of interspecies prion infection is varied and governed by a so-called "species barrier," the integrity of which is inversely proportional to the strength of the interaction between host PrP^C and incoming PrP^{res}. Among tested species, rabbits have been shown to be unusually resistant to prion disease inoculation, as attempts to transmit Kuru, CJD, sheep scrapie, TME, and mouse-adapted scrapie have failed.^{18,19} While subsequent groups have confirmed that the rabbit is not absolutely resistant to prion infection, there is general agreement that they are only minimally susceptible.^{20,21} The degree of primary sequence homology is important in determining the robustness of the species barrier and the rabbit prion protein shows relatively low sequence homology to other species prion proteins. To illustrate this, Figure 3 shows an amino acid sequence alignment²² of human and rabbit prion proteins. Based upon this work, we propose that a rabbit PrP-based HetPrP treatment strategy may be more effective than HaPrP at inhibiting prion disease.

While IC injection of HetPrP was used in our study, the clinical evolution of this approach necessitates a more effective and simpler means of delivery. One such approach would be delivery via the blood stream to deliver HetPrP to all areas of the brain as previous studies have demonstrated the ability of PrP to cross the blood-brain-barrier.²³ In addition, it may be possible to use peptides derived from HetPrP rather than whole proteins. Indeed Chabry et al., showed *in vitro* inhibition of PrP conversation with synthetic peptides derived from mouse and hamster PrP.^{24,25} Another such possibility for HetPrP treatment is the adoption of a gene therapy-based approach using lentiviral vectors. There is increasing precedence for gene therapy based approaches to prion diseases. Mallucci et al., generated an adult-onset PrP knockout mouse model with delayed, neuron-specific deletion of PrP^C which mitigated the clinical and neuropathologic consequences of prion disease.²⁶ In an alternate gene therapy study, the same group used RNAi-driven gene silencing to reduce PrP^C expression. Using lenti-shRNA directed against PrP^C, treated mice experienced a significant down-regulation of PrP^C expression and a delay in prion disease progression.²⁷ Moreover, Toupet et al demonstrate that chronic injection of a recombinant lentiviral vector expressing a

dominant negative prion protein directly into the brains of prion disease infected mice at 80 and 90 d post-infection was shown to reduce astrocytic gliosis and extend survival.²⁸

These initial studies demonstrate proof of principal that HetPrP may serve as useful therapeutic agents for prion diseases. However, further studies are warranted to optimize both the form of HetPrP as well as its mode of delivery. The increasing awareness of the role of protein misfolding in many neurodegenerative processes makes the development of an effective treatment strategy for prion diseases a high priority. Strategies that work for treating prion diseases may also be effective when applied to other neurodegenerative diseases.

ABBREVIATIONS

TSEs	transmissible spongiform encephalopathies
CJD	Creutzfeldt-Jakob disease
GSS	Gerstmann-Straussler-Scheinker syndrome
FFI	fatal familial insomnia
BSE	bovine spongiform encephalopathy
CWD	chronic wasting disease
denoted PrP ^C	cellular prion protein
commonly denoted as PrP ^{res} or PrP ^{Sc}	misfolded isoform
HetPrP	heterologous prion proteins
HaPrP	hamster prion protein

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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