Therapeutics and Prion Disease: Can Immunisation or Drugs be Effective?

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Abstract: Prion diseases are of considerable importance because of the threat of a variant form of Creutzfeldt Jakob disease that has emerged in recent years. Pre-clinical diagnosis of prion diseases still remains poor and effective therapies also do not exist at present. This review examines research on possible therapeutic strategies that might have potential benefits if applied before neurodegeneration has occurred.

Keyword: Antioxidants, immunisation, prion disease, therapeutics, transmissible spongiform encephalopathy (TSE).

INTRODUCTION

Prion diseases such as Creutzfeldt-Jakob disease (CJD) are fatal, neurodegenerative disorders for which there is, as yet, no known therapy. These diseases are defined by their ability to be transferred by experimental infection both within and between species and by the pathological damage they produce in the central nervous system (CNS). Prion mediated neuropathology is typically characterised by four features: spongiform change, astrocytosis, deposition of abnormal prion protein in the central nervous tissue and neuronal loss. Before the beginning of the 1980s, prion diseases were mostly diagnosed on the basis of spongiform degeneration of the neuropil layer in the brain of affected individuals. Following intensive research from Stanley Prusiner's laboratory in the 1980s, diagnosis also came to include the deposition of an abnormal, host-encoded protein, PrP^{Sc} (abnormal prion protein), in the CNS and lymphoreticular system [1, 2, 3]. This abnormal protein is a misfolded version of a normal host protein and is associated with the plaques and fibrillar structures observed in histological sections of infected brain tissue. The normal host protein, PrP^{c}, is a glycosyl-phosphatydilinositol (GPI)-linked, copper binding membrane protein of up to 220 amino acid residues in length, adopting a largely alpha-helical conformation [4]. Conversely, the abnormal PrP^{Sc} is predominantly in a beta-sheet conformation [5] and adopts a fibrillar structure in vitro, similar to the deposits found in prion infected CNS tissue [6].

The extent of PrP^{Sc} deposition is very variable, ranging from the little or no deposition found in some cases of experimental sheep and goat scrapie [7, 8], to the dense networks of amyloid fibres seen in the cerebella of variant CJD patients [9]. In spite of the variation in PrP^{Sc} distribution, many researchers now believe this protein is itself the disease agent and that disease development does not follow the usual pattern of infection by, for example, bacteria or viruses [10, 11, 12, 13]. For a successful prion infection to occur, the normal host protein has to convert to the abnormal form. The most convincing evidence showing that PrP^{c} expression is essential for production of the infectious prion agent comes from work with murine models in which PrP^{c} expression was ablated [14]. Without PrP^{c} expression, the mice were completely resistant to prion challenge [15]. The reason for this was that there was no PrP^{c} to be converted to PrP^{Sc}. Both in vivo and in vitro evidence from a large number of sources now clearly shows that neuronal expression of PrP^{c} is the first essential component of prion disease [15, 16, 17, 18, 19]. Although misfolding of the cellular prion protein PrP^{c} into an alternative form, denoted is a key event in prion infections, the normal function of PrP^{c} remains to be agreed up. There are four main hypotheses: 1) PrP^{c} is involved in signal transduction, but the nature of the signal and its function remain uncertain [20]. 2) PrP^{c} is an adhesion molecule because it sticks to a number of proteins [21, 22]. However, there are many of these proteins and the potential physiological role of any of these interactions is unclear. 3) The protein is a mediator of Cu transport or sequestration [23]. This theory takes into account the protein's Cu binding ability. Evidence from other groups suggests that Cu causes uptake of PrP^{c} into cells [24, 25]. Potentially, any protein that binds Cu at the cell surface will cause uptake of Cu into a cell, but there is clear evidence that cells do not require PrP^{c} expression for Cu uptake [23] and there are other known uptake pathways for Cu for cells [26]. Therefore, this is not likely to be the protein's main or only function. 4) PrP^{c} is an antioxidant protein [27, 28, 29]. Of these proposed functions, only the latter takes into account both the protein's ability to bind Cu and is supported by a large body of evidence from a number of independent laboratories [30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40] In particular, it has been shown that PrP^{c} can act as a superoxide dismutase of high activity [27].

GENERAL APPROACHES TO THERAPY

Therapeutic strategies to combat prion disease are urgently needed. Diagnosis of prion diseases would allow the possibility of treating the conditions before the symptoms became too severe but reliable pre-clinical diagnostic tests do not exist at present. Therefore the only real hope for patients is to find ways of inhibiting the neuropathological damage, particularly neuronal degeneration and death, caused by the disease. Thus immediate treatment of CJD patients would have to inhibit neuronal death and the

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first step in the development of such strategies is the identification of target points at which intervention can be directed. Several points of intervention can be considered.

The first of these is the formation of PrP^{Sc} and its interaction with cells and the normal protein PrP^{c}, which results in its loss. Expression of PrP^{c} is necessary in order for the host to generate PrP^{Sc}. Also, in the absence of neuronal expression of PrP^{c} there is no observed toxicity of PrP^{Sc}[41, 42]. There is evidence that PrP^{Sc} interacts directly with PrP^{c} and other proteins to initiate cell death [43]. It is currently unclear as to whether changes to or loss of the normal function of PrP^{c} in prion disease is associated with or causal to neuronal death. However, it is possible that the loss of function due to its conversion to the abnormal isoform might be directly involved in the causation of neuronal death. Although, PrP-knockout mice are normal [14, 44] and don't show any spontaneous pathology it is possible that compensations occurring in the knockout prevent these changes, but similar changes do not occur in prion disease and so there is a net loss of a protective function. Such changes in PrP-knockout mice have been noted [45] and are particularly related to changes in antioxidant defence mechanisms. Therefore possibly the use of compounds that mimic the normal function of PrP^{c} might have a beneficial effect in prion disease.

This common theme in the pathogenesis of these disorders and the extracellular localization of the accumulating abnormal protein make them highly amenable to therapeutic approaches based on experimental manipulation of protein conformation and clearance. A number of different approaches under current development include drugs which affect the processing of the precursor proteins drugs the clearance of the amyloidogenic protein, and which inhibit or prevent the conformation change and immunological approaches. Particularly interesting are compounds termed 'beta-sheet breakers' that directly target the abnormal conformational change both for beta-amyloid protein- and PrP^{Sc}-related deposits. In addition, immune system activation can serve as beta-sheet breakers and/or to increase the clearance of the disease-associated proteins. These conformation-based approaches appear to hold the best promise for therapies for this devastating group of disorders.

Another point of intervention would be the initiation of cell death signalling cascades. There is some evidence from peptide models that the intracellular signalling leading to neuronal cell death uses known pathways [46]. However, these pathways have not been clearly defined. Defining them further would potentially unmask specific proteins that could be inhibited in their action such as BAX. Global inhibition of BAX or similar proteins might then suspend cell death.

Other factors may also be involved which could be targets for intervention. These include possible oxidative stress generated in the brain or the interaction of the abnormal protein with cells such as microglia, which have been suggested to be involved in the cell death mechanism [47]. A defined strategy might require a cocktail of compounds or effects at multiple targets, which might be necessary to ensure inhibition of neuronal death.

Therefore anything that can act at these sites could inhibit neuronal death. This implies that in order to address strategies to inhibit neuronal death in patients with CJD studies must be carried out to test if intervention at the targets described above will be effective.

In summary the range of possible therapeutic or preventative strategies for prion disease include:

1. Create agents that remove PrP^{Sc} or abolish its formation
2. Block PrP^{Sc} interaction with cells.
3. Alter expression of PrP^{c}. Requires analysis of factors that regulate the levels of PrP expression.
4. Map the intracellular signalling pathways that lead to neuronal death. Then use agents which inhibit cell death through the identified pathway.
5. Prevent changes to cells resulting in loss of PrPc activity

This list is not meant to be exhaustive but is to illustrate the diversity of approaches that are possible. Many of these are currently being investigated.

Many researchers are currently seeking inhibitors that block PrP^{Sc}. Creating agents which remove PrP^{Sc} and strategies which prevent it interacting with cells are very similar and may be considered as one kind of strategy.

Switching off the expression of PrP^{c} might prove to be the most effective way of inhibiting neurotoxicity as it would be very specific for prion disease. It has been shown in granulocytes cell lines that all-trans retinoic acid causes down regulation of PrPc expression [48]. It is unknown if this compound or other retinoids will down regulate PrP^{c} expression in neurones or other cells lines but this is currently being investigated. In addition it also known that exposure to copper and oxidative stress also alter the expression of PrP^{c} [49]. However, strategies to bring about effective down regulation of PrP^{c} expression require much greater knowledge of how PrP^{c} expression is regulated. Therefore a detailed analysis of the regulation of PrPc expression would be worthwhile. Mapping the intracellular pathways leading to death would require a re-analysis of the mechanism of toxicity of or peptide fragments. Finally, preventing changes resulting in the loss of PrP^{c} activity depends on knowing what the function of PrP^{c} is. As we have considerable evidence that PrP^{c} is an antioxidant investigations are taking place into whether antioxidants or non-mammalian PrP^{c} can prevent the toxicity of PrP^{Sc}.

IMMUNISATION STRATEGIES AGAINST PRION DISEASE

The sequence identity of PrP^{c} and PrP^{Sc} results in the immune system displaying natural tolerance to PrP^{Sc}. Thus, no antibodies against prions can be detected in experimentally infected animals or humans [50, 51] and a lack of inflammation is a characteristic feature of these diseases. Consequently, the search for an effective immunisation strategy against prion disease is proving to be a challenge.

Immunologically based strategies are currently being developed to target prion replication at the early, lymphatic stage of disease or to develop a strategy that provides total resistance. Several recent reports have indicated that

antibodies directed against PrP^{c} might eliminate PrP^{Sc} from scrapie infected cells in vitro [52]. Transgenic mice expressing fragments of anti-PrP antibodies are able to prevent the spread of parenteral prion infection [53]. These findings suggest that early immunotherapeutical intervention is not unattainable and suggests that prion disease pathogenesis might be more generally reduced using anti-prion protein antibodies. Immunization with PrP peptides was also shown to provoke an immune responses in some mouse strains [54] though the mechanism is not yet fully understood. This strategy remains to be tested in other, more conventional prion disease models.

There is increasing recognition that numerous neurodegenerative conditions have the same underlying pathogenetic mechanism, namely a change in protein conformation, where the beta-sheet content is increased. In Alzheimer's disease (AD), for example, amyloid deposition in the form of neuritic plaques and congophilic angiopathy is driven by the conversion of normal soluble amyloid-beta peptide (sA beta) to A beta plaques; while in prion disease the critical event is the conversion of normal prion protein, PrP^{c}, to the disease-associated form, PrP^{Sc}. Because of this similarity between AD and prion diseases, similar therapeutic strategies are being tried for both diseases. Both active and passive immunization were proved effective in transgenic mouse models of AD [55, 56, 57] and the concept was also extended to prion diseases.

The effectiveness of monoclonal antibodies (mAb) to inhibit prion replication in cell culture models with clearing of prion infectivity systems was well demonstrated [58, 59] and the real challenge of the in vivo vaccination approach for the prion disease lay in breaking the natural immune tolerance towards PrP^{Sc}. This was achieved by immunizing CD-1 mice with recombinant murine PrP and Freund adjuvant [60]). The vaccine delayed the onset of the disease if use both as a preventive measure and as a rescue treatment when serial vaccinations were started after peripheral prion exposure. No toxicity or autoimmune reaction was observed in these experiments. The delay in onset of neurological symptoms in scrapie infected mice strictly correlated with the anti-PrP antibody titres that developed in the vaccinated animals [60, 61] suggesting that a humoral response is of great importance for delaying full blown disease symptoms. This was further confirmed by passive immunization experiment [62]. CD-1 mice were infected intraperitoneally with prion extract and furthermore receive weekly 50μg doses of anti-PrP antibodies. These mice demonstrated significant delay in the onset of disease symptoms compared to mice that receive doses of murine IgG.

The difference in the effectiveness of various antibody clones was also observed. MAb 8B4, raised against prion protein residues 34-52 of PrP, seemed to be more effective at the same dose than mAb 8H4 (against residues 175-185), whereas mAb 8F9 was the least effective. Ten percent of infected animals treated with mAb 8B4 did not develop signs of disease within 300 days of observation, whereas non treated animals displayed symptoms of the disease 150 days post inoculation (dpi) and were dying between 210-220 dpi. White et al. [63] who experimented with various protocols of the passive immunization demonstrated that this approach could be successful in mice if applied within 30 days after intraperitoneal inoculation. There was no benefit with passive immunization if it was started after the onset of neurological symptoms or in mice inoculated intracerebrally with prion extract, as the peripherally administered antibodies have virtually no penetrance through the blood-brain barrier. By increasing the dose of mAb to 2mg twice a week White et al. [63] were able to prevent neurological symptoms in all treated animals during 500 days of observation. Two mAb clones were used in this study: ICSM 18 (146-159 PrP residues) and ICSM 35 (91-110 PrP residues). Although both clones were equally effective in terms of preventive clinical symptoms, trace amounts of PrP^{Sc} in the spleen of ICSM 18 treated mice was detected, but not in ICSM 35 treated mice. This was consistent with previous observations that some mAb clones are superior to others for preventing prion replication, with those raised against the N-terminus of PrP molecule being the most successful. Approaches currently under development include mapping a portion of PrP^{Sc}, which is the most crucial for its replication using various mAb clones.

Promising results of these in vivo studies have produced a great deal of hope that successful treatment for human prion disease may be within reach. Various protocols could be considered. For example, carriers of the PrP gene mutation (PRNP), which may require life long protection from PrP^{Sc} seem to be good candidates for active immunization approaches. Similarly, active immunization may be offered to people living in areas with high incidence of animal prion disease, which are transmissible to humans. This would include bovine spongiform encephalopathy, and possibly to chronic wasting disease of deer and elk [64, 65] Because these infections occurs through an oral route a mucosal vaccine can be designed to raise humoral immunity neutralizing prion inoculum within the alimentary tract thus preventing it from entering into the circulation [61, 66].

Although, no side effects of active immunization in animals have been noticed recent experience in a human AD vaccine trial indicated the need for extensive safety tests prior to clinical trials [67]. A key issue will be to limit the cytolytic T-cell response, which has been associated with potential toxicity in the form of encephalitis in the AD trials, while maintaining the maximal humoral immune response necessary for the vaccine to work. On the other hand, passive immunization approach seems to be a logic application for subjects who have been accidentally exposed to prions disease. Although it would potentially involve multiple infusions of monoclonal antibodies, this approach is toxicity related to cell medicated immunity [68]. Using non-human primate models of prion disease, an optimal clinical protocol has to be develop for mAb dosing, timing of treatment and selection of appropriate humanized mAb clones for the prevention of prion replication and clearing the immune system of PrP^{Sc}. Evaluation of remission can be achieved through lymphatic tissue biopsy or through imaging techniques utilizing ligands targeting the β-sheet rich structure of PrP^{Sc}. Such approaches have been proven successful for the detection of amyloid-β plaques in AD transgenic mice [69, 70], and similar ligands for prions are being developed [71].

A recent report from the laboratory of Adriano Aguzzi [72] has shown the great potential that a immunological approach has for preventing prion disease. In this report

transgenic mice were developed that express a gamma immunoglobulin Fc fragment fused to two PrP molecules. In wild-type mice infected with mouse scrapie, the expression of this fusion protein (PrP-Fc(2)) delayed PrP^{Sc} accumulation, agent replication, and onset of disease. This suggests that enhancing the immune systems response to changes in PrP will slow down or stop disease progression. Furthermore, mice expressing PrP-Fc(2) but lacking endogenous PrP^{c} were resistant to scrapie. This again suggests that activation of the immune system might help to prevent the conversion process in prion disease. There are no immediate diagnostic or therapeutic benefits from this work as, like many studies based on experimental mice, the same system cannot be replicated in humans and use of this system would require diagnosis of prion disease before onset of the irreversible symptoms. However, it does suggest that enhanced activation of the bodies own defences might be beneficial in treating these diseases.

PHARMACOTHERAPEUTICS

There are also, at present, no effective pharmacotherapeutic strategies against prion disease. One reason for this is that the pre-clinical diagnosis of these diseases is still inadequate and at present any treatment would only be given to a patient at an advanced stage of disease, thus proving ineffective. Effective drug treatments would have to halt neurodegeneration at a much early stage. Nevertheless research into a number of potentially interesting compounds is progressing even though they mostly have limited use once the disease has spread to the CNS. The potential effectiveness of these drugs and the mechanism of their action can be related to a great deal of work which has been done on understanding the neurodegenerative mechanisms in prion disease, both in vitro and in vivo.

There is much evidence to suggest that the accumulation of PrP^{Sc} is not the only cause of neuronal degeneration and death in prion disease. One additional factor acting in this context is oxidative stress [73]. Oxidative stress accompanies the changes in the nervous system. A recent report has suggested from a single case that the use of antioxidant therapy slowed disease progress [74]. However, the patient eventually died. Therefore, although promising use of antioxidants needs further investigation at a more basic level. Numerous groups have demonstrated that the synthetic peptide PrP106-126 causes oxidative stress in vitro, disturbs the expression of antioxidant proteins and that the toxicity of PrP106-126 can be inhibited by antioxidants [17]. Cultures infected with PrP^{Sc} are more susceptible to the toxicity of reactive oxygen species [75] and oxidative damage is involved in the mechanism by which PrP106-126 induces apoptotic cell death in a culture system. Microglial activation and the trauma caused by either the presence of PrP^{Sc} in the brain or the degeneration of the brain tissue are sufficient to cause increases in oxidative stress and it follows that there are significant changes in the antioxidant pathways with the onset of disease. Furthermore, it was shown recently that PrP^{c} has superoxide dismutase (SOD) activity [27] providing further evidence that variations in antioxidants occur in the disease.

Apoptosis has been described in several in vivo prion disease models [76, 77] and has also been observed in cell cultures treated with neurotoxic prion fragments, such as PrP106-126 [78]. Brown et al. [17] proposed that the neurotoxic mechanism of PrP106-126 is rather complex involving a direct and indirect component, and that this can be used as an accurate model of what happens in vivo during prion infection. The direct component is the necessity for neurones to become sensitive to oxidative stress (for example when the activity of PrP^{c} is compromised) and the indirect component involves an increased production of reactive oxygen species. The source of reactive oxygen species can be microglial cells, although microglia per se are not essential for the mechanism to act as long as some alternative source of oxidative stress exists. Microglial cells are activated by the presence of either PrP106-126 or PrP^{Sc}. This finding has been confirmed by a number of groups [17, 18, 79, 80]. Activated microglia release cytotoxic substances such as superoxide and other reactive oxygen species or tumour necrosis factor (TNF-α). Many experiments from different laboratories have shown that they enhance the toxicity of PrP^{Sc} [18] and that they are activated before the onset of neurodegeneration in mouse scrapie [18, 81]. The effect is reproducible and confirms that an indirect influence of substances released by microglia contributes to the neurotoxic mechanism in prion disease.

Studies with antioxidants in cell culture systems have indicated that they are able to block neuronal loss [17]. Thus treating prion infected individuals with antioxidants might prove effective. Levels of antioxidant molecules in the blood and cerebrospinal fluid (CSF) of humans and animals with prion diseases are increased at a pre-clinical stage and might indicate the protective nature of these molecules. Either exogenous application of antioxidants or upregulation of their endogenous expression might prove effective in halting neurodegeneration.

OTHER TREATMENTS

Trials using the fungicide Amphoterecin B and its less toxic derivative MS-8209, have shown that the incubation period in hamster scrapie models can be prolonged [82, 83, 84]. Treatment of mice with pentosan polysulphate enables them to survive lethal doses of infectious prion material. The treatment is effective even when administered several weeks after parenteral infection, although it is no longer effective once the disease has reached the CNS [85]. Cyclic tetrapyrroles such as porphyrins and phthalocyanins have been shown to inhibit the PrP^{Sc} formation [86]. They were also recently shown to prolong the survival of rodent scrapie models if treatment is given simultaneously with infection [87]. IDX is an anthracycline, which has also been found to prolong the survival of prion infected Syrian hamsters. This compound must be given intracerebrally as it does not cross the blood-brain barrier [88]. The anti-inflammatory agent dapsone has also been investigated in rat CJD models but its effectivenessis still questionable due to conflicting results [89. 90]. Acridine and phenothiazine derivatives are currently believed to be the most likely candidates for intervention therapy in CJD cases. The combined use of the antimalarial drug, quinacrine with the antipsychotic, chlorpromazine, was shown to inhibit the conversion of PrP^{c} to PrP^{Sc} in cell culture systems [13]. Although no data has been published on the use of these drug combinations in animal models,

they are already licensed for human use because they are able to cross the blood-brain barrier. Indeed, quinacrine has been tested in humans with rather tragic consequences. Although, it has been shown to inhibit the toxic effect of a prion peptide [91], quinacrine is toxic to the liver and therefore is only of value at present as a lead compound.

CONCLUSION

There are currently no treatments for prion disease. Despite this here are many avenues opening up for further investigation. Some compounds are clearly effective in animal models and an immunological approach might provide insight into new ways to treat these diseases. Additionally, antioxidants are possible new direction yet to be explored. Regardless of the possibilities discussed as far as treatments, the real important step forward in therapeutics for prion diseases will depend on the development of diagnostic tools to identify individuals harbouring the disease before the onset of symptoms such as neurodegeneration which are irreversible.

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