

Review Article

Therapies for human prion diseases

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Abstract: The pathological foundation of human prion diseases is a result of the conversion of the physiological form of prion protein (PrP^c) to the pathological protease resistance form PrP^{res}. Most patients with prion disease have unknown reasons for this conversion and the subsequent development of a devastating neurodegenerative disorder. The conversion of PrP^c to PrP^{res}, with resultant propagation and accumulation results in neuronal death and amyloidogenesis. However, with increasing understanding of neurodegenerative processes it appears that protein-misfolding and subsequent propagation of these rouge proteins, is a generic phenomenon shared with diseases caused by tau, α -synucleins and β -amyloid proteins. Consequently, effective anti-prion agents may have wider implications. A number of therapeutic approaches include polyanionic, polycyclic drugs such as pentosan polysulfate (PPS), which prevent the conversion of PrP^c to PrP^{res} and might also sequester and down-regulate PrP^{res}. Polyanionic compounds might also help to clear PrP^{res}. Treatments aimed at the laminin receptor, which is an important accessory molecule in the conversion of PrP^c to PrP^{res} – neuroprotection, immunotherapy, siRNA and antisense approaches have provided some experimental promise.

Keywords: Prion diseases, Creutzfeldt-Jakob disease, treatments, neurodegenerative diseases, protein misfolding, protein propagation

Introduction

Prion disease is thought to arise from the transformation of normal host-encoded prion proteins (PrP^c) to aberrantly folded protease resistant isoforms (PrP^{res}) [1, 2] (**Figure 1**). This abnormal prion propagation and accumulation throughout the central and peripheral nervous system results in an ultimately fatal neurodegenerative disease process characterised by rapidly progressive dementia [3]. Exposure to exogenous PrP^{res} through contaminated biomaterials, the food chain (nv CJD) and mutations in the PrP gene have been identified as causative agents [4]. However, the aetiology of the majority of prion diseases in neurological practice is tragically sporadic with no cause ever identified. This devastating clinical scenario signals the need for the development of therapeutic strategies focused upon symptomatic management, prolongation of survival and ultimately reversal of neurodegenerative processes. This article reviews current therapeutic strategies and recent advances in the treatment of prion diseases.

Therapeutic possibilities

The mechanisms involved in prion pathogenesis remain enigmatic [5] which has unfortunately hindered the development of effective therapeutic agents [2]. However it is evident that prions do not require nucleic acids or other cofactors to transmit disease [6, 7] and clinical manifestations cannot ensue without the initial presence of PrP^c [8]. However, the importance of understanding the role of PrP^c transformation and accumulation is not only relevant to prion-mediated neurodegeneration.

Neurodegeneration caused by templated conformational change of proteins appears to be a generic mechanism of pathogenesis [9, 10]. Several *in vivo* and *in vitro* findings have confirmed the role of mutant tau, amyloid- β and α -synuclein in the development of regional pathology and disease progression in Alzheimer's disease, frontotemporal dementias and Parkinson's disease [11, 12]. The simultaneous existence of cellular dysfunction together with rouge protein propagation and accumulation

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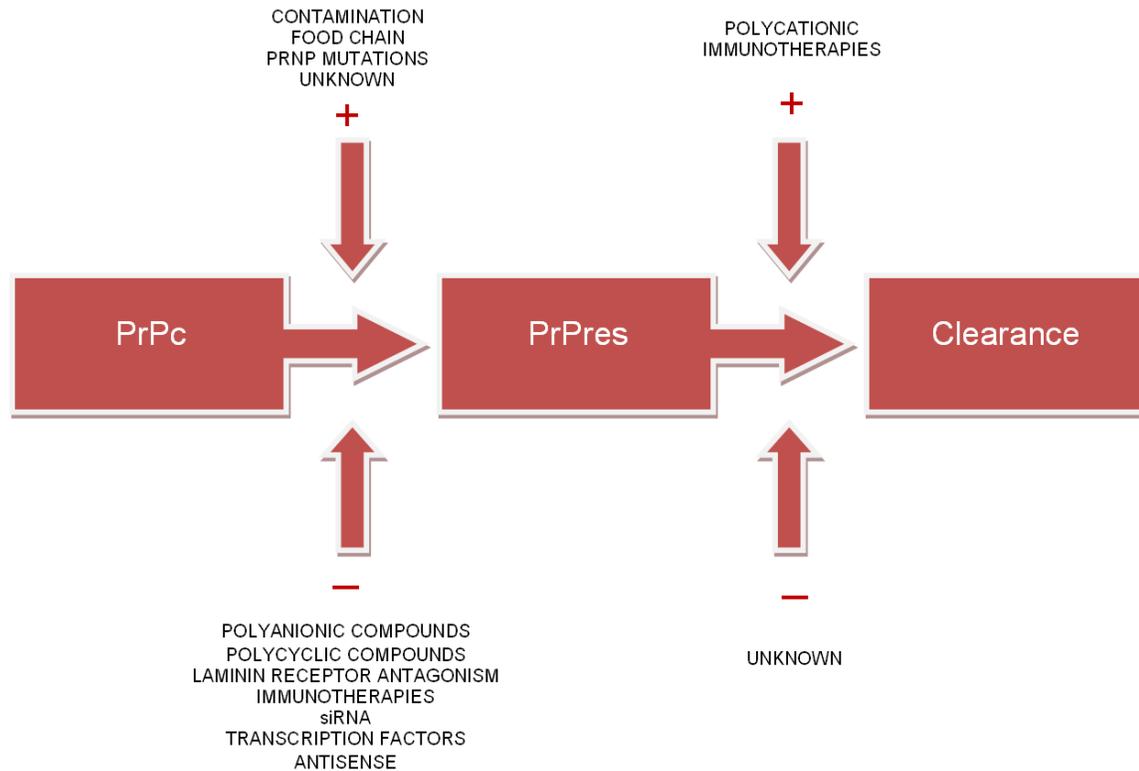


Figure 1. Therapeutic approaches to human prion diseases.

are a feature of these disease patterns though do not equally impinge on the rate of neuronal death [9]. While transmissibility seems to be an exclusive feature of prion diseases [10], the generic process of regional neuronal destruction might mean that developing a therapeutic agent for prion disease may have wider-implications for other diseases [13].

The current foundations of therapeutic efforts centre upon the assumption that disease processes stem from the transformation of PrP^c to PrP^{res} and subsequent accumulation of this protease-resistant isomer [6]. Direct inhibition of this conversion, degradation of PrP^{res}, interference with important accessory molecules (Fab, glycosaminoglycans) or altering PrP^c expression and/or cell surface localisation remain important target strategies [5, 14-16].

Laminin, a high affinity prion receptor (LPR/LR) may be a suitable medication target. Interestingly, it seems that LPR/LR acts as a receptor for both the cellular prion protein and the infectious PrP^{res} [17] and might further play an essential role in PrP^{res} binding and intracel-

lular internalisation. Indeed it has been observed that siRNA against LRP mRNA inhibits PrP^{res} accumulation in infected N2a neuroblastoma cells [18]. Resultantly, ligands targeting this receptor must be explored. Additional investigation into the contribution of lipid rafts, sphingolipid rich molecular carriages and the location of PrP^c transformation present further therapeutic options.

Additional findings demonstrate the transcriptional activation of PrP^c may result from the direct binding of p53 to the suspected promoter region [8]. The generation of A β oligomers via γ -secretase action in turn, activates p53. Understanding this complex interaction suggests the use of γ -secretase inhibitors as a strategy to not only modulate PrP^c transcription, but also PrP^{res} accumulation [8]. In fact Spilman et al [19] demonstrate a reduction of neocortical and hippocampal PrP^{res} accumulation in mice with the oral administration of γ -secretase inhibitors and quinacrine. However, the sole use of γ -secretase inhibitors in this study did not realise any therapeutic or pathological benefit.

Several studies have illustrated the role of tau proteins in exacerbating β -amyloid related cytotoxicity and neurodegeneration in dementia [20, 21]. In fact, Nussbaum and colleagues [21] demonstrate that knocking out tau provided complete protection against neuronal loss and glial activation from toxic A β species in mice. Subsequently, these findings have provoked interest into the relevance of tau in prion and other neurodegenerative disease processes. Findings of *in vivo* and *in vitro* studies suggest that β -amyloid interacts with PrP^{sc} [22]; resulting in kinase activation and tau hyperphosphorylation [23]. More recently Chen et al [24] highlighted this interaction, demonstrating that PrP^{sc} over expression down-regulated tau protein at the transcriptional level whilst A β oligomer binding alleviated the induced tau reduction. Furthermore, subsequent treatment with Fyn pathway inhibitors reversed the PrP^{sc}-induced tau reduction; suggesting that the Fyn pathway may regulate A β -PrP^{sc}-tau signalling. Increases in total tau protein levels have been observed in advanced prion disease and were found to be a significantly superior disease marker when compared to the 14-3-3 protein in sporadic Creutzfeldt-Jakob Disease (CJD) [25].

Recent evidence also places focus upon α -synuclein; a major component of filamentous inclusions defining a large group of neurodegenerative diseases including Parkinson's disease, Lewy body dementia and multiple-system atrophy [12]. Lesions produced by α -synuclein spread progressively throughout the brain, correlating closely with clinical deficits experienced. Findings from several studies suggest that pathological α -synuclein may be transmissible [12, 26]; demonstrating that the introduction of exogenous α -synuclein fibrils induces Lewy-body pathology in cultured neurons [27]. More recently, Masuda-Suzukake and colleagues [12] demonstrated that intracerebral injections of sarkosyl-insoluble α -synuclein from brains of patients with Lewy body dementia or recombinant α -synuclein from wild-type mice induce hyperphosphorylated α -synuclein pathology *in vivo*. Further biochemical analysis revealed the prion-like transformation of α -synuclein, with subsequent deposition and propagation throughout neuronal tissue [12].

Aside from medications, other theoretical possibilities for the treatment of prion diseases exist. There has been some experimental data

to suggest the use of monoclonal antibodies against PrP for prophylaxis [5]. However, the *in vivo* stimulation of immune competent cells which recognise and neutralise abnormally folded prion isoforms needs to be investigated. Delivery systems using lentiviruses or adenoviruses with anti-prion components including antibodies, short-interfering micro RNAs or Antisense RNAs remain considerations.

Therapeutic challenges

Investigating therapeutic possibilities for rare diseases can often provide valuable insights for more common conditions [13]. Neurodegeneration caused by the underlying mechanism of protein misfolding seems to be a truly generic phenomenon [9] and therefore identification of an anti-prion agent may have wider therapeutic applications and consequences. However, therapeutic progress is hampered by the scarcity of natural history and clinical progression data in addition to the lack of validated outcome measures [6, 13]. Complicated with the heterogeneity of disease manifestation and the rapidity of deterioration experienced by patients, developing clinically and ethically adequate trial designs is challenging [13].

Early spongiform change and synapse loss often remains subclinical [28]. While the characteristics of a lesion resulting from α -synuclein and tau propagation often correlate well with clinical progression [12] the extent of prion-induced neuronal death may be difficult to detect clinically early. Subtle motor dysfunction leading to a patient's initial presentation may result from quite advanced and irreversible neuronal cell death [28, 29]. Coupled with a typically lengthy diagnostic and investigative period, the trajectory to irreversible neurodegeneration makes early treatment problematic. While current treatment options cannot provide reversal of symptoms or significant prolongation of survival, it is difficult to decipher whether these options are truly ineffective or are simply administered too late in the clinical course to offer any real benefit [28].

Establishing an accurate and early diagnosis may therefore be the key to therapeutic success and imperative to the continuation of research progression [13]. An accurate diagnosis is difficult to defend in the absence of acceptable criteria; and therefore, any research

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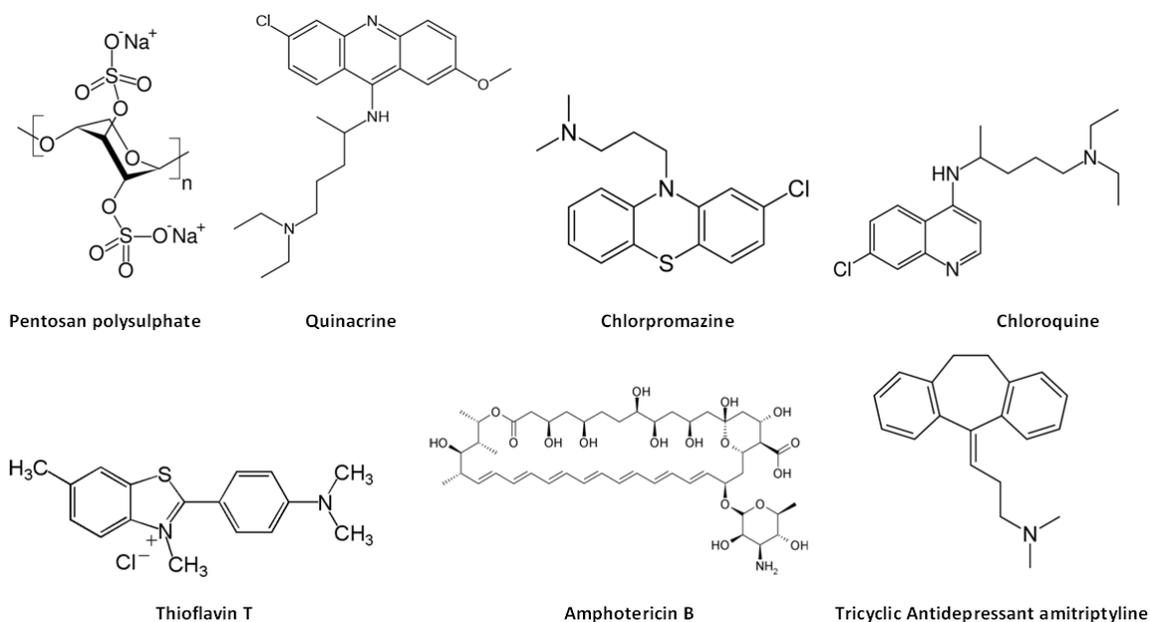


Figure 2. Structure activity relationship of compounds used to treat human prion diseases: some of these agents share cyclic benzene ring structures with aliphatic side chains at middle ring moieties.

findings necessitate additional scrutiny when prion propagation may not be the cause of a patient's neurodisability. The rapid progression of neurological deficits characteristic of prion disease places survival as the key outcome measure in current therapeutic trials [13]. However, this does not factor the molecular variability of differing subtypes of prion diseases [29], the clinical progression of the disease nor has the scope to capture the profound physical and cognitive impairments that many patients face [13, 29].

Prophylactic treatment may be indicated in individuals susceptible to developing genetic prion disease. This subgroup could provide a rare opportunity to investigate the potential benefits of agents long before neuronal death has occurred whilst also offering the insight of longitudinal follow-up.

Therapeutic experiences

Pentosan polysulfate

Pentosan polysulfate (PPS) is a large polyglycoside molecule, demonstrating weak heparin-like activity [30] (Figure 2). PPS is presumed to act in competition with endogenous heparin sulphate proteoglycans as co-receptors for PrP on the cell surface [31]. When directly adminis-

tered into the cerebral ventricles of PrP^{res} infected mice, PPS significantly prolongs the incubation period of early disease processes and inhibits the formation of PrP^{res} in cell culture. Albeit promising, findings to date fail to illustrate any reversal of clinical manifestations or recovery of pre-existing neurological deficits in patients.

Administration of continuous intraventricular PSS infusion (1-120 μ /kg/day) was commenced in a 68 year old patient with sCJD 10 months following first symptomatic onset [32]. While the patient's existing deficits did not improve, there was a noted stabilization in her neurological deterioration for four months (2 to 8 months following PSS infusion) and her survival of a total 27 months surpassed mean survival periods reported elsewhere in the literature. Parry et al [33] also described prolonged survival of 51 months in a 22 year-old male with vCJD using continuous intraventricular PPS at a dose of 32 mg/kg/day for 31 months.

Furthermore, a prospective study conducted by Tsuboi et al [30] showed a prolonged, mean survival time of 24.2 months in four patients following intraventricular PSS infusion (120 μ /kg/day). One patient is still alive six years after continuous infusion, though no major neurological improvement has been reported. Indeed

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given this extraordinary prolongation of life, the likelihood of prion pathology must be questioned.

The development of subdural effusions of variable degrees and intraventricular hematomas were common adverse effects [30, 32].

Quinacrine

Quinacrine is an acridine derivative which has shown success in cellular models. Of interest, the drug has been shown to inhibit PrP^{res} formation in scrapie-infected neuroblastoma cells [34]; a function which is thought to arise from the inclusion of a nitrogen side chain to its quinoline ring [35]. Due to its use as an anti malarial drug, immediate trials in humans have been advocated [36] though *in vivo* findings are yet to identify any benefit to quinacrine treatment.

Furukawa et al [37] demonstrated a transient response in visual and auditory hallucinations in a small number of patients. Recovery of voluntary limb and pursuit eye movements were reported in a 37 year old female with CJD (secondary to a cadaveric dura matter graft) following administration of 300 mgs of quinacrine per day. The periodic sharp waves resolved. However, just two weeks after the onset of therapy the patient deteriorated and positive sharp waves reappeared [38].

A further four patients with sporadic CJD received 300 mgs of quinacrine; akinetic mutism improved within two weeks for one patient. The remaining patients were deemed "insensible" before treatment, though showed improvements in their interactive responses with eye contact, voluntary movement, and responses to verbal and visual stimuli after 1-2 months. Follow-up beyond two months was not provided [39]. Conversely a study conducted one year later revealed a slight but non-significant improvement with quinacrine use in 32 patients with sporadic CJD. No pathological evidence of benefit was observed [40].

Collinge and colleagues [41] illustrate that while 300 mgs per day of quinacrine was tolerated; it was not an effective therapeutic agent and did not influence the natural history of the disease. In fact, of the 107 participants with CJD included in this study only 26 survived and were suitable to be treated with quinacrine. The

adjusted mortality was lower for the quinacrine treated group, but confounded by disease severity. After adjustment there was no difference. Four patients had a transient improvement in neurological scales; two had serious adverse side effects.

The therapeutic scope of quinacrine has possibly been hindered by a focus upon mono therapeutic approaches [42]. Synergistic anti-prion therapy has been trialled with Chlorpromazine, a phospholipase A₂ inhibitor, which reduces inflammatory oxidative stress and may ease neurodegenerative processes [43]. Benito-Leon [44] treated two familial insomnia patients with quinacrine and chlorpromazine without benefit. In fact, the condition of the patients worsened. The same treatment course was trialled in a 54-year-old woman with a Heidenhain variant of prion disease and conversely, the patient remained stable and survived an additional 19 months [45]. Clinical improvement following six month treatment with quinacrine and chlorpromazine was also reported in a 46-year-old woman with iatrogenic CJD [46].

In vitro demonstration of cholesterol biosynthesis up-regulation following prion infection in various neuronal cells [47] has highlighted a possible association between prion infection and/or susceptibility with altered cholesterol homeostasis. Founded upon this notion, Orru and colleagues [48] demonstrated that the addition of cholesterol ester modulators (progesterone, pioglitazone, Verapamil and everolimus) to quinacrine and chlorpromazine, enhancing their anti-prion effects by reducing their EC₅₀ 10-fold. While the exact mechanism is poorly understood, such results warrant further investigation.

Thioflavine

Thioflavine is used in tissue stains as a means of identifying amyloidogenic proteins and is characterised by positive birefringence. Thioflavine and related chemicals can inhibit PrP^{res} production [49]. Thioflavine has also been shown *in vitro* to inhibit PrP^{res} formation along with A β 1-42 [50]. Thioflavine has also been shown to interfere with PrP185-288 aggregation and diminishing fibril assembly [51]. There is no human data on the effects of thioflavine in prion diseases.

Amphotericin B

In 1987 it was shown that amphotericin B, a macrolide polyene antibiotic, considerably delayed the incubation period in scrapie inoculated hamsters [52]. In 1992, Xi et al [53] showed that scrapie infected hamsters given amphotericin B had a delayed accumulation of PrP^{res} by 30 days, without affecting scrapie replication. The hamsters treated with amphotericin B developed prion disease later than those untreated. Demaimay et al [54] showed that late treatment with amphotericin B prolonged the viable time of scrapie infected mice – between 80 and 140 days post inoculation with reduction in the accumulation of PrP^{res}.

A synthetic derivative of amphotericin B was shown to prevent replication of the scrapie agent at the inoculation site. This site contained astrocytes, where the abnormal PrP was produced. Grigoriev et al [55] speculated that the lysosomal system could be a target of amphotericin B. *In vitro* studies have also shown that amphotericin B prevents the conversion of PrP^c [alpha helical] to PrP^{res} (pathological beta sheet formation) [56].

In 1992, two patients with CJD were unsuccessfully treated with amphotericin B. One, a 71-year-old-woman, was hospitalised 3 months after the onset of dementia. The amphotericin B was given by intravenous infusion, starting 5 days after admission with a dosage of 0.25-1 mg/g. The amphotericin B was administered 6 days a week and the maximum dose reached in 8 days. After 20 days of treatment the patient's clinical situation worsened and she died. The second patient was a 50-year-old woman who was seen 5 months after the onset of CJD at which time she was bed-ridden with akinetic mutism and myoclonic jerking. The EEG revealed periodic triphasic complexes. Amphotericin B was started one week after admission and at the doses described above, with a maximum dosage achieved in 8 days. The treatment was continued 6 days a week by slow intravenous infusion. The patient's condition deteriorated and she died 8 months after the onset of the disease. Amphotericin B had no neurological effects on both of these patients and did not prevent progression [57].

Tetracyclines

Tetracyclines interact with PrP^{sc}, destabilizing the structure of amyloid fibrils and facilitating

the proteinase K digestion of PrP peptides [58, 59]. Tetracyclines appear to not only bind to PrP aggregates, but to other neurotoxic peptides inhibiting nerve and glial cell toxicity in addition to astroglial proliferation [58, 59]. De Luigi and colleagues [42] conducted a series of landmark experiments to not only highlight the efficacy of tetracyclines in early disease; but to assess therapeutic benefit when administered in latent diseases stages. In the first experiment, Syrian hamsters were peripherally infected with a 263K scrapie inocula intramuscularly and received a single dose of 10 mg/kg of Doxycycline at the same infection site within one hour. This single dose significantly ($p=0.031$) increased the median survival by 64% (from 217 days in controls vs 355 days for treated). A single intracerebroventricular infusion of 25 µg / 20 µl of Doxycycline or Minocycline entrapped in liposomes, was administered 60 days following inoculation when 50% of animals showed initial symptoms of the disease. Even at this advanced stage of neurodegeneration, survival rates increased by 8.1% and 10% respectively.

While these results stem from observational data, it is clear that both compounds significantly delayed the onset of clinical signs and prolonged survival [58]; warranting further investigation. Furthermore, it is suggested that tetracyclines inhibit the pathogenic PrP misfolding and thus, reduces the infectivity of the propagating prion. The implications of these findings also suggest that this approach may extend to other neurodegenerative processes reliant on rogue protein misfolding [58]. The long-standing safety profile and success of tetracycline use within other areas of clinical practice further render this therapeutic approach as a hopeful candidate in future human-trials.

Tricyclic antidepressants

Tricyclic antidepressants, like Desipramine, are heterocyclic compounds **Figure 2** which have been shown to abolish prion infectivity in cell culture [60] The anti-prion effect was related to the redistribution of cholesterol from the plasma membrane to the intracellular compartment. This leads to membrane destabilization. In this study there were ultra-structural changes in the endosomal compartment. When these authors synthesized a novel compound of quin-

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acrine and desipramine (quinapyramine) it had a synergistic effect in preventing prion infectivity. Of interest, the anti-prion effects of desipramine, quinacrine, quinapyramine were synergistic with simvastatin, a HMG CoA reductase inhibitor.

There is no human data on the effects of tricyclic antidepressants on prion diseases. Caution must be exercised in the use of tricyclic antidepressants as a reversible toxic neurological syndrome resembling prion diseases has been described [61].

The study of Klingenstein et al [60] raise the possibility that future treatments of prion disease might involve a combination of drugs including a tricyclic, an acridine derivative like quinacrine, and a statin.

Lithium chloride

Used primarily to treat mania or bipolar affective disorder, lithium chloride has been found to induce autophagy, enhancing clearance of mutant huntingtin and α -synucleins [1]. Application of this principle to prion-infected neuronal and non-neuronal cells, demonstrated an intense reduction in PrP^{Sc} accumulation as a result of lithium induced autophagy. When combined with Rapamycin, the anti-prion effects of lithium chloride were further enhanced [1]. This synergistic combination was also observed in a Huntington's disease fly model [62]. Further testing is required, but such findings further highlight the possible generic phenomenon of protein-based neurodegeneration.

Immunotherapies

In 2004 it was shown that humoral immune responses to native eukaryotic prion protein correlate with anti-prion protection [63]. The difficulty with immunological approaches to prion therapy is overcoming self tolerance to the physiological prion PrP^C. *In vitro* and *in vivo* experiments have shown that antibodies to PrP^C decrease or prevent the transformation to PrP^{Res} [64, 65]. Active and passive immunisation might stimulate antibody induced phagocytosis, antibody disruption of peptide aggregates, mobilisation of toxic soluble peptides, stimulation of cell mediated immunity and other mechanisms [66].

Monovalent antibody fragments to various parts of the prion protein have been shown *in vitro* to prevent the oligomerization of the prion protein. These regions are: codons 90-110, the helix region codons 145-160, the extreme C-terminal codons 210-220 and the octarepeat region [67]. Sakaguchi and Arakawa [68] suggested that tolerance could be overcome by immunizing the PrP fused to bacterial enterotoxins or delivered using an attenuated *Salmonella* strain.

Campana et al [69] showed that brain delivery of prion specific proteins using single chain variable fragments with adeno-associated virus transfer delayed the onset of prion disease in infected mice. The single chain variable fragment of the PrP complex including Arg (PrP 151) linked to the adeno-associated virus was the key residue anchoring PrP^C to the cavity of the antibody. Federoff [70] postulated that active immunisation can disrupt A β 1-42 formation in mice and passive immunisation of a recombinant adeno virus anti-prion can attenuate disease progression and prolong life in experimental models of prion diseases. This mounting body of experimental evidence will in the future lead to immunological trials of both active and passive immunisation in prion diseases as is being performed in Alzheimer's disease. Full length antibodies do not cross the blood brain barrier.

Other possible therapies

Bellingham et al [71] have shown that prion gene expression is regulated by transcription factors SP1 and a metal transcription factor-1. The Menkes protein is a major copper efflux protein. Fibroblasts that have the Menkes deletion have an increase in copper levels. If the cells have Menkes protein over-expression, then copper is reduced. In the cell lines with low copper, cellular prion protein and mRNA level is reduced. siRNA knockouts of SP1 and MTF1 reduced prion PrP protein and gene expression *in vitro* suggesting that these transcription factors might be future targets for prion therapy.

The LRP/LR laminin receptor is a 37 kDa / 67 kDa that is important in cell adhesion, apoptosis, virus binding and prion binding function. This multi-faceted and multi-functional protein is found at the cell membrane, cytoplasm and

nucleus. *In vitro* experiments blocking the LRP/LR receptor with antibodies, or siRNA agonists to LRP mRNA or LRP decoy mutants block or downregulate the receptor and reduce prion infectivity. Of interest, PPS and other heparin-like moieties bind to this receptor [72].

Lentiviral vectors expressing small interfering siRNAs directed against the laminin receptor precursor mRNA prolonged the preclinical phase of scrapie infected mice [73]. Stereotactic intracerebral micro injection into the hippocampus with recombinant lentiviral vectors expressing siRNA to LRP7 and 9 were effective in prolonging the pre-clinical phase and modulating clinical disease in scrapie infected mice.

More experimental work on the exploration of transcription factors and the laminin receptor is required to justify human studies.

Superoxide dismutase (SOD) is a free radical scavenger and recent experimental studies in a rodent model, using an agent with similar actions to SOD, extended the life of scrapie infected animals raising the possibility of a free radical and oxidative stress attack on prion diseases [74].

Conclusion

There are difficulties in developing new treatments for prion diseases. Double-blind randomised controlled trials with adequate power seem impossible. Current therapeutic experiences are based on small numbers of patients and having control subjects raise ethical difficulties in a disorder with a poor prognosis. Short survival time and variable natural history are an added difficulty for therapeutic studies. However, these limitations should not discourage the search for prion disease treatments.

Recent findings suggest a common thread of neurodegenerative pathogenesis involving misfolding of prion, tau, α -synuclein and β -amyloid proteins which, with increasing comprehension of these processes, might lead to generalised therapeutic modalities.

There is a need for prion disease biomarkers which might best indicate clinical responses to treatment. Predictive gene testing is possible and families with pre-manifest prion disease exist. Potential treatments are a possibility for

these individuals, as currently conducted for inherited Alzheimer's disease.

These limitations suggest that in the future an international collaboration might be necessary to pool therapeutic experiences and coordinate therapeutic studies similar to study groups in other disorders with small numbers of patients like the Huntington's Study Group (HSG): a Prion Diseases Study Group (PSG)?

Disclosure of conflict of interest

None.

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