The search for a cure

Prion eliminating compounds

Lee J. Byrne and Mick F. Tuite
(University of Kent, UK)

Prions are remarkable infectious agents that cause fatal neurodegenerative diseases in humans and other mammals. There is increasing activity, but, as yet, limited success, in the search for therapeutic compounds that can be used to treat these diseases. This is, in part, because a number of significant problems exist, hampering the route to rational drug screening strategies.

CJD (Creutzfeldt–Jakob disease) and its BSE (bovine spongiform encephalopathy)-related form, variant CJD (vCJD), are high profile and invariably fatal human prion diseases.

Yet in spite of the relative rarity of these awful neurodegenerative diseases in the human population, there is considerable clinical and public interest in the discovery of chemical agents that can be used to slow down, or even halt, the progress of these diseases. There are, however, a number of significant problems that hamper the anti-prion drug-discovery process:

- The nature of the infectious agent, the prion, is not fully established, other than that it is associated with a conformationally altered form of a cellular protein (PrP).
- For a compound to be effective, it must be able to cross the blood–brain barrier to reach the brain.
- The mechanism of prion-mediated pathogenesis is poorly defined; for example, we do not know what causes the neurons to die in CJD/vCJD patients.
- The disease is only evident after the onset of severe clinical symptoms, by which time major neurological damage will have occurred and will perhaps be beyond repair.
- The number of patients that can take part in clinical trials is very limited; at the time of writing (March 2005) there are only five living vCJD sufferers in the UK[1].

Key words: amyloids, prion diseases, PrP, self-propagating prions, vCJD.

Perhaps consequently, no therapeutic compounds with clinically proven efficacy against CJD/vCJD have yet to enter the clinic, although the last four years has seen the emergence of several candidate compounds that show promise[2]. Only in the case of one such compound, quinacrine, has a randomized controlled human trial been set up in the UK[3].

In this article we will consider what prion-eliminating therapeutic compounds have been uncovered to date, either by rational or empirical approaches, and consider how new anti-prion compounds can be best identified and validated. We will also discuss how recent studies on chemically induced elimination of fungal prions can contribute to this drug-discovery process.

Identifying therapeutic targets for intervention

Most modern drug-discovery programmes start by identifying specific cellular processes or molecular targets against which inhibitors can be sought. Prion diseases are characterized by an accumulation of PrP^{Sc}, the protease-resistant conformer of PrP, and since the accumulation of PrP^{Sc} is associated with pathogenesis, the most logical target to focus on initially is the conversion of PrP into PrP^{Sc}, i.e. replication of the infectious prion. The disaggregation of the characteristic amyloid deposits (Figure 1), which most likely represent 'dead-end' products of the prion replication cycle, may also constitute a valid target, since they may contribute to the neurodegenerative process either directly or indirectly (although they may not themselves be infectious).

PrP^{C}, the normal form of PrP, is a plasma-membrane-associated protein that is found primarily in the neurons and glia of the brain and spinal cord, as well as in some peripheral tissues and leucocytes, but its cellular role remains obscure. Gene knockout experiments show that PrP is not essential to the prolonged viability of an animal and thus compounds directed at the biological function would appear to be non-starters (even if we knew what the biological function of PrP was!). However, the intracellular trafficking of PrP^{C} might be a rational

target, since this could lead to a block in the conversion to PrP^{Sc} [4].

The search for prion eliminating compounds

A number of compounds have been identified that inhibit the accumulation of the protease-resistant form of PrP (usually referred to as PrP-Res since this experimentally induced form does not necessarily have the infectivity associated with PrP^{Sc}-containing preparations) either in an *in vitro* assay using recombinant PrP, or in prion-infected cultured neuroblastoma cells. Any anti-prion effect such compounds may have is probably caused by preventing the interaction of PrP^{Sc} with its normal cellular form, PrP^{C}, that lies at the heart of the PrP prion-conversion cycle. However, one cannot rule out indirect effects through other cellular mechanisms.

To identify new anti-prion agents in the extensive chemical libraries that are now available requires the establishment of simple cell-based assays that can be screened in a high-throughput mode. The more successful of the assays so far developed screen for compounds that inhibit PrP^{Sc} accumulation in prion-infected neuroblastoma cells[5] (Figure 2). Briefly, neuroblastoma cells are infected with one of two different prion strains and are then incubated with the test compound for 5 days. After the 5 days, the cells are lysed, and the resulting lysates are treated with proteinase K to remove any protease-sensitive PrP^{C}, before being applied to a PVDF membrane. Exposure of the membrane to an anti-PrP antibody is used to identify any non-digested PrP-Res in the lysate, and a significant reduction in the intensity of the signal detected is used to demonstrate the efficacy of the test compound at preventing PrP-Res formation.

Using the neuroblastoma cell-based assay, Caughey's group tested 2000 compounds and identified a total of 15 compounds active against both prion strains with an $IC_{50} \leq 1$ μM, but without observed cellular toxicity[5]. The compounds identified included several natural products, as well as polyphenols, anti-malarial compounds and anti-histamines. To test the molecular action of these compounds, a solid-phase cell-free conversion reaction, in which hamster PrP^{Sc} was used to induce the conversion of radio-labelled recombinant hamster PrP^{C}, revealed that three of the compounds (tannic acid, katacine and 2′,2′′′-bisepigallocatechin) could directly inhibit the conversion with an IC_{50} of 100 nM. The remaining compounds were unable to inhibit this reaction up to concentrations of 100 μM, indicating that their anti-prion effect was unlikely to be via the modulation of the interaction between PrP^{Sc} and PrP^{C}. All of the compounds identified in Caughey's screen already have FDA (Food and Drug Administration) approval or are dietary constituents, making further testing in humans and animals possible.

However, there is a cautionary note: subsequent studies by Caughey and co-workers highlighted the importance of testing putative anti-prion compounds that emerge from such cell-based screens, in transgenic animal models[6]. In their follow-up study, they showed that a group of compounds that blocked the formation of PrP-Res in cultured neuro blastoma cells had no significant effect on the incubation periods in prion-infected rodents. This did not reflect a failure of these compounds to cross the blood–brain barrier, because they were administered intracerebrally prior to experimental infection.

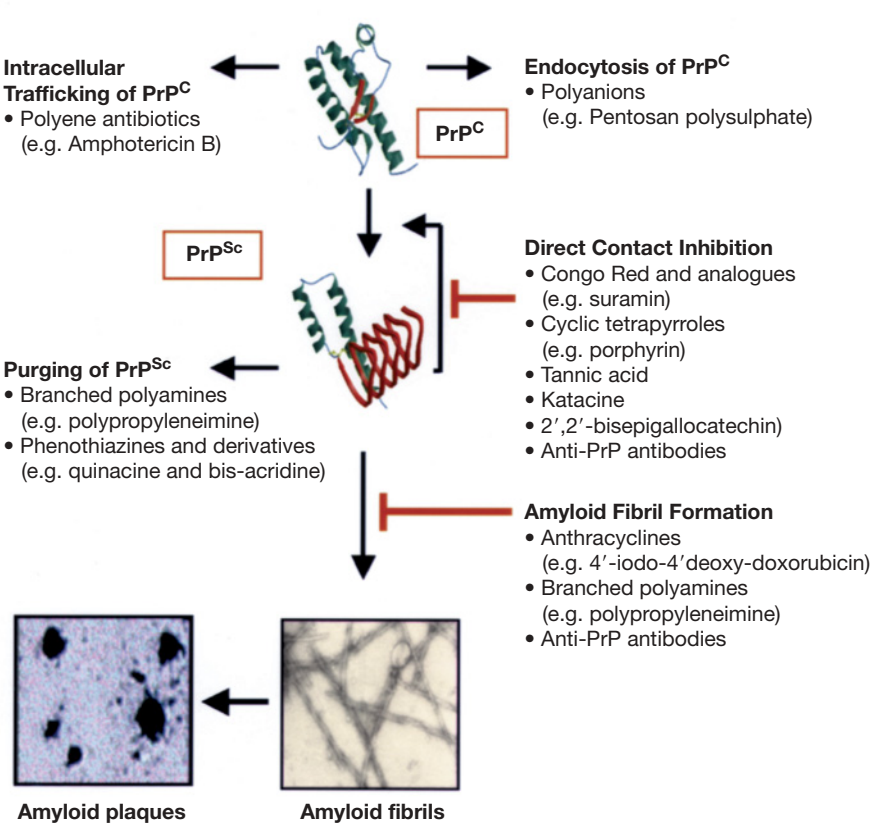

Figure 1. Targets for drug intervention in prion propagation and pathogenesis. Compounds that have emerged as potential anti-prion compounds and their likely target(s) are shown. Images reprinted from Trends in Molecular Medicine, Vol 7, Soto et al., Prions: disease propagation and disease therapy by conformational transmission, pp 109–114, Copyright 2001, with permission from Elsevier.

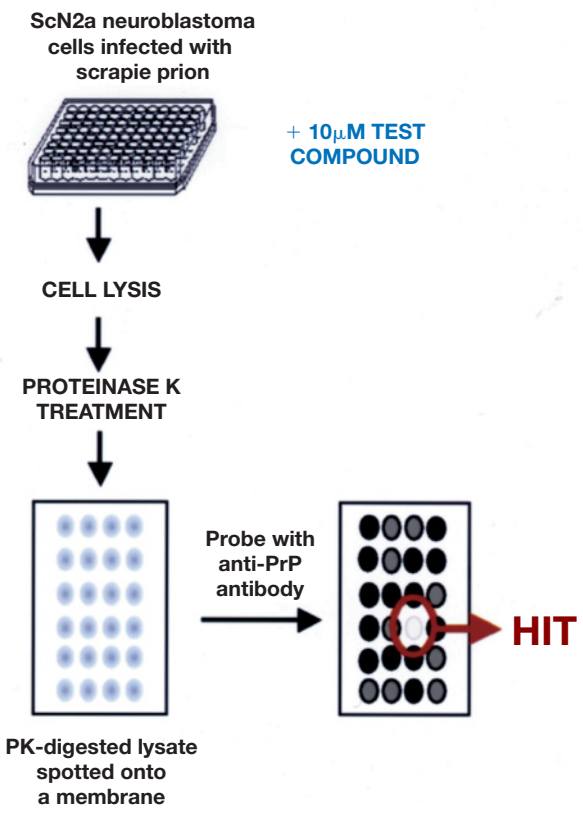

Figure 2. An animal cell-based assay that allows for high-throughput screening of compounds with anti-prion activity. The neuroblastoma cell line ScN2a, grown in a microtitre format, is used as the basis for this assay. Compounds that inhibit the propagation of the protease-resistant form of PrP (i.e. hits) result in no signal upon Western blot analysis of treated lysates.

Inhibitors of prion replication

One of the first anti-prion compounds to emerge from the cell-based screens was CR (Congo Red), a dye that is widely used as a diagnostic stain for amyloids. CR irreversibly blocks the seeded formation of PrP^{Sc} in cultured neuroblastoma cells[7]. Analogues of CR, sulphated polyanions, polyene antibiotics, cyclic tetrapyrroles, branched polyamines, anthracyclines and phenothiazines have similar activity. CR, its analogues and the cyclic tetrapyrroles (such as porphyrin and phthalocyanine compounds) are compounds that inhibit the direct interaction between the two forms of the protein, whereas polyanions, such as pentosan polysulphate, and the polyene antibiotics, such as amphotericin B, work indirectly. Polyanions have also been found to encourage the endocytosis of PrP^{C} from the plasma membrane, perhaps removing PrP^{C} before it can interact with PrP^{Sc}. Similarly, polyene antibiotics appear to modulate the intracellular trafficking of the PrP protein, thereby preventing interaction of PrP^{c} with PrP^{Sc} [3].

A number of compounds have emerged from these assays, but their underlying mechanism remains to be established. In particular, branched polyamines, such as polypropyleneimine, can reduce prion infectivity and purge PrP^{Sc} from infected neuroblastoma cells[8]. The high surface density of primary amino groups and the highly branched structure of such branched polyamines are important for the removal of PrP^{Sc}. Although the precise mechanism of action is not entirely understood, branched polyamines do render PrP^{Sc} susceptible to proteolytic degradation.

Several other compounds clear PrP^{Sc} from infected neuroblastoma cells by promoting amyloid fibril disaggregation. For example, anthracyclines (such as 4′-iodo-4′-deoxy doxorubicin, IDX) bind to and promote amyloid fibril disaggregation. The phenothiazines (e.g. chlorpromazine) and phenothiazine-derivative compounds (e.g. quinacrine and bisacridine analogues) have also been found to clear PrP^{Sc} from infected neuroblastoma cells, but how they achieve this is not known.

Eliminating yeast prions

In addition to infectious prions in mammals, self-propagating prions also exist in lower forms of eukaryotic life. In particular, the budding yeast *Saccharomyces cerevisiae* has at least three different prions: [PSI^{+}], [*URE3*] and [RNQ^{+}], all of which satisfy the key criteria that have been used to define a prion[9]. In the case of two of these prions, we know the cellular function of the prion protein and the phenotypic consequence of switching to the prion form. The [PSI^{+}] prion is the alternative conformer of Sup35p, a subunit of the translation termination factor, and leads to a nonsense suppression phenotype. [*URE3*] is the alternative conformer of the Ure2p protein that regulates nitrogen catabolite repression and facilitates the utilization of poor nitrogen sources in the presence of ammonium ions. As with PrP, both native conformers of Sup35p and Ure2p are converted into their prion forms on contact with its corresponding prion form, and since the underlying mechanism by which yeast prions are replicated appears to be broadly similar to how PrP^{Sc} is replicated, then studying compounds that

block yeast prion replication might facilitate anti-prion drug discovery.

There are some very effective yeast prion-eliminating compounds, the most remarkable being the protein denaturant guanidinium hydrochloride (GdmCl). At millimolar levels, GdmCl rapidly clears all three prions from actively growing yeast cells within 24 hours. However, this is achieved indirectly via inhibition of Hsp104p[10], a molecular chaperone that is essential for the continued propagation of yeast prions. To date, no equivalent molecular chaperone has been shown to play a key role in mammalian prion propagation, but the findings in yeast indicate the potential to develop prion-replication inhibitors that do not act directly on the prion protein itself.

A significant advantage of using yeast prions in this context is that, unlike PrP, the cellular function of the native conformer is known and therefore a screen can be tailored to reflect the loss of function within the cell that is conferred by the presence of the prion. Furthermore, yeast prions are non-toxic, and, rather than being detrimental to the cell, they may actually provide some survival advantages over non-prion-containing yeast cells.

The mechanistic similarities between PrP^{Sc} and yeast prion propagation have led to the development of a successful screen using a yeast cell-based assay for chemicals which eliminate both the [*PSI*$^+$] and the [*URA3*] prions from yeast[11]. Of the 2500 compounds screened by Bach et al.[11], only six were active against both yeast prions and, of these, five were novel kastellpaolitines (KP1–5), and the sixth, phenanthridine, was a previously known molecule. To show the applicability of the yeast-based assay to the discovery of compounds active against mammalian prions, two anti-mammalian prion compounds, quinacrine and chlorpromazine, were tested and shown to exhibit anti-prion activity against the [*PSI*$^+$] prion. The addition of one of the kastellpaolitines (KP1) and a chemically modified form of phenanthridine, namely 6-aminophenanthridine (6AP), also led to a decrease in the formation of PrP-Res in the cell-based assay.

Antibodies as prion-eliminating agents

In addition to chemical compounds with anti-prion activity, there have been several reports on the use of antibodies to purge PrP^{Sc} from infected cells. Interest in antibodies as anti-prion agents was first stimulated following the demonstration that anti-PrP antibodies can cause a 100-fold reduction in the infectivity of a prion inoculum[12]. Subsequent work, by a number of groups, has shown that antibodies can abolish prion replication and clear pre-existing PrP-Res from infected neuroblastoma cells, suggesting the possibility of effectively curing an established infection and therefore offering a novel therapeutic strategy.

In vivo immunization of prion-infected mice with monoclonal anti-PrP antibodies can also reduce PrP^{Sc} levels and prion infectivity in the tissues of the infected mice, thereby prolonging their survival when compared with untreated controls[13]. However, despite halting prion replication well after the infection has established, the anti-PrP antibodies were unable to prevent terminal illness if treatment was begun after the manifestation of clinical symptoms. Currently the mechanism of prion-inhibition by anti-PrP antibodies remains obscure, although the most likely reason is that anti-PrP antibodies sterically interfere in the $PrPC:PrP^{Sc}$ interaction and thus prevent conversion of PrP^{c} into the prion form. Nevertheless, anti-PrP antibodies provide a promising means of halting or slowing prion disease progression, although early diagnosis is crucial before the onset of irreversible damage.

The clinical outlook

A number of natural and synthetic chemical compounds, as well as antibodies, have emerged as offering potential for therapeutic use in treating the prion diseases of humans and animals. As in all drug development programmes, there are a number of biological and regulatory challenges ahead, most notably in testing these compounds, or their analogues, in controlled clinical trials. Yet, even without clear experimental evidence that they can be of benefit in the established disease, some of the compounds emerging from drug screens are being used to treat vCJD patients. Most notably, it has been reported recently that a number of vCJD sufferers are being treated with pentosan polysulphate, but, because this compound does not cross the blood–brain barrier, it must be injected directly into the brain[14]. It remains to be seen whether pentosan polysulphate or quinacrine will enter the clinic as effective treatments for prion diseases. The clinical use of anti-prion agents may also be extended to treat other 'protein-folding' or amyloid-based diseases, such as Huntington's, Alzheimer's and Parkinson's diseases, whose sufferers are counted globally in millions.

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Lee J. Byrne received his PhD from the University of Kent before moving to St. Jude Children's Research Hospital in Memphis, TN, where he worked as a postdoctoral research associate. He has since returned to the University of Kent to investigate yeast prions in Mick Tuite's laboratory, and, in so doing, collaborates with both bioscientists and statisticians at Kent.

L.J.Byrne@kent.ac.uk

Mick Tuite is Professor of Molecular Biology and current Director of Research in the Department of Biosciences at the University of Kent. He has had a long-standing interest in the study of the [*PSI*$^{+}$] prion of yeast, an interest that began while he was a DPhil student with Brian Cox in Oxford's Botany School. Prior to taking up his post at Kent in 1983, he undertook postdoctoral research at both Oxford University and the University of California at Irvine.

M.F.Tuite@kent.ac.uk