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# Prion protein as a target for therapeutic interventions*

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*Abstract*: Transmissible spongiform encephalopathies (TSEs), currently known as prion diseases, are neurodegenerative disorders of the central nervous system (CNS) caused by an elusive infectious agent called "prion" (proteinaceous infectious particle). These disorders include: kuru, Creutzfeldt–Jakob disease (CJD) and its variant (vCJD), Gerstmann–Sträussler–Scheinker (GSS) disease and fatal familial insomnia (FFI) in humans, scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) or mad cow disease, and chronic wasting disease (CWD) in cervids. According to the widely accepted "prion hypothesis", prion is an aggregate of the abnormal isoform of prion protein ($PrP^{Sc}$). Prion protein is a cell-derived glycoprotein (this normal isoform is called $PrP^{c}$) encoded by a gene on chromosome 20 in humans (*PRNP*). In familial forms of TSEs, mutations within the ORF of *PRNP* are linked to the phenotypic expression of the disease. TSEs are important from public health perspective, and "mad cow disease has created the greatest threat to the safety of human food supply in modern times. vCJD threatens the safety of the blood supply worldwide". Thus, to search for effective therapy is more than an urgent task. In TSEs, aggregates of $PrP^{Sc}$ accumulate in the brain in a form of plaques, or synaptic deposits. The conversion of $PrP^{c}$ into $PrP^{Sc}$ and subsequent deposits of $PrP^{Sc}$ are targets for therapeutic interventions. These include: tricyclic compounds—acridine and phenothiazine derivatives; quinacrine; anti-$PrP^{Sc}$ antibodies; dendrimers; polyethylene antibiotics (amphotericin B, MS-8209); pentosan polysulfate; and dextran sulfate. All these compounds are active in many in vitro and in vivo assays, but not proved definitely active in humans. Thus, albeit interesting and promising, the chemotherapy of TSEs is still in the infant phase.

## INTRODUCTION

Transmissible spongiform encephalopathies (TSEs) or prion diseases are a group of neurodegenerative disorders that include kuru; Creutzfeldt–Jakob disease (CJD); Gerstmann–Sträussler–Scheinker (GSS) disease and fatal familial insomnia in humans; natural scrapie in sheep, goats, and mufflons; transmissible mink encephalopathy in ranch-reared mink; chronic wasting disease of mule deer and elk in the United States; bovine spongiform encephalopathy (BSE) or "mad cow disease" and its analogs in several exotic species of antelopes and wild felids in zoological gardens; and feline spongiform encephalopathy in domestic cats [1].

These disorders are caused by a still not completely understood pathogen variously referred to as a "prion" (predominantly) or a "virus" (now rather infrequently and usually with adjectives *slow*, *unconventional*, or *atypical*), "agent", or "virino". Despite wide acceptance for the prion theory and its

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vindication by a Nobel Prize, these names still reflect different views on the molecular structure of the pathogen and, by the same token, our ignorance of its nature. The vast majority who prefer to view this pathogen as composed "predominantly or exclusively" of one pathologically folded (or misfolded) protein, $PrP^{Sc}$, use the term "prion"; hence, the term "prion diseases".

## PRION PROTEIN, ITS GENE, THE "PRION HYPOTHESIS"

PrP (prion protein) is a highly conserved sialoglycoprotein encoded by a cellular gene mapped to chromosome 20 in humans and 2 in mouse. The gene is ubiquitous; it has been cloned in numerous mammalian species including marsupials and there are analogs of this gene in birds, reptiles, amphibians, and even fish. PrP27-30 was first discovered as a protein copurifying with infectivity in extracts derived from brains inoculated with the 263K strain of scrapie agent, which led to the conclusion that PrP is a part of the infectivity.

The "prion" hypothesis, which is deeply rooted in this association between PrP and infectivity, was formulated by S. B. Prusiner in 1982 [2]. The hypothesis postulated that the scrapie agent was a proteinaceous infectious particle (actually, it should read "proin", but Prusiner thought that "prion" sounds better than "proin"), because infectivity was dependent on protein but resistant to methods known to inactivate nucleic acids.

Like all amyloid proteins, PrP27-30 is a proteolytic cleavage product of a precursor protein, $PrP^{Sc}$. However, $PrP^{Sc}$ is not the *primary* product of the cellular gene. It has the amino acid sequence, and postranslational modifications (like glycosylation and the attachment of GPI, glycophospholipid inositol anchor) identical to those of $PrP^{c}$, but strikingly different physicochemical features. In particular, $PrP^{c}$ is completely degraded by a limited proteolysis (e.g., with proteinase K, PK), while $PrP^{Sc}$ is only partially degraded, yielding a core protein (PrP27-30) which may be visualized by electron microscopy as scrapie-associated fibrils (SAFs) also known as prion rods. To become $PrP^{Sc}$, $PrP^{c}$ must be first transported to the cell surface and then through the endosomal-lysosomal pathway.

To analyze any drug therapeutic regimen, an outline of **peripheral** (in natural condition, the TSE agent or prion enters the body from the outside; i.e., by an oral route and the GI tract) pathogenesis is necessary. Following peripheral inoculation (oral route, skin abrasions), the agent travels to the lymphoid tissue including the spleen; from those, it is transported by several different autonomic nerves into the central nervous system. In a typical experiment, a drug is administered around (before, at, or after) the time of infection and the incubation period (IP) is measured. Alternatively, chronically infected neuroblastoma cells ($N_2Sc$) are tested for the amount of $PrP^{Sc}$.

## THERAPEUTIC INTERVENTIONS

The epidemics of vCJD in the United Kingdom (currently, 144 cases) and a great media attention produced a strong pressure not only to search for the effective treatments with new drugs, but also to test those that proved promising in experimental studies, even to bypass phases 1 and 2 of clinical trials. It must be kept in mind, however, that the incubation period of vCJD is long (>10 years) and that the affected brain is extensively damaged toward the clinical phase of the disease. As a result, any drug regimen instituted at that time may prove ultimately to be a failure.

Several classes of drugs have been used in TSE; these were recently summarized (Table 1) [3], and only some of these will be discussed in this brief review.

**Table 1**

| Compound | Activity |
|---|---|
| Polyanions (dextran 500, pentosan polisulfate) | Blocks peripheral replication; used in experimental trial of vCJD |
| Congo red and other sulfonated dyes | Binds to both $PrP^{c}$ and $PrP^{Sc}$ |
| Tetrapyrroles | Prolongs IP; used in humans, but not in CJD |
| Anthracyclines | Abrogates IP and $PrP^{Sc}$ accumulation in vivo; binds different classes of amyloid. |
| Tetracyclic compounds | Prolongs IP in a strain-specific manner and abrogates both $PrP^{Sc}$ accumulation and $PrP^{Sc}$ PK resistance |
| Polyene antibiotics | Prolongs IP in a strain-specific manner and abrogate $PrP^{Sc}$ accumulation |
| Branched polyamines (dendrimers) | Disaggregates $PrP^{Sc}$ aggregates and reduce its β-sheet content |
| Acridine derivatives (quinacrine) | Strain-dependent activity |
| Phenothiazine derivatives (chloropromazine) | Blocks $PrP^{Sc}$ formation in a strain-dependent manner; used in humans as antimalarial drug |
| β-breakers | Reduces $PrP^{Sc}$ β-sheet content; used in other amyloidoses (i.e., Alzheimer's disease) |
| Anti PrP antibodies | Blocks $PrP^{c}$ to $PrP^{Sc}$ conversion |
| Gleevec (Fyn tyrosine kinase inhibitor) | Abrogates $PrP^{Sc}$ $T_{1/2}$ |

## Tricyclic compounds derivatives of acridine and phenotiazine

The first compound tested was chloropromazine (Fig. 1), followed by quinacrine (Fig. 2), a well-known antimalarial drug [4].

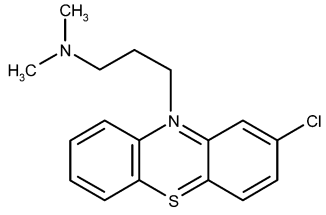


**Fig. 1** Chloropromazine.

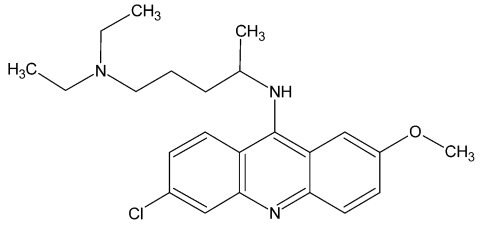


**Fig. 2** Quinacrine.

The acridine-derivatives block $PrP^{Sc}$ formation in scrapie-infected neuroblastoma cells ($N_2Sc$) in vitro, and its administration in vivo prolongs the incubation period. In some experiments, $N_2Sc$ cells were even cured of $PrP^{Sc}$ (and infectivity). As quinacrine has been used for over 50 years, it was possible to bypass phases 1 and 2 of clinical trials and administered it to CJD patients in the United States. In the MRC Prion Unit, the complex clinical trial of a quinacrine (300 mg/day) is going on:

- for those CJD patients whose family agreed to a double blind trial, an immediate vs. delayed administration of quinacrine is being tested;
- for those without a family consent, a randomized trial is performed (quinacrine vs. placebo); and
- for those patients whose family not agreed for a randomized trial, an open trial is being tested.

The effects of that trial are based on a potential disappearance of $PrP^{Sc}$ from tonsils and an abrogation of hyperintensive signal in the pulvinar region on MRI.

## Antibiotics

Three classes of antibiotics were tested:

- tetracycline derivatives (Fig. 3)
- anthracyclines (anticancer compounds) of the ring reminiscent of that of tetracyclines
- polyene antibiotics (amphotericin B) (Fig. 4)

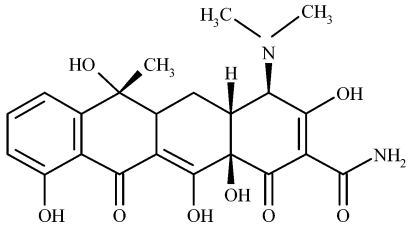


**Fig. 3** Tetracycline.

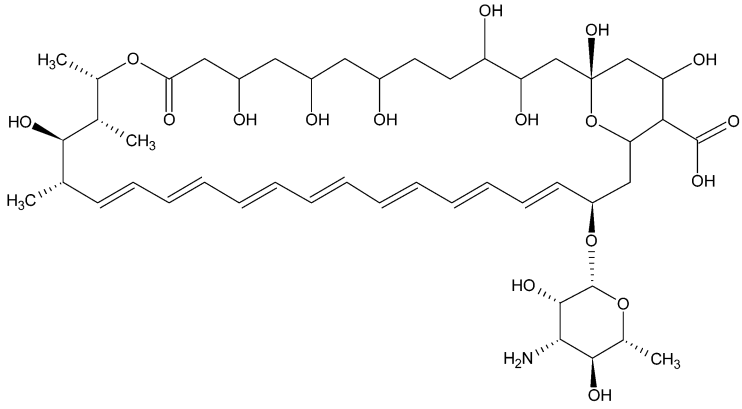


**Fig. 4** Amphotericin B.

## Tetracyclic compounds

Tetracyclic compounds bind both $PrP^{Sc}$ and PrP peptides (including PrP 106-126) and abrogate $PrP^{Sc}$ aggregation and its PK (proteinase K) resistance [5]. In scrapie-affected mice, tetracycline prolongs IM [6].

## Anthracyclines

The compound tested was iododeoxyrubicine (IDX) [7] a potent anticancer drug. It binds amyloid fibrils of different molecular composition, including $PrP^{Sc}$ [8] and prolongs IP in scrapie-affected mice.

### Polyene antibiotics (amphotericin B)

This compound was shown to prolong IP in scrapie-affected hamsters and to abrogate $PrP^{Sc}$ accumulation [9,10]. The activity of amphotericin B is strain-dependent. The drug is active toward both the Fujisaki strain of GSS in mice and the 263K strain of scrapie in hamsters, but not toward 139A-H, ME7, and 87V strains of scrapie [11]. Interestingly, it is active following i.c. inoculation. Even more active was amphotericin B-derivative MS-8209 [12], which prolongs IP and abrogates accumulation of $PrP^{Sc}$ and GFAP. MS-8209 delayed the development of spongiform change and gliosis in scrapie-affected hamsters. As efficiency of those compounds is inversely correlated with the $PrP^{Sc}$ concentration in TSE-affected brains (more $PrP^{Sc}$ correlated with better outcome), MS-8209 was less efficient in BSE-infected mice than in scrapie-infected hamsters.

### Organic polyanions (pentosan sulfate)

Recently, pentosan sulfate (Fig. 5), a very anionic compound, was used in a vCJD patient administered by an infusion pump directly into the ventricular system. That compound, along with dextran sulfate-500, has been used for many years both in vitro and in vivo experiments [13].

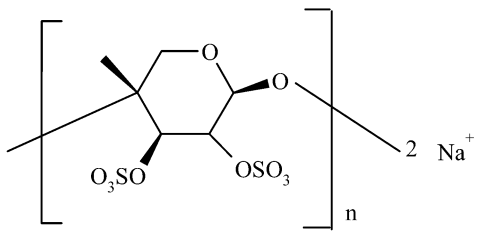


**Fig. 5** Pentosan polysulfate.

As with many other compounds, DS 500 prolongs IP following i.p. or i.v., but not i.c. inoculation [14]. DS 500 abrogates the infectivity titre in the spleen, but its activity is clearly strain-dependent, which may reflect differences of the kinetics of infectivity (e.g., neuroinvasion). A potent drug of this group is pentosan sulfate, which prolongs IP and abrogates accumulation of $PrP^{Sc}$. Interestingly, the kinetics of pentosan sulfate delivery influences the outcome; faster administration proved better [15].

### Dendrimers

Dendrimers are branched polyamine spherical molecules of nm-range diameter, which abrogate $PrP^{Sc}$ accumulation in scrapie-infected neuroblastoma cells and cured $N_2Sc$ cells in vitro [16].

### Antibodies

Anti-PrP antibodies block the conversion of $PrP^{c} \rightarrow PrP^{Sc}$ in vitro [17]. Anti-PrP FAB fragments cured $N_2Sc$ cells in vitro or prevent $PrP^{Sc}$ formation.

### β-breakers

β-breakers are peptides of sequence partially homologous to the sequence of PrP, but with substitutions unable to fit in β-sheet structure [18].

PrP 115 – GAA AAG AVVGGLG 122

β-breaker sequence PrP 13:

DAP AA PA GPAVPV

PrP 13 blocks $PrP^{c} \rightarrow PrP^{Sc}$ conversion and prolongs IP in scrapie-affected mice.

### Signaling cascade inhibitor

Gleevec is a potent inhibitor of tyrosine kinase linked to a Fyn signaling cascade. It has been used as anticancer drug to treat CML leukemia, GIST (gastrointestinal stromal tumor), and glioblastoma multiforme. This compound does not influence $PrP^{Sc}$ accumulation, but it abrogates its T 1/2.

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