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RECENT ADVANCES IN PRION CHEMOTHERAPEUTICS

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Abstract

The transmissible spongiform encephalopathies are rapidly progressive and invariably fatal neurodegenerative diseases for which there are no proven efficacious treatments. Many approaches have been undertaken to find ways to prevent, halt, or reverse these prion diseases, with limited success to date. However, as both our understanding of pathogenesis and our ability to detect early disease increases, so do our potential therapeutic targets and our chances of finding effective drugs. There is increasing pressure to find effective decontaminants for blood supplies, as variant Creutzfeldt Jakob Disease (vCJD) has been shown to be transmissible by blood, and to find non-toxic preventative therapies, with ongoing cases of Bovine Spongiform Encephalopathy (BSE) and the spread of Chronic Wasting Disease (CWD). Within the realm of chemotherapeutic approaches, much research has focussed on blocking the conversion of the normal form of prion protein (PrP^C) to its abnormal counterpart (PrP^{res}). Structurally, these chemotherapeutic agents are often polyanionic or polycyclic and may directly bind PrP^C or PrP^{res}, or act by redistributing, sequestering, or down-regulating PrP^C, thus preventing its conversion. There are also some polycationic compounds which purport to enhance the clearance of PrP^{res}. Other targets include accessory molecules such as the laminin receptor precursor which influences conversion, or cell signalling molecules which may be required for pathogenesis. Of recent interest are the possible neuroprotective effects of some drugs. Importantly, there is evidence that combining compounds may provide synergistic responses. This review provides an update on current testing methods, therapeutic targets, and promising candidates for chemical-based therapy.

Keywords

prion; PrP; chemotherapeutic; decontamination; prophylaxis; polyanion; conversion

INTRODUCTION

The accumulation of protease-resistant prion protein (PrP^{res}) aggregates is one of the neuropathological hallmarks of transmissible spongiform encephalopathies (TSEs). PrP^{res} is a corrupted form of the normal, protease-sensitive, prion protein (PrP^C), and is the primary protein component of the transmissible prion agent [1,2]. As such, it has been a major target for therapeutic intervention. Unfortunately, our incomplete understanding of prion disease pathogenesis has hindered the development of successful treatments. PrP^{res} accumulates as a result of the repeated conformational conversion of α -helical PrP^C to highly β -sheet PrP^{res}, likely through an autocatalytic seeded polymerization mechanism. Membrane interaction facilitates this transformation [3], and the site of conversion of wild-type PrP^C within prion-infected cells is likely to be the plasma membrane or endocytic vesicles [4,5,6]. However, large extracellular plaques of PrP^{res} can develop in mice whose PrP^C lack GPI anchors and

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are therefore not attached to the membrane [7]. It is also not known how PrP^{res} formation and/or accumulation leads to pathology. Large aggregates of PrP^{res} are neither necessary nor sufficient to cause clinical disease in all cases, and it may be that soluble [8,9], more protease-sensitive [10,11], oligomeric forms of prion protein (PrP) are more relevant to mechanisms of infection, conversion [9] and toxicity [12].

Early treatment regimes, including various prophylactic compounds and immunotherapies, have sought efficacy through neutralization of infectious sources, blockade of infection via the most common peripheral routes, and/or blockade of neuroinvasion. Effective therapies targeting later disease, which are initiated after the appearance of clinical signs, will most likely involve some combination of inhibiting pathogenic PrP formation, destabilizing or enhancing clearance of existing pathogenic PrP, blocking neurotoxic effects of the infection, and/or promoting the recovery of lost functions in the central nervous system (CNS). This article reviews the recent progress in chemotherapeutic approaches to TSE prevention and treatment.

MODELS FOR STUDYING CHEMOTHERAPEUTIC CANDIDATES

In vivo tests provide the most rigorous evaluations of anti-TSE treatments, but are slow, costly, and impractical for screening purposes. A variety of relatively high throughput, low cost, cell culture models [13,14], and some yeast models [15], have enabled the identification of a number of different classes of anti-prion compounds which then have shown efficacy in animal models. Also, *in vitro* assays have allowed investigation of the mechanisms of prion inhibition. In many cases, anti-prion compounds which bind to PrP^c cause it to cluster and internalize, thus rendering PrP^c inaccessible or incompatible for conversion to PrP^{res} [16,17,18,19].

Noncellular *in vitro* assays have also been developed to assess a wide range of potentially effective compounds. These methods generally assess the competitive binding of PrP^c and PrP^{res} or the prevention of PrP amyloid fibril formation. Recent techniques include surface plasmon resonance [20], fluorescence correlation spectroscopy [21], semiautomated cell-free conversion [22], and a fluorescence-polarization-based competitive binding assay [23]. Computer “in silico” modelling is also being used to predict binding molecules [24,25].

Ultimately, promising treatments discovered *in vitro* require testing in animals and humans. Of the many compounds studied in rodent models, few have made their way into human trials or case reports, and the effectiveness of treatment administered at the onset of clinical symptoms, or when there is significant neuropathology, is low. However, many compounds show some prophylactic or early treatment effect in TSE-infected animals, and are therefore relevant to decontamination and early therapy efforts. These drugs need not be permeable to the blood-brain barrier since they can target the peripheral replication of the agent, before neuroinvasion, which is relevant for the many prion diseases that arise following oral or other peripheral exposures. Of course, the option for early treatment has been hindered by a lack of early diagnosis, but with the recent development of new sensitive detection assays [1,26,27,28,29], there is hope for early preclinical TSE diagnostics, and more effective screening and testing of at risk individuals. This, coupled with the rising concern of blood transmission of variant Creutzfeldt Jakob Disease (vCJD), the occurrence of Bovine Spongiform Encephalopathy (BSE) in livestock, and the spread of Chronic Wasting Disease (CWD), lends tremendous relevance to such chemoprophylaxis compounds and potential decontaminants in the management of prion diseases.

TARGETTING PrP CONVERSION

A major focus of drug screening efforts has been the PrP conversion reaction. Many inhibitors prevent conversion by directly binding and blocking interactions between PrP^C and PrP^{Sc}. Others affect conversion by interfering with important accessory molecules, or by altering PrP^C expression and distribution [16]. Many different chemical classes of compounds have been screened and tested *in vitro*, and additional *in vivo* data are available for some (Table 1), including early or prophylactic treatments and later stage therapies.

Binding PrP^C and/or PrP^{res}

Polyanions—Sulphated glycans and other polyanions are some of the oldest known inhibitors of PrP^{res} formation [30,31]. Their mechanism of action is likely competitive inhibition of physiological cofactors that can bind PrP and promote conversion, including RNA [2,32,33,34], or endogenous sulfated glycosaminoglycans (GAGs) [19,30,31,32,35,36]. The usefulness of these compounds is limited by potential anti-coagulant activity and/or poor blood brain barrier permeability. However, this latter drawback can be circumvented through intraventricular infusion in animal models [37].

Pentosan polysulphate (PPS) is a classic sulphated glycan which prevents new PrP^{res} accumulation [30,31] and is regularly employed to cure cell culture models of prion infection. It prolongs incubation times in rodent-adapted scrapie models [38,39,40,41], and has significant effect when administered via an intraventricular cannula and osmotic pump [37]. Its efficacy has recently been demonstrated in a CWD-infected deer-cell model [42], as well as in a mouse model of BSE [43]. There is also a significant amount of data from case reports of intraventricular PPS used in human prion diseases, with mixed results.

Several other polyanions have been studied. Fucoidan, a complex sulphated fucosylated polysaccharide, is an edible component of seaweed which has *in vitro* and *in vivo* anti-prion effects [44]. Phosphorothioate oligonucleotides, particularly those of 17 or more bases, have striking prophylactic effects in mice inoculated subcutaneously or intraperitoneally [45], and have reduced anti-coagulant effect compared with pentosan polysulfate. Copaxone, a polymer of alanine, glutamate, lysine, and tyrosine, is an approved treatment for multiple sclerosis, but can also prolong the incubation periods in rodent-adapted scrapie if it is mixed with the inoculum prior to infection, or if it is given at the time of inoculation [46].

Sulphonated dyes and similar compounds (Congo red, suramin, curcumin)—

Congo red was the first identified inhibitor of PrP^{res} accumulation [47]. For a review of its role in neurodegenerative diseases see reference [48]. Its ability to stack and mimic larger polyanions [16,49,50], likely accounts for its ability to inhibit PrP^{res} formation through competition with sulphated glycans for PrP^C binding [35]. Inhibition may also result from an overstabilization of PrP^{res} [51]. *In vitro*, Congo red and analogues thereof decrease PrP^{res} and scrapie infectivity in cells [30,47,51,52,53,54,55,56], block cell-free conversion reactions [53,54,57,58], and decrease surface PrP^C in normal cells [19]. *In vivo*, Congo red prolongs incubation times in hamsters [59,60], but its clinical use is limited by the potential teratogenic and/or carcinogenic activity of the benzidine moiety, and by its poor blood brain barrier permeability.

Polysulphonated naphthyl urea (suramin) is a symmetrical aromatic which is structurally similar to Congo red and can decrease PrP^{res}, decrease surface PrP^C, and cause aggregation of recombinant PrP [61]. It has a modest early treatment effect on incubation times in hamsters [41], [61]. Suramin analogues with naphthalene- or benzene-sulphonic acid substitutions also decrease PrP^{res} accumulation and induce PrP aggregation, but have no effect on PrP^C expression [62].

Diferuloylmethane (the spice curcumin), has a related structure which lacks sulphonates and the benzidine moiety, is non-toxic, has good blood brain barrier permeability, and can inhibit PrP^{res} formation *in vitro* [63]. It binds a β -sheet rich form of PrP 90-231, as well as α -helical intermediates, but not the native α -helical form of this N-terminally truncated PrP fragment [64]. Whether curcumin can bind to native full-length PrP^C remains to be determined. While high dose treatment was ineffective *in vivo* [63,65], low doses (50 mg/kg/day) have recently been shown to prolong survival in intracerebrally inoculated mice that were treated starting at 100 days post-inoculation [65].

Cyclic tetrapyrroles (porphyrins, phthalocyanines)—These compounds have highly conjugated planar aromatic ring systems that bind transition metal ions and can be circumscribed by anionic, cationic, or uncharged peripheral substituent groups. Examples include phthalocyanine tetrasulphonate (PcTS), deuteroporphyrin IX 2,4-bis-(ethylene glycol) iron(III) (DPG₂-Fe³⁺), meso-tetra(2-N-methylpyridyl)porphine iron(III) (TMPP-Fe³⁺), tetra (4-N,N,N-trimethylanilinium)porphine (Fe-TAP), and tetra (4-sulphonatophenyl)porphine (Fe-TSP). They inhibit PrP^{res} accumulation *in vitro* [66] and significantly increase survival times *in vivo* when given early in the disease [17,42,67,68], probably by stacking and directly interacting with the flexible amino-terminal domain of PrP molecules [16,69]. Of note, Fe-TSP was more effective in treating mice inoculated intracerebrally when it was used in combination with pentosan polysulphate [17], thus highlighting a possible role for combination therapy. Tetrapyrroles may also be useful in non-rodent models; the compound indium(III) meso-tetra(4-sulphonatophenyl)porphine chloride (In-TSP) has recently been shown to affect CWD-infected deer cell cultures [42].

Another new player on the porphyrin stage is the natural cyclic tetrapyrrole, hemin, which inhibits PrP^{res} formation in cell culture (D. A. Kocisko and B. Caughey, unpublished data). The finding that hemin binds PrP^C and causes its clustering and internalization in cell culture [70], raises the possibility that there are physiologically significant hemin-PrP^C interactions, and suggests that prion inhibition may result from sequestration of PrP^C into a non-convertible state.

Lysosomotropic factors (quinacrine, quinoline, acridines, and phenothiazines)—Quinacrine, chlorpromazine, quinine and related molecules have inhibitory effects on PrP^{res} formation *in vitro* cells [71,72,73], and variable effects *in vivo* [37,74,75,76]. Quinacrine has received attention in human clinical trials, but a retrospective analysis of human CJD patients treated with quinacrine, compared to untreated control cases, demonstrated no benefit [77]. Interestingly, the combined use of quinacrine plus desipramine or the HMG-CoA reductase inhibitor simvastatin [78] or the recombinant mutant PrP peptide rPrP-Q218K [79], may be more effective than quinacrine alone (see below). Also, intraventricular cannula treatment of mice with quinine and biquinoline partially reduced pathology in the hemisphere with the cannula, suggesting that this route of administration might enhance efficacy for some of these drugs [80]. One compound, the anti-malarial mefloquine, looked promising in cell culture, but lacked efficacy in tg7 mice that were inoculated intraperitoneally [81].

Tetracyclic compounds—The experiments published to date on tetracycline and doxycycline look promising in that pre-incubation with inocula reduces infectivity [82,83] and detectable PrP^{res} [82,84], and the results of *in vivo* studies are pending. However, the related molecule minocycline did not demonstrate therapeutic effect [65].

Other amyloidophilic compounds—Recently, anti-prion effects have been studied in a new group of amyloidophilic compounds [85]. Prion-strain dependent effects were seen in

cell culture and in intracerebrally inoculated mouse models, where oral drug administration, started early in the disease course, extended incubation times.

Pyridine dicarbonitrile compounds—Certain PrP mutants resist conversion, and a computational search for molecules that spatially resembled these mutants led to the discovery of several compounds with inhibitory effects in cell culture [24]. A pyridine dicarbonitrile substructure was found to be common to these agents [25], and further screening of these types of compounds discovered two which, at 50 nM concentration, could reduce PrP^{res} in cell culture to < 30% of controls [86]. No *in vivo* data is currently available.

Peptide aptamers and β -sheet breakers—In cell culture models and cell-free conversion systems, peptides of PrP residues 106-141, 119-136, 166-179, and 200-223 can block PrP^{res} formation [87,88,89]. Subsequently, searches have been made for other peptides which might interfere with conversion. One group placed random 16-mer sequences in a constant scaffold of thioredoxin A from *E. coli*, and selected those which recognized PrP sequence 23-231. Cell culture studies demonstrated that inhibition of PrP^{res} formation occurred in those which targeted PrP^c in the endoplasmic reticulum or lysosome [90]. Another group sought peptides which represented a portion of the target protein with prolines inserted to block incorporation into a β -sheet structure. iPrP13, one such β -sheet breaker, had some prophylactic effect *in vivo* [91]. However, there may be some strain or model dependence, as there was effect in a Chinese Hamster Ovarian cell model expressing mutant PrP, but no effect on infected N2a cells [92].

Redistribution / sequestration of PrP^c

Cholesterol-depleting agents—PrP^c is primarily located in areas of membrane rich in sphingolipids and cholesterol, called lipid rafts, and conversion of membrane associated PrP^c depends on interactions with PrP^{res} in the same membrane [3]. Therefore, agents which affect membrane cholesterol levels could alter PrP^c distribution and thereby affect PrP^{res} formation. Mevinolin, an HMG CoA reductase inhibitor which blocks cholesterol synthesis, does reduce cell surface PrP^c and leads to the accumulation of PrP^c within the Golgi apparatus [93]. Amiodarone and progesterone cause cholesterol redistribution and can clear PrP^{res} from cells [78]. Lovastatin and squalastatin, statin drugs which inhibit cholesterol synthesis, reduce PrP^{res} accumulation in cell culture, an effect which is lost upon addition of cholesterol [94,95]. Inhibitors of cholesterol esterification have also been shown to block PrP^{res} accumulation, consistent with a correlation between cholesterol ester levels and susceptibility of cells and sheep to scrapie infection [96]. However, such *in vitro* effects of cholesterol modulators do not always translate into *in vivo* results; inhibitors of 7-dehydrocholesterol reductase and 24-dehydrocholesterol reductase prevented PrP^{res} formation in cell culture, but had no effect on scrapie incubation times, or on brain cholesterol levels *in vivo*, regardless of whether treatment was initiated prior to or at the time of inoculation [97].

In contrast, the statin drug simvastatin can cross the blood-brain barrier and give some benefit *in vivo*, despite its short half-life of 1.5 hours. While low doses (1 mg/kg/day) were only effective if begun at the time of intracerebral inoculation [98], high doses (100 mg/kg/day) started 100 days after intracerebral inoculation prolonged survival in mice [99]. Interestingly, in both cases there were no effects on PrP^{res} levels, and cholesterol levels were only reduced in the liver, not the brain. Thus, it may be that the benefits seen were from anti-inflammatory effects more than cholesterol lowering [99]. Regardless of the statin mechanism of action, its usefulness may be enhanced in combination therapy, as using simvastatin with quinacrine can produce a synergistic inhibitory effect [78].

Polyene antibiotics—The antifungal drug amphotericin B reduces PrP^{res} formation in cell culture, but is not curative [100]. It and its less toxic analog MS-8209 offer some early treatment benefits in hamsters [101,102,103,104,105,106,107,108], and some late treatment effects in C57BL/6 mice that have been inoculated intracerebrally [109,110], likely by perturbing the raft membrane domains with which PrP^c is associated [100,111]. However, the effect may be somewhat strain-specific [104,105,112], and amphotericin B has been unsuccessful in the treatment of humans with CJD [113]. Another analogue, mepartricin, has efficacy only against intraperitoneal inoculations. Most recently, filipin, a relative of amphotericin B, has been shown to reduce PrP^{res} in cell culture [111], but no *in vivo* data is available at this time.

Suppressing PrP^c expression

Targeting PrP^c expression may hold promise for later stage therapy, given that PrP^c is required for susceptibility to prion disease [114], that turning off neuronal PrP^c expression has no observable consequence in adult mice [115], and that a reversal of neuropathological [116] and behavioral [117] abnormalities has been observed in scrapie-inoculated mice when neuronal expression of PrP^c was eliminated, despite the continued presence of PrP^{res} deposits and accumulation of infectivity [116]. Effective methods for reducing PrP^c expression in cell culture include the use of small interfering RNA (siRNA) to the PrP gene (Prnp) [118], or specific Prnp nucleotides 392-410 [119]. Recently, a lentivirus vector, designed to express a short hairpin RNA corresponding to Prnp nucleotides 512-532, had significant effect *in vitro* and in a chimeric mouse infected with scrapie [120].

Other / unknown mechanisms

Copper, chelators—The role of copper in the functioning and conversion of normal PrP, which has several copper binding sites, has been much debated [121,122,123,124,125,126,127,128]. The proteinase K resistance of PrP^{Sc} is enhanced *in vitro* as copper concentrations are increased, suggesting that decreasing copper may be beneficial therapeutically. Copper chelators have had promising effects in cell culture, and examples such as D(-)-penicillamine [129] and clioquinol [130] have shown modest effects *in vivo*. It should be noted, though, that their effectiveness may correlate more with superoxide dismutase ability than chelating properties [131]. Also, clioquinol has structural similarity to quinacrine, raising questions regarding its actual mechanism of action. The chelator chrysoidine inhibits PrP^{res} in cell culture, without reducing PrP^c levels or destabilizing PrP^{res} [132], but again, chelation may not be its sole mechanism of action. Further confusing the issue is the recent observation that providing infected mice with copper in their water was sufficient to prolong their incubation periods [133].

Dimethylsulfoxide (DMSO)—This organic solvent inhibits PrP^{res} accumulation in cell culture [134] and lowers the infectivity titre in brain homogenate [135], but has significant toxicity issues in long term use [136]. Also, studies vary in their support for any treatment effect *in vivo* [136,137].

Antivirals—Early attempts to treat human CJD patients with a wide variety of antiviral agents were unsuccessful, except for some temporary modest improvements reported in two of three CJD patients intermittently treated with vidarabine (adenine arabinoside) [138]. Subsequent studies of rifampicin, cytosine arabinoside, adenosine arabinoside, isoprinosine, amantadine, methisazone, phosphonoacetic acid, virazole, methisoprinol, β -propiolactone, and thiamphenicol have failed to show any effect in mice [101,139,140,141,142].

Targeting accessory molecules and pathways to conversion

Laminin receptor precursor protein (LRP/LR)—The 67 kDa laminin receptor and its 37 kDa precursor bind PrP^C [143,144], and possibly PrP^{res} [145], and play roles in PrP^{res} propagation [146]. Several techniques have been used to target LRP/LR, including small interfering RNAs (siRNAs), antisense RNA, LRP antibodies [146], and more recently, recombinant single chain antibodies (scFvs) [147]. These approaches have been well reviewed in reference [148].

Cell signalling pathways—A variety of enzyme and cell signalling inhibitors have been screened and tested for their anti-prion abilities. The mitogen-activated protein kinase ½ (MEK½) inhibitor SL327 [149], the cysteine-protease inhibitor L-trans-epoxysuccinyl-leucylamido (4-guanidino) butane (E64d) [71], and inhibitors of phospholipase A₂ (PLA₂) and platelet activating factor (PAF) [150], all decrease PrP^{res} in cell culture. The p53 inhibitor pifitrin-α also reduces PrP^{res} *in vitro*, but has no anti-prion effect *in vivo* [137]. The tyrosine kinase inhibitor STI571, also known as imatinib mesylate, has efficacy *in vitro* and *in vivo*, likely via targeting lysosomal degradation pathways. It cured cells of PrP^{res} after 10 days of treatment [151,152], decreased spleen PrP^{res}, and delayed neuroinvasion if it was administered early after intraperitoneal inoculation. However, even intracerebral delivery of this drug could not clear PrP^{res} from the brain [152].

Enhanced PrP^{res} clearance

Polycations—Anti-prion tendencies *in vitro* have been reported in a group of cationic polyamines which may exert their effects, in part, via enhanced PrP^{res} clearance [153]. Polypropyleneimine generation 4.0, polyethyleneimine, and polyamidoamide generation 4.0, are components of Qiagen SuperFect transfection reagent which prevent PrP^{res} formation [153] and remove infectivity from cell culture [154], possibly acting at the level of the lysosome [154]. Phosphorus dendrimers, generation 4, are less toxic, more bioavailable, and can reduce the splenic content of PrP^{res} in mice inoculated intraperitoneally [155]. Spermine and spermidine reduce the tRNA-induced polymerization of alpha-PrP, suggesting they might influence prion infection [156]. DOSPA is a lipopolyamine which enhances PrP^{res} clearance in cells and also blocks de novo formation. Its activity is dependent on membrane association [157].

NEUROPROTECTION

Compounds without direct effects on PrP^C, PrP^{res}, or conversion, may have therapeutic potential as neuroprotective agents or symptomatic treatment.

Analgesics

Flupirtine maleate is a triaminopyridine analgesic which has been tested in a prospective double-blind treatment trial of 28 human CJD and found to significantly improve cognitive functioning, although the study was not powered to detect any differences in survival [158]. It has anti-apoptotic effects in cell culture models of β-amyloid [159] and prion protein fragment PrP106-126 [160], likely mediated through up-regulation of the proto-oncogene bcl-2 and normalization of glutathione levels [159,160].

Cannabis

Cannabidiol is a nonpsychoactive constituent of cannabis which readily crosses the blood brain barrier, targets the brain, and has minimal toxicity. It can inhibit PrP^{res} accumulation in cells, reduce PrP^{res} toxicity in primary neuronal cultures, and prolong survival in intraperitoneally inoculated mice given early treatment, while having no effect on cell-free

conversion, PrP^c expression, or PrP^{res} stability [161]. Interestingly, brain PrP^{res} levels were reduced, while spleen levels were not. It was proposed that cannabidiol's anti-prion effect may actually be one of neuroprotection, possibly via inhibition of PrP^{res}-induced microglial cell migration, or its ability to antagonize the NMDA receptor.

Antioxidants

Many studies have found associations among PrP^c, prion infection, and oxidative stress [162,163,164,165,166,167,168,169], and some have proposed that PrP^c has superoxide dismutase (SOD) activity [170]. After the initial appearance of PrP^{res} deposits in BSE-infected mice expressing bovine PrP^c, brain levels of the endogenous lipophilic antioxidants coenzyme Q 9 and 10 steadily increase, suggesting that the anti-oxidant system may be overwhelmed in prion infection [171]. Antioxidant therapy may therefore be of some benefit. Some pyrazolone derivatives, derived from the free radical scavenger edaravone, can inhibit PrP^{res} in cell culture at a concentration as low as 3 nM [172]. However, no *in vivo* data is available, and given that there was no correlation between inhibitory activity and the compounds' SOD, copper oxidizing, or copper binding activities, it is unclear whether antioxidant properties were responsible for the effect.

Ginkgo Biloba

Egb761, an extract of leaves from Ginkgo biloba, has been explored as a treatment for improving cognition in dementia patients, and can interfere β -amyloid formation, aggregation, and toxicity [173,174,175]. The mechanism of action is unclear, but bilobalide, a component of Egb761, appears to be an antagonist of the GABA(A) receptor, whose $\alpha 5$ subunit, found predominantly in the hippocampus, has been implicated in the processes of learning and memory. Mice whose $\alpha 5$ subunits are blocked with drugs [176] or who are genetically engineered without this subunit [177] have enhanced spatial learning. Because the loss of spatial learning is an early sign in some rodent-adapted scrapie models, it was theorized that an upregulated GABA(A) receptor might play a role in prion pathogenesis, and that its modulation might affect disease outcome. However, intraperitoneal Ginkgo biloba administered at 1250 mg/kg/day (corresponding to 45 mg/kg/day bilobalide) starting at 21 days post-inoculation had no effect on the survival times of intracerebrally inoculated tg7 mice (Sim, Morrey, and Caughey, unpublished data).

COMBINATION THERAPY

Given the partial efficacies of treatments targeting PrP conversion, PrP^{res} clearance, PrP^c expression, and neuroprotection, it makes sense to try combining therapies and look for cooperative or synergistic effects. Precedence for this can be found a few examples. A more than additive improvement of survival was discovered with a combination of pentosan polysulphate and Fe(III)*meso*-tetra(4-sulfonatophenyl)porphine (Fe-TSP) in tg7 mice inoculated intracerebrally with scrapie [45]. Weekly intracerebral treatments more than doubled survival times when treatment was initiated 2 weeks post-inoculation. In another instance, quinacrine enhanced PrP^{res} inhibition in cell culture when used in combination with the conversion-resistant mutant rPrP-Q218K [79,178]. Quinacrine also produced more than additive inhibition when cells were co-treated with simvastatin or desipramine. This latter observation led to the creation of a more potent cell culture inhibitor of PrP^{res}, called quipramine, by covalently linking the acridine scaffold of quinacrine with the iminodibenzyl scaffold of desipramine [78].

CONCLUSIONS

As new techniques are developed for earlier and more sensitive detection of PrP^{res} [1,26,27,28,29], and as we continue to learn more about prion pathogenesis, many compounds with prophylactic and early treatment potentials may become practical for clinical use, in addition to decontamination purposes. Combining several arms of therapy which block conversion, promote PrP^{res} clearance, and ameliorate prion disease pathogenesis, may greatly enhance our abilities to significantly influence clinical outcomes.

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Table 1

Chemical-based therapeutic and prophylactic agents.

Compound	In vitro	In vivo (early)	In vivo (late)	Comments	References
POLYANIONS					
Heteropolyanion-23	+	+	-		[30,141,179,180]
Dextran sulphate	+	+	-	Prolongs incubation after ic inoculation if given within 2 hours (hamsters).	[30,38,41,180]
Pentosan polysulphate (PPS)	+	+	+	Intraventricular infusion prolongs incubation (ig7 mice). Used in humans. Inhibits Pp ^{res} formation in cell culture but can stimulate cell-free conversion. PPS + Fe-TSP has more than additive effects in vivo.	[30,32,35,37,38,39,40,41,181]
Heparan sulphate	+	+	-	Inhibits Pp ^{res} formation in cell culture but can stimulate cell-free conversion.	[30,31,32,35]
Heparan sulphate mimetics, e.g. HM2606, CR36	+	+	-		[43,182,183]
Fucoidan	+	+		Non-toxic. Oral administration. Strain dependent.	[44]
Phosphorothioate oligonucleotides	+	+			[45]
RNA aptamers	+	-	-		[184,185]
Copaxone	+	+	-		[46]
SULPHONATED DYES AND RELATED COMPOUNDS					
Congo red	+	+		Only modest prophylactic effects in vivo. Subinhibitory concentrations stimulate Pp ^{res} in cell-free conversion. Possible teratogen and/or carcinogen.	[30,47,51,52,53,54,55,56,57,58,59,60]
Suramin	+	+		Only modest prophylactic effects in vivo.	[41,61]
Curcumin	+	+	+	Low dose effective 100 dpi in intracerebrally inoculated mice.	[63,65]
CYCLIC TETRAPYRROLES					
PcTS, DPG ₂ -Fe ³⁺ , TMPP-Fe ³⁺	+	+	-		[66,67,68]
In-TSP	+				[42]
Fe-TAP, Fe-TSP	+	+	+	Fe-TSP & PPS have more than additive effects in vivo.	[17]

Compound	In vitro	In vivo (early)	In vivo (late)	Comments	References
QUINACRINE, QUINOLINE, ACRIDINES, PHENATHIAZINES					
Quinacrine	+			Quinacrine + desipramine or simvastatin better than quinacrine alone in cells. Quinacrine + PrP-Q218K enhances inhibition in cells. No human benefit seen to date; trial ongoing. Increases amount of PrP ^{Pres} in the spleen. Crosses blood-brain barrier.	[71,72,73,75,77,78,79]
Chlorpromazine	+	+			[72,76]
Quinine and biquinoline	+	+	-		[80]
Mefloquine	+	-	-		[81]
OTHER AMYLOIDOPHILIC COMPOUNDS	+	+		Oral administration.	[85]
PYRIDINE DICARBONITRILES	+				[24,25,86]
PEPTIDE APTAMERS AND B-SHEET BREAKERS					
Peptide aptamers	+				[90]
β -sheet breaker peptides	+	+		Tested by mixing with inoculum only. May be strain or cell model specific.	[91,92]
CHOLESTEROL DEPLETING AGENTS					
Lovastatin, squalestatin	+				[94,95]
Simvastatin	+	+	+	Effect may be unrelated to cholesterol lowering; brain cholesterol levels do not drop.	[78,98,99]
Amiodarone, progesterone	+				[78]
Mevinolin				Causes sequestration of PrP ^C in Golgi apparatus. No infectivity experiments.	[93]
7-dehydrocholesterol reductase and 24-dehydrocholesterol reductase inhibitors	+			No effect even if given prior to inoculation.	[97]
POLYENE ANTIBIOTICS					
Amphotericin B, MS-8209	+	+	+	Human treatments ineffective.	[100,101,103,104,105,106,107,108,109,110,111,113]
Mepartricin	+	+	-		[103]
Filipin	+				[111]
COPPER/CHELATORS					
D(-)penicillamine	+	+			[129]

Compound	In vitro	In vivo (early)	In vivo (late)	Comments	References
Clioquinol		+		Structurally similar to quinacrine.	[130]
Copper	#	+	-	Can promote or inhibit PrP conformational conversions.	[121,122,123,124,125,126,127,128,133]
Chrysoidine	+			Mechanism of action may not relate to chelating properties.	[132]
DMSO	+	-		Conflicting treatment outcomes.	[134,135,136,137]
ANTIVIRALS					
Adenine arabinoside		+	+	2 out of 3 CJD patients had moderate temporary improvement if treated early in course. Mouse studies failed to show effect.	[138]
INTRACELLULAR MECHANISMS					
MEK½ inhibitors (SL327)	+				[149]
Cysteine-protease inhibitors (E64d)	+				[71]
Phospholipase inhibitors	+				[150]
P53 inhibitors (Pifitrin- α)	+	-	-		[137]
Tyrosine kinase inhibitors (STI571, imatinib mesylate)	+	+	-		[151,152]
POLYCATIONS					
Polypropyleneimine gen. 4.0, polyethyleimine,polyamidoamide gen. 4.0	+				[153,154]
Phosphorus dendrimers generation 4	+	+			[155]
Spermine, spermidine	+				[156]
DOSPA	+				[157]
NEUROPROTECTION					
Flupirtine maleate	+		+	Tested in human trials; some improvement in cognition. May be neuroprotective via up-regulation of bcl-2 and normalization of glutathione levels.	[158,159,160]
Cannabidiol	+	+	-	No apparent interaction with PrP ^{sc} or PrP ^{Pres} . May be protective via inhibition of PrP ^{Pres} -induced microglial cell migration, or antagonism of the NMDA receptor.	[161]
Antioxidant pyrazolone derivatives	+			Properties other than antioxidant ones may be responsible for effect.	[172]

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- + Effect demonstrated
- Effect not present
- # Induces conversion in vitro