Novel therapeutic uses for porphyrins and phthalocyanines in the transmissible spongiform encephalopathies

Commentary

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Abbreviations

BSE	bovine spongiform encephalopathy
CJD	Creutzfeldt-Jakob disease
nvCJD	new variant CJD
PrP	prion protein
TSE	transmissible spongiform encephalopathy

Introduction

Until recently the transmissible spongiform encephalopathies (TSEs), a group of infectious and fatal neurodegenerative diseases, were a fascinating set of diseases of unusual etiology that had relatively little impact on human health (see [1] for a general review). The most common human form Creutzfeldt-Jakob disease (CJD), while a devastating illness to contract, is rare and occurs world-wide at an incidence of about 1 in every 1–2 million people. Rarer still are cases of iatrogenic CJD, associated primarily with corneal or dura mater transplantation, and familial CJD, which has been identified as a heritable disease in only a few families. Even the risk to humans from animal TSE diseases was considered minimal since there had never been any evidence that scrapie, the well-known sheep TSE, could infect humans.

The situation changed dramatically with the onset of the bovine spongiform encephalopathy (BSE) epidemic in cattle in the United Kingdom in the mid-1980s. Concerns that humans could be at risk via exposure to cattle byproducts were apparently confirmed when a previously unknown form of CJD (new variant CJD or nvCJD) was described in young people in England [2]. It was hypothesized that the most likely cause of nvCJD was exposure to BSE contaminated materials; a hypothesis that has since been supported by several different studies [3–5]. At present, 40 cases of nvCJD have been confirmed and there is some concern that the number of cases could be on the rise [6]. Thus, with the potential exposure of millions of people to BSE and the onset of nvCJD the need for anti-TSE drugs has become acute.

Identification of anti-TSE drugs

The unusual nature of TSE diseases, which are infectious but have no known viral or bacterial components, has made standard antiviral and antibacterial approaches to disease treatment especially difficult. The situation is further complicated by the fact that there is little or no immune response to infection. Over the past 25 years a variety of different classes of compounds have been tested as inhibitors of TSE disease. These include antiviral, antibacterial and antifungal drugs, hormones, immunosuppressants and sulfated polyanions (see [7,8] for reviews). Several different compounds from within these classes have been shown to significantly delay disease in mouse and hamster models of scrapie (Table 1). As shown in Figure 1, the structures of these compounds are varied suggesting that there is not a common mechanism of action. Inhibition can be dependent upon the dose, route of inoculation and strain of scrapie agent, as well as upon the dose, route of inoculation, and time of administration of the inhibitory compound. Unfortunately, the difficulty with almost all of these anti-TSE compounds is that most have the greatest inhibitory effect if given near the time of infection. This severely limits the effectiveness of these compounds in treatment of human TSE disease where the time of infection is generally unknown, no early diagnostic test is available, and the disease itself is difficult to detect until well after the onset of clinical symptoms [7].

Identification of therapeutic drugs for the TSE is further complicated by a lack of understanding of many of the key aspects of TSE pathogenesis, such as the exact nature of the infectious agent, the manner in which the agent enters target cells, or the mechanism by which neurodegeneration is induced. Although complete understanding of disease pathogenesis is certainly not a prerequisite for drug development, it does help to identify potential targets for therapeutic intervention that could then be used as a basis for rational drug design.

Fortunately, there is one obvious target for therapeutic intervention in TSE diseases. Spongiform encephalopathies derive their name from the major pathological changes observed in the brain which can include a characteristic spongiosis as well as neuronal loss and gliosis. However, the most consistent finding in TSE diseases is the accumulation in these affected areas of an abnormal form of the host prion protein, PrP. Normal PrP, a glycoprotein that is expressed on the cell surface in a wide variety of tissues, is both detergent soluble and sensitive to digestion with proteinase K (PrP-sen). PrP-sen is the precursor to PrP-res, the abnormal form of PrP associated with disease. Although PrP-res has the same amino acid sequence as PrP-sen, it is quite different from PrP-sen in terms of three-dimensional structure (i.e. conformation), and thus forms insoluble aggregates and is partially resistant to digestion with proteinase K. The fact

Table 1

Compounds that inhibit PrP-res formation *in vitro* and TSE disease *in vivo*.

Compound tested	Type of compound	IC_{50}*	Disease inhibition†	Reference‡
Pentosan polysulfate	Sulfated glycan	1 ng/ml	Yes	[20]
Congo red	Disulfonated dye	8 ng/ml	Yes	[16]
Dextran sulfate 500	Sulfated GAG	9 ng/ml	Yes	[21]
Phthalocyanine tetrasulfonate	Cyclic tetrapyrrole	0.5 μg/ml	Yes	(a)
Deuteroporphyrin IX 2,4 bis(ethylene glycol) iron (III)	Cyclic tetrapyrrole	5 μg/ml	Yes	(a)
4'-iodo-4'-deoxy-doxorubicin	Anthracycline	NT	Yes	[22]
Amphotericin B	Polyene antibiotic	>10 μg/ml	Yes	[23]
Chondroitin sulfate	Sulfated GAG	>10 μg/ml	No	[21]
Dextran	Neutral glycan	>10 μg/ml	No	[24]

*Concentration of inhibitor at which the amount of PrP-res in mouse scrapie-infected murine neuroblastoma cells is decreased by 50%. Data derived from [14,25]. †The conditions under which disease inhibition occurs and the extent of inhibition vary depending upon the compound and the *in vivo* model system used. ‡References to the original studies. [a]SA Priola, unpublished data.
GAG, glycosaminoglycan; NT, not tested.

that PrP-res is found in TSE-infected tissues and is closely associated with tissue preparations enriched for infectivity has led to the proposal [9] that PrP-res itself is the infectious protein agent first hypothesized for the TSE over 30 years ago [10]. Although this hypothesis has yet to be definitively proven and remains a point of contention in TSE research, there is a significant amount of information available about PrP-sen and PrP-res, and the key role of PrP in disease pathogenesis is undeniable. Thus, one obvious approach to identifying anti-TSE agents is to find compounds that prevent formation of PrP-res.

Two different *in vitro* assay systems have been used to identify inhibitors of PrP-res formation. The first assay uses mouse scrapie-infected mouse neuroblastoma cells. These cells replicate mouse scrapie infectivity and make mouse PrP-sen and mouse PrP-res [11]. Potential anti-TSE compounds can be assayed by simply adding the compound to the cell medium and measuring the amount of PrP-res in the cells [12]. The second system is a cell-free conversion system that models the conversion of PrP-sen into protease-resistant abnormal PrP in a test tube [13]. In this assay, radiolabeled PrP-sen is combined with PrP-res plus any potential anti-TSE compound and the amount of radiolabeled PrP-res generated is measured [14]. Inhibition of PrP-res formation in either assay system has correlated well with inhibitors previously known to effect *in vivo* TSE disease (Table 1) and has thus provided strong evidence that the conversion of PrP-sen into PrP-res is a critical event during disease pathogenesis.

Identification of compounds that inhibit PrP-res formation *in vitro* has already led to the discovery of anti-TSE drugs and allowed some studies on their mechanism of action. For example, Congo red was first identified as a potent inhibitor of PrP-res formation using mouse scrapie-infected neuroblastoma cells [15] and was subsequently shown to inhibit TSE disease in animals [16]. Further studies on chemically modified forms of Congo red in both the mouse scrapie-infected tissue culture cells and the cell-free conversion system were able to identify structural determinants in Congo red that influenced PrP-res formation [17]. Therefore, these *in vitro* assays can be used not only to identify potential anti-TSE drugs prior to *in vivo* testing but also to address the mechanisms behind both formation and inhibition of PrP-res.

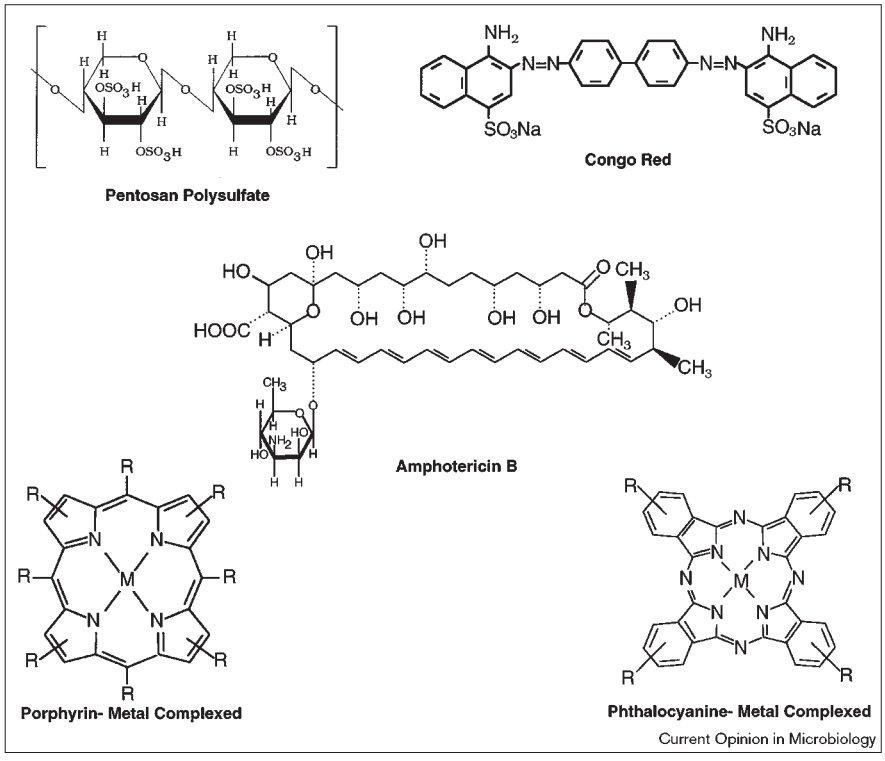
Porphyrins and phthalocyanines

An examination of the extensive literature on the interactions of porphyrins and phthalocyanines with proteins provided a basis for a search for effective inhibitors of PrP-res formation among these compounds. Porphyrins and phthalocyanines (Figure 1) are cyclic tetrapyrroles, a large class of compounds that include the biologically important hemes and chlorophylls [18]. Some of these compounds are known not only to bind strongly and selectively to a protein but also to induce changes in the protein's conformation [19]. Since the conversion of PrP-sen into PrP-res involves changes in protein conformation the possibility that a compound from this class might serve as an inhibitor of the conversion process, and thus an inhibitor of TSE disease, was examined. In fact, when over 30 different porphyrins or phthalocyanines were tested for their ability to inhibit PrP-res formation in scrapie-infected mouse neuroblastoma cells and the cell-free conversion assay, two-thirds were able to inhibit formation of PrP-res by greater than 90% at concentrations of $\leq$10 μM [14]. The fact that these compounds can inhibit PrP-res formation suggests that they are potential inhibitors of TSE disease. This is indeed the case as recent studies have shown that treatment with some porphyrins or phthalocyanines can more than double the lifetime of scrapie-infected animals (SA Priola, unpublished data). These recent findings render the porphyrins and phthalocyanines a new and promising class of anti-TSE agents.

The cyclic tetrapyrroles have a number of potential advantages. Practically speaking, many of the porphyrins and

Figure 1

Structures of some inhibitors of TSE disease. Five different inhibitors are shown. Only a basic structure is shown for the porphyrins, and phthalocyanines as structures may vary widely. The 'R' may represent a variety of different groups; a line from the 'R' into a ring denotes attachment of the R group at any point on the ring. The 'M' in the middle of the macrocylcic ring structures can represent a wide variety of metals (e.g. iron or aluminum), which can bond via central nitrogens with the porphyrin or phthalocyanine structure. When metal-free, porphyrins and phthalocyanines have two central nitrogens with hydrogen bound.



phthalocyanines have demonstrated low toxicities *in vivo* and are sufficiently inexpensive to make their use feasible either prophylactically or therapeutically. Their potential for crossing the blood–brain barrier, an important consideration in the TSE, can be enhanced by the lipophilic character of the macrocyclic ring (Figure 1), by the type of substituents that can be attached to the ring, as well as by known techniques for introducing porphyrins and phthalocyanines into liposomes. Some porphyrins have already been successfully used therapeutically in humans in many ways including photodynamic therapy (e.g. gliomas), radiotherapy-based cancer treatments, and as a treatment for jaundice. A large number of structurally diverse porphyrins and phthalocyanines are currently available and their chemistry has been examined in detail. Known synthetic methods permit the introduction of a wide variety of groups at the periphery of the macrocyclic ring as well as several different metals at the central nitrogens (Figure 1).

Using the porphyrins and phthalocyanines as anti-TSE agents represents a new therapeutic application of these compounds that appears to rely on one of the basic biological roles of porphyrins. Namely, to affect changes in protein conformation. It is well known from many studies that subtle changes in protein structure effect porphyrin structure and vice versa. The changes that can occur in the three-dimensional structure of porphyrins are remarkably well understood as a result of the many crystal structures available. Thus, it is possible that the mechanism of action of the compounds as inhibitors of PrP-res formation could be elucidated from their use as reporters of changes in protein structure. This in turn could lead to a greater understanding of the conformational changes that occur during the conversion of PrP-sen to PrP-res.

Conclusions

The ability of porphyrins and phthalocyanines to inhibit abnormal PrP formation coupled with their chemical properties makes it possible that a compound could be designed to specifically inhibit TSE disease or even other amyloid diseases such as Alzheimer's disease. In the case of the TSE, drugs could be designed with a low toxicity and a long half-life *in vivo* that could be used prophylactically to prevent formation of PrP-res. Other compounds, which cross the blood–brain barrier and prevent further formation of abnormal protein, could be designed for treatment after disease onset. It is clear that there are a number of potential uses for porphyrins and phthalocyanines in TSE disease research and the challenge in the future will be to realize this potential.

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