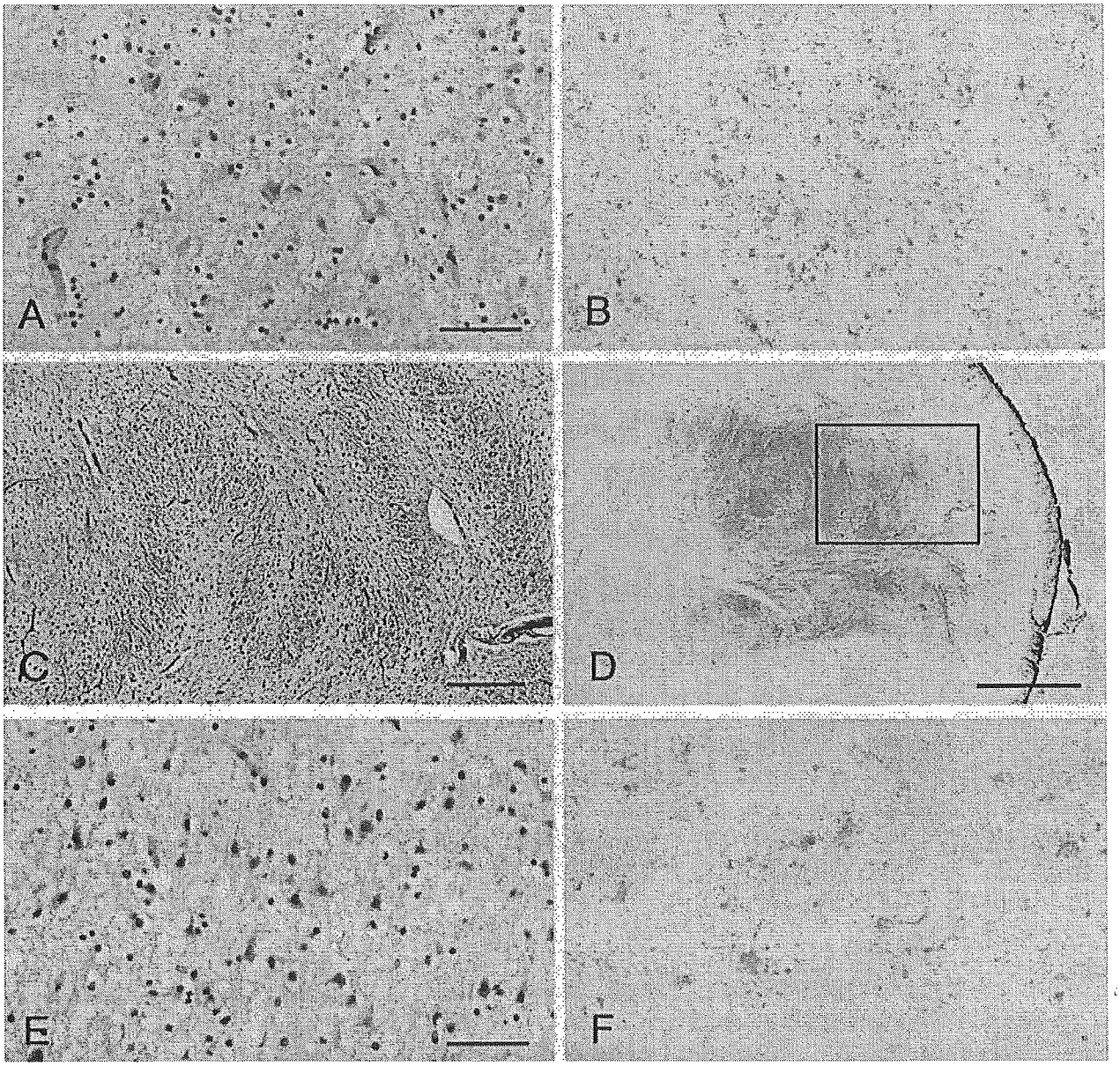

Figure 4. Neuronal loss and gliosis revealed by haematoxylin and eosin stain (A, E), Bodian's stain (C) or immunohistochemistry for glial fibrillary acidic protein (GFAP) (B, D, F). A, B: centromedial nucleus of the thalamus. C, D: inferior olivary nucleus. Neuronal loss is more evident in the medial part (left side of panel C, which represents the rectangular area depicted in panel D). E, F: cerebral cortex (frontal lobe). Both spongiform change and gliosis are remarkable. Bars: 50 μm (A, B, E, F), 200 μm (C), 1 mm (D).

amus or inferior olivary nucleus (Figure 5C,D). In the cerebellar molecular layer, punctate deposits of PrP were focally observed (Figure 5A), and the regions with these deposits were coincident with the extent of spongiform change. Likewise, fine granular deposition of PrP was also detected together with spongiform degeneration in the cerebral cortex (Figure 5B). The distribution of PrP deposits appeared to be more broad and noticeable in the cerebral cortex than in the cerebellum. As a unique finding, the anti-PrP antibody revealed swollen and/or frizzled axons in the deeper parts of the cerebral white matter, in the corpus callosum, or at the borders of the thalamus and caudate nucleus (Figure 5E). Axonal transported substances, APP (Figure 5E, inset) and SNAP-25 (data not shown) were also detected immunohistochemically in those axons.

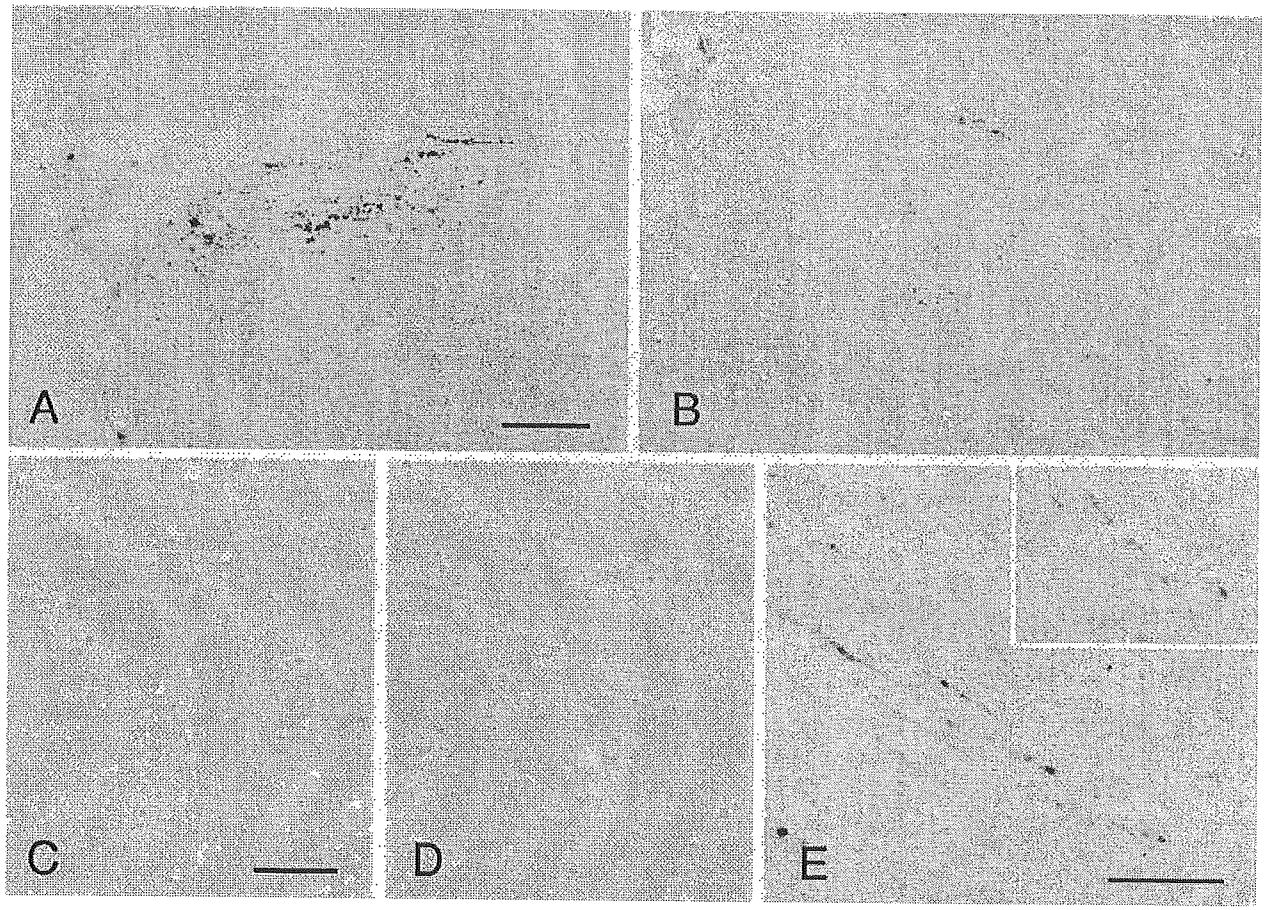

Figure 5. Immunohistochemistry for prion protein (PrP) deposition. A: cerebellum. B: frontal cortex. C: centromedial nucleus of the thalamus. D: inferior olivary nucleus. PrP deposition can not be detected in the thalamus or the inferior olivary nucleus, but coarse or fine granular PrP deposition is visible within the cerebral cortex and the cerebellar molecular layer. E: axons with swollen and/or frizzled features can be detected in the white matter at the border of the thalamus. These axons are also immunostained with anti-APP (amyloid precursor protein) antibody (inset). Bars: 50 μm (A, B, E), 100 μm (C, D).

Although the conventional method of Western blot analysis for PrP^{res} failed to detect any particular signal (data not shown), by application of phosphotungstic acid precipitation that preferably concentrates PrP^{res} but not cellular PrP [12], Western blot analysis of the extract from the frontal cortex of this case revealed a detectable amount of PrP^{res} (Figure 6). The molecular weight of non-glycosylated form of PrP was about 19 kDa (PrP^{res} type 2 pattern) and also the PrP^{res} glycoform ratio was compatible with that of FFI, which has been previously reported [14]. The extract from the cerebellum showed no significant signal in Western blot analysis even with phosphotungstic acid precipitation (data not shown).

The disease of this case was successfully transmitted to some of the mice inoculated with tissue homogenate from the frontal cortex. The incubation time was 571.6 ± 61.1 days (5/7 of the inoculated mice developed TSE) in the NZW mice and 736 ± 64.4 (5/8) in the Tg7 mice, respectively. Although not all the mice developed TSE, diseased mice demonstrated lethargy in the terminal stage rather than excitability. In the TSE-developed mice pathological examination of the brain showed that spongiform change and gliosis were prominent in the cerebral cortex in addition to the thalamus (Figure 7). Immunohistochemistry for PrP revealed that diffuse granular PrP deposition was present within the deep layer of the cerebral cortex as well as in the lateral portion of the thalamus (Figure 7B,E).

Discussion

It is established that there is an overlapping spectrum between classical FFI and CJD in association with PRNP

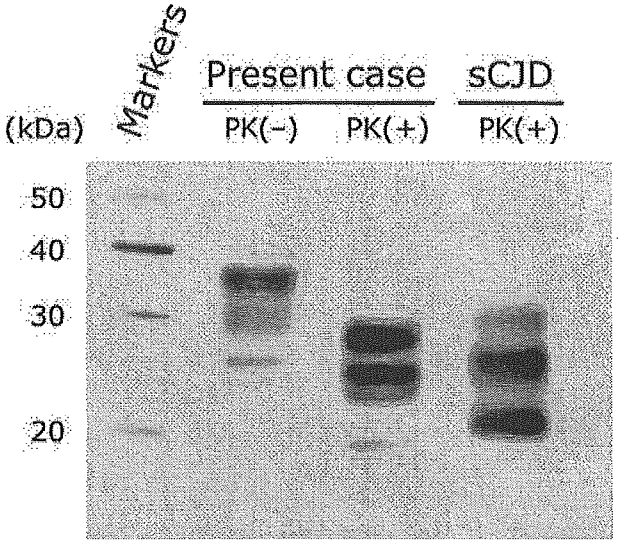

Figure 6. Western blot analysis for protease-resistant prion protein (PrP^{res}). Brain homogenate of the frontal cortex of this case is treated with or without proteinase K (PK), and then PK-digested sample is followed by the 40-times concentration with phosphotungstic acid precipitation for PrP^{res}. The abnormal PrP molecules in the frontal cortex of this case migrate as PrP^{res} type 2. PrP molecules in the lane sporadic Creutzfeldt-Jakob disease (sCJD) are also shown as a standard type 1 PrP (MM1). Molecular sizes (kDa) are indicated on the left.

D178N [6]; however, this case adds to our knowledge about this disease. Although the present case had FFI genotype, the clinical features were initially characterized by prominent cerebellar ataxia, and the neuropathological findings were also atypical in the following respects. First, PrP deposition and spongiform change in the cerebral cortex were more conspicuous than in the thalamus or inferior olivary nucleus, both of which are extremely vulnerable sites for FFI. It has been previously reported that heterozygotes Met/Val at codon 129 result in a longer clinical course than homozygotes [4], and it is therefore possible that the lesions seen in the cerebral cortex were more prominent simply because of the longer course of illness in this patient. However, a further noteworthy point about this case is rather that there was no PrP deposition either in the thalamus or in the inferior olivary nucleus.

Second, immunohistochemical examination detected a peculiar deposition of PrP within the molecular layer of the cerebellum. The localized lesions of granular deposits of PrP and spongiform change in the cerebellar molecular layer seemed to be similar to those reported in a patient from an Austrian FFI family [15]. The cerebellar ataxia of this case could have attributed to the loss of granular neurones and degeneration of Purkinje's cells, in addition to the lesions of the inferior olivary nucleus, although the pathology related to PrP deposition could have also been responsible.

A third atypical feature is that the neuronal loss in the thalamus was most noticeable in the centromedial nucleus. A previous study revealed that severe atrophy of the anterior ventral and dorsomedial thalamic nuclei was consistently observed, whereas that of other thalamic nuclei was less severe and they were inconsistently affected [2]. In this case, the medial portion of the thalamus was indeed damaged crucially, but the principal lesion was different from the typical pathology of FFI.

In addition, an interruption of axonal transport was suggested. Some of the axons were swollen and associated with PrP accumulation, and both APP and SNAP-25 were also accumulated in those axons. APP and SNAP-25 are presynaptic protein and APP is considered as the most effective marker for axonal injury [16]. Aberration in recruitment of PrP might be involved in the pathogenesis of TSE, as described previously [17,18].

This case showed a small amount of specific PrP^{res} in the cerebral cortex but not in the cerebellum as detected by Western blotting. The ratio of PrP^{res} quantity in those regions was visually correlated with that of immunohistochemical reactivity for PrP. Although fresh frozen samples from the thalamus or the inferior olivary nucleus were not obtained for Western blot analyses, we suspect that PrP^{res} in such regions would be too sparse to be detected by Western blotting even in combination with phosphotungstic acid precipitation. The type 2 migration pattern and the glycoform ratio of PrP^{res} in this case were compatible with those in the typical FFI [14]; however, it remains to be elucidated whether these abnormal proteins that can be classified in the same PrP^{res} type may have different influences on the neurodegeneration processes.

Finally, the transmission study revealed that a pathogen in the frontal cortex of this case might be different from that of an FFI case previously reported by Dr Tateishi and his colleagues [13]. NZW mice infected with a thalamic tissue sample of a typical FFI case exhibited excitability as the principal clinical sign and demonstrated PrP deposition predominantly localized within the thalamus. On the other hand, NZW mice infected with a frontal cortical tissue sample from the present case showed lethargy as a clinical sign, and demonstrated diffuse PrP deposition within the deep layer of the cerebral cortex, as well as in the lateral portion of the thalamus. The PrP deposition

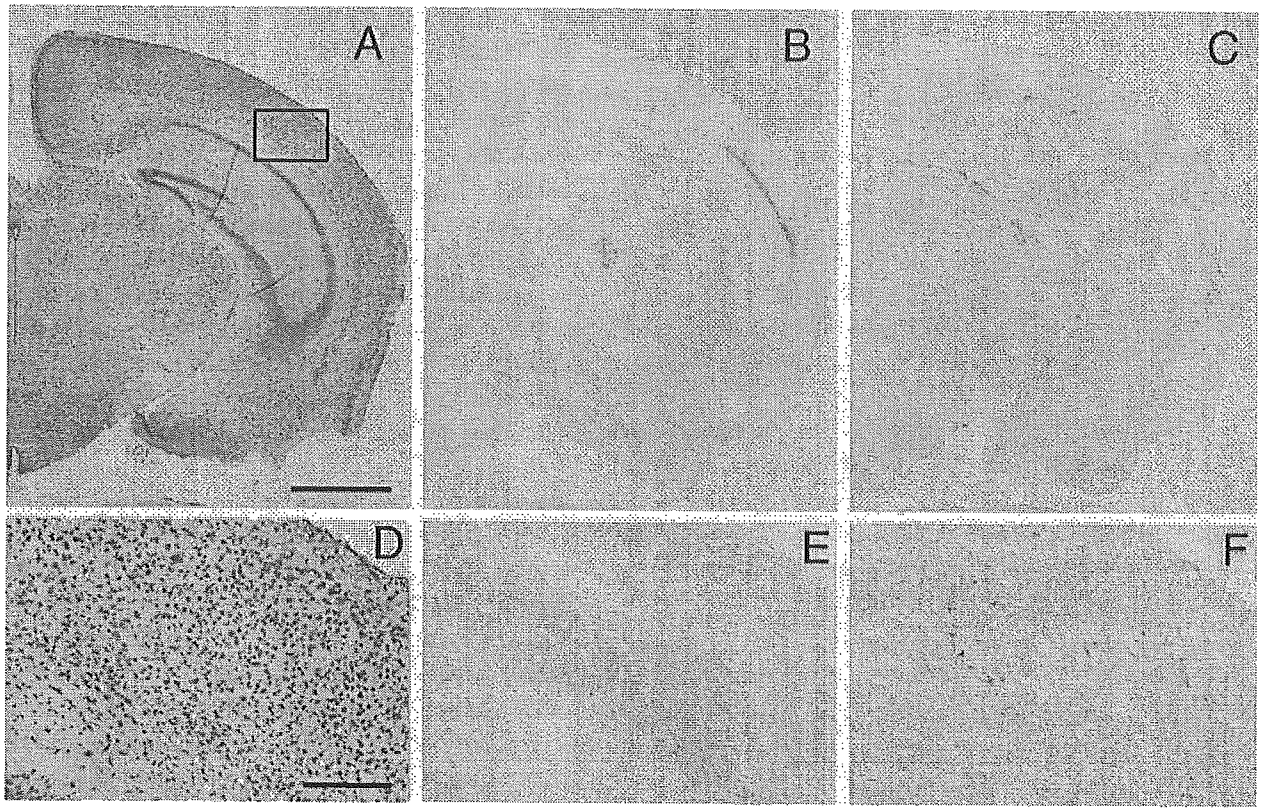

Figure 7. Histological profiles of the mice inoculated with the patient's brain material. A, D: hematoxylin and eosin stain. B, E: prion protein (PrP). C, F: glial fibrillary acidic protein. Spongiform change, PrP deposition and astrocytic gliosis can be observed within the deep layer of the cerebral cortex as well as in the lateral portion of the thalamus. D–F: high power magnifications of the cortical lesions represent the rectangular area depicted in panel A. Bars: 1 mm (A–C), 150 μm (D–E).

pattern of this mouse was distinctive against that of mouse models with other scrapie strains, thus refuting the possibility of contamination. It is not clear whether there were more than two pathogen strains in the brain and whether the strains were dependent on the brain areas. Because we have not examined transmissibility of this case systematically and not obtained frozen materials for Western blot analysis, this aspect still awaits further clarification.

In conclusion, the present case which had FFI genotype showed atypical features, especially with regard to the PrP deposition pattern; there was no deposition within the thalamus or inferior olivary nucleus. Diversity in disease phenotype among patients with the same genotype suggests that some other unidentified factors as well as abnormal PrP deposits or other as yet unknown genetic factors may be responsible for the pathogenesis of the disease. In this study we have shown that variation in pathogen strains may also be one such factor and this factor could have greatly affected the pathogenesis in the present case of FFI.

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Treatment Options in Patients with Prion Disease - the Role of Long Term Cerebroventricular Infusion of Pentosan Polysulphate

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Summary. Prion diseases (PrD), also known as transmissible spongiform encephalopathies, are believed to be caused by accumulation of an abnormal isoform of the prion protein (PrP^{sc}) in the central nervous system. Creutzfeld-Jacob disease (CJD) in its sporadic and variant form is the most frequent and clinically important PrD. At present there is no proven specific or effective treatment available for any form of CJD, although some oral agents, such as quinacrine or flupirtine, are being investigated in clinical trials.

Pentosan polysulphate (PPS), a large polyglycoside molecule with weak heparin-like activity, has been shown to prolong the incubation period of PrP^{sc} infection when administered to the cerebral ventricles in a rodent scrapie model. PPS also prevents the production of further PrP^{sc} in cell culture models. However, PPS penetrates poorly the blood-brain barrier and only a minor fraction of orally administered drug may reach the CNS. These properties of PPS prompted its cerebroventricular administration in patients with vCJD and other PrD, such as iatrogenic CJD and Gerstmann-Sträussler-Scheinker syndrome (GSS). Long-term continuous infusion of PPS at doses of up to 110 μg/kg/d did not cause serious drug-related side effects. Follow-up CT and MRI imaging demonstrated that brain atrophy may progress further during PPS administration, while the neurological status may remain stable. Proof of clinical efficacy has not been the aim of the current clinical studies of PPS, however one patient with vCJD survived for 23 months after initial symptoms and 39 months after diagnosis, while the median duration of illness with vCJD is 13 months (range 6-39).

Some lessons have been learned from the early studies of application of PPS in PrD patients. Surgery in a brain affected by PrD may result in a higher rate of surgical complications than might be expected in analogous cases with other conditions. Secondly, efficacy of PPS or any other treatment option in advanced PrD cases will be very difficult to assess, due to the lack of specific and objective criteria for measurement of response. Overall survival may remain therefore one of the few objective ways of assessing outcome in treated patients. Finally, if clinically significant benefits to patients are to be expected, PPS administration should start as early as possible in the course of the respective disease and before irreversible loss of neurological function has occurred. Further clinical, neuroradiological and laboratory investigations of cerebroventricular PPS administration in the setting of a prospective clinical study will be essential for the assessment of possible clinical benefits of PPS in PrD.

Key words. GSS syndrome, pentosan polysulphate, prion disease, sporadic CJD, transmissible spongiform encephalopathy, quinacrine, variant CJD

Introduction

Significant research interest has been attracted recently by human pathological conditions related to transmitted or intrinsically generated pathologic prion protein. Prion diseases (PrD) have thus a common and unique biological background, but a variable clinical manifestation of the presumably common pathogenetic mechanism. PrD, also known as transmissible spongiform encephalopathies (TSE), are fatal neurodegenerative disorders with different clinical forms including sporadic, inherited, and acquired diseases, the latter including transmissible and iatrogenic forms [1]. All forms of PrD have in common an abnormal metabolism of the prion protein, PrP^{c}, which results in the production of uncleavable, protease-resistant isoforms, PrP^{sc}, accumulating mostly in the CNS and causing neuronal dysfunction and eventually death. Prion diseases can affect both humans and animals and include such conditions as Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker (GSS) syndrome, kuru, and fatal familial insomnia (FFI) in humans, bovine spongiform encephalopathy (BSE) or "mad cow disease" in cattle, scrapie in sheep, and chronic wasting disease in mule, deer, and exotic ungulates [1].

The normal physiologic isoform of the prion protein, PrP^{c}, is found in the body of all mammals. The *Prnp* gene encodes a polypeptide which undergoes glycosylation in the rough endoplasmic reticulum and then gly-

cosyl modification in the Golgi apparatus of the cell. Because of this glycosylation, it is expected that the glycosyl structure of the protein in different cells would be different [2]. The glycosylated PrP^c protein, associated at the C-terminus with a glycosyl phosphatidyl-inositol (GPI), is transported to the surface of the cell membrane. The half-life of PrP^c on the membrane of cells grown in culture is 3-6 hrs, after which it is internalised and degraded in the endolysosome compartment [3, 4]. A shorter peptide form of PrP^c may be recycled to the surface of the cell before lysosomal destruction. Conversion between PrP^c and PrP^{sc} occurs likely during the internalisation process. The specific physiologic function of PrP^c is largely unknown, although studies suggest it may play a role in copper binding and oxidative metabolism [5], interactions with the extracellular matrix, apoptosis, and signal transduction [6]. It is evolutionary conserved, which suggests an important physiologic role, but mice lacking PrP^c appear to grow and function normally [7].

The pathologic form of the prion protein, which is the causative agent "prion" of all PrD, is an abnormally folded isoform of the cellular prion protein PrP^c, known as PrP^{sc}. PrP^{sc} accumulates mostly in the brain of affected mammals [8, 9]. The underlying pathological process involves a post-translational conformational change PrP^c into PrP^{sc} [10, 11]. PrP^c is usually present in an α-helix conformation, in pathologic conditions only a small fraction of PrP^c is folded as α-helix, while the vast majority is present in an unfolded β conformation (β-helix PrP^{sc}). The precise molecular mechanism responsible for this unfolding process is not known, but two models are favoured (for review see [12]). One model suggests that PrP^{sc} acts like a crystal seed for the further addition of converted PrP^{sc} molecules and the subsequent formation of PrP^{sc} aggregates [13], whereas the other model postulates conversion intermediates involving a putative PrP^c-PrP^{sc} heterodimer complex [8, 14]. The claim that the PrP^{sc} is an infective agent involves the demonstration that the PrP^{sc} form can itself modify the structure of PrP^c to PrP^{sc} *in vitro*, however, this modification is inefficient [15]. Recent results suggest that single-stranded RNA molecules are necessary for PrP^{sc} amplification, and that RNA from invertebrates fails to support pathologic prion amplification *in vitro* [16].

PrP^{sc} is only found in infective animals, and mice without PrP^c production cannot become infected or infective [7]. PrP^{sc} appears to accumulate within lysosomes, growing insoluble crystalloid fragments. Initially, PrP^{sc} builds up intracellularly and then it is seen extracellularly as amyloid in histopathologic sections stained with Congo red. Microglia is activated by contact with amyloid plaques and insoluble extracellular PrP^{sc}, which results in local production and release of cytokines, reactive oxygen species, and

glutamate [17, 18]. These compounds give rise to specific local neuronal damage and apoptosis, seen as spongiform defects in the brain. Apoptosis and oxidative damage in neurons seem to follow their local exposure to cytokines [19, 20], and physiological neuronal activity is expected to be severely impaired well in advance of histopathologic changes. The progressive, slow build-up of PrP^{sc} may mean that only tissues where cells are not involved in a continuous turnover are likely to exhibit functional and morphological damage. Although cells of the immune system are also infected by PrP^{sc}, their cellular turnover is considered to prevent the body showing any immunodeficiency; whereas neurons infected with and accumulating PrP^{sc} are damaged but not replaced, and hence long term neurological deficits become clinically manifest.

While the prime target of PrP^{sc}-caused damage seems to be neuronal, massive neuronal loss is not always seen in PrD. On the other hand, activation of astrocytes occurs very early in the course of prion infection of the CNS in a consistent fashion. It can be reproduced easily in experimental models and leads to significant physiological effects such as impairment of the blood-brain barrier [21]. In addition, astrocytes are one of the few cell types capable of supporting prion replication [22]. Microglial cells are another cell type increasingly implicated in brain damage due to prion infection. Experiments indicate that activation of microglia may be essential in causing neuronal damage in PrD, and that this phenomenon is dependent on the expression of PrP^{c} [17]. Moreover, microglial activation and accumulation in affected brain areas precede neuronal cell death and parallel the temporal and spatial pattern of PrP^{sc} deposition [23]. Histologically, common late stage lesions in the CNS are neuronal loss, spongiosis and astrogliosis, accompanied by an accumulation of microglia and, occasionally, the presence of amyloid plaques and various small deposits of prion protein [24]. For a definitive diagnosis of human PrD, histopathologic assessment of the CNS is essential [25].

Sporadic CJD was originally described in 1921 and occurs mostly in individuals between 40-80 years of age, with an incidence of approximately one case per million per year. Patients suffering from CJD show a wide spectrum of clinical symptoms within a few distinctive forms of the disease [26]. While most of the CJD cases at present are sporadic, CJD may also occur as a familial form in no more than 10% of sporadic cases [27]. It follows an autosomal dominant pattern of transmission, with 70% of the patients having mutations in codons 178 or 200 of the *Prnp* gene.

Iatrogenic transmission of CJD has been proven in more than 200 cases in relation to corneal transplants, dura mater grafts, and hormones purified from human glands [28, 29].

FFI and GSS are also inherited by autosomal dominance. Both are very rare, with no more than 10 known families with FFI and 50 with GSS [30, 31]. GSS, unlike other PrD, may have a longer clinical course [32]. It is characterised by missense mutations of the *Prnp* gene, by specific neuropathological lesions and multicentric amyloid plaques.

The most recently recognized form of PrD in humans, new variant CJD (vCJD), was first described in 1996 as linked to BSE [33]. What distinguishes vCJD from sporadic cases is that the age of patients is much lower (vCJD age range 19-39 years, versus sCJD age range 55-70 years) and the duration of illness is longer (vCJD 7.5-22 months, versus sCJD 2.5-6.5 months). Variant CJD displays a distinct pathology characterised by abundant florid plaques surrounded by vacuolation [34]. Most cases of vCJD have been observed in the UK. In addition, all investigated cases of vCJD showed homozygosity of methionine at codon 129 [35, 36].

The clinical features of PrD are extremely heterogeneous and may include rapidly progressive dementia, psychiatric symptoms (mostly in vCJD, less in CJD), cerebellar syndrome (in kuru, GSS, CJD), movement disorders (myoclonus, dystonia, chorea, mostly in vCJD), encephalopathy (in CJD), pyramidal signs, cortical blindness, and sensory symptoms (hyperpathia, mostly in vCJD) [26, 32, 37, 38].

Treatment options in prion disease

PrD are still uniformly fatal, some within weeks to months from diagnosis, while vCJD patients may survive for more than a year, and GSS patients for up to 6 years. No specific treatments for PrD are known, although some prophylactic and neuroprotective agents have been proposed on the basis of cell culture experiments and animal studies [39-42]. Animal studies indicate that substantial neuropathological changes in PrD are already present before the onset of symptoms and are spatially related to PrP^{sc} deposits. Ideally, an effective intervention should start during the preclinical stage of disease, and be aimed at preventing PrP^{sc} neuroinvasion or propagation in the CNS. Unfortunately, no tests are available currently to detect asymptomatic PrD, except for carriers of pathogenic mutations of the *Prnp* gene.

Inhibition of PrP^{sc} formation may be achieved, at least in laboratory experiments, through one of the following strategies:

- Abrogation of PrP^{c} synthesis or prevention of its transport to the cell surface
- Stabilisation of the PrP^{c} structure to make its conformational change unfavourable

- Sequestration of PrP^{sc}
- Reversion of PrP^{sc} to a protease-sensitive form
- Interference with the interaction between PrP^{c}, PrP^{sc}, and other macromolecules involved in the conversion process (for review see [43]).

However, most compounds that have shown some effectiveness in cell culture or in animal models of PrD only work when administered at the time of infection or shortly thereafter. The heterogeneity and complexity of PrD suggest that a combination of several compounds with different modes of action may be necessary for their prevention and treatment. Preclinical diagnostic tests for PrD are urgently needed and deemed crucial in the success of an early treatment.

Antibiotics, dyes, and NMDA receptor ligands

The polyene macrolide antibiotic, amphotericin B [44, 45], and its less toxic derivative, MS-8209 [46], have been shown to delay scrapie agent propagation and PrP^{sc} accumulation in mice or hamsters. The amyloid-binding dye, Congo red, is able to inhibit PrP^{sc} accumulation and replication, most likely by overstabilising the abnormal conformational isoform [47, 48]. The anthracycline 4-iodo-4-deoxy- doxorubicin has been found to delay hamster scrapie progression via binding to amyloid fibrils [49]. Suramin, a highly sulphated urea based compound, and dapsone, a sulphone, were also tested against mouse scrapie and found to increase the incubation period when given continuously [50, 51]. Porphyrins and phthalocyanins as sulphated forms also were shown to inhibit the production of PrP^{sc} in neuroblastoma cell cultures [52, 53].

The neurotoxic effect displayed by PrP^{sc} and its fragments was found to be prevented *in vitro* by antagonists of NMDA receptor channels, such as memantine [17, 54]. Moreover, flupirtine, a triaminopyridine compound clinically used as non-opiod analgesic drug, which acts like an NMDA receptor antagonist, but does not bind to the receptor, was found to display a strong cytoprotective effect on neurons treated with PrP^{sc} or with a toxic fragment [54, 55]. A double-blind placebo-controlled study has been carried out in 28 CJD patients [56]. Patients treated with flupirtine showed significantly less cognitive changes (dementia) than placebo patients, which led the authors to conclude that flupirtine may have beneficial effects on the cognitive function of patients with CJD [56]. The study did not investigate other aspects of neurological deterioration in progressive CJD, the results appear therefore of limited usefulness.

Active and passive immunisation

Several studies have suggested that antibodies (Ab) might have beneficial anti-prion properties in infected cells [42, 57]. Auto-antibodies can be induced in PrP^c-expressing mice and have the potential to cure cells after prion infection [58, 59]. Furthermore, in transgenic mice expressing both PrP^c and a defined anti-PrP^c antibody, prion infectivity within the spleen is significantly reduced [60]. Peretz et al. (2001) investigated seven different recombinant antibodies raised to various parts of the normal PrP^c protein [61]. They exposed a mouse neuroblastoma cell line (ScN2a) infected with PrP^{sc} to varying concentrations of each Ab and measured the amount of PrP^{sc} protein. The most potent Ab prevented conversion of PrP^c to PrP^{sc} and also cleared pre-existing PrP^{sc} in a dose-dependent manner. Removal of Ab after 2 weeks of treatment left cultures free of prion infectivity for an additional 4 weeks [61].

Recent work by White et al. (2003) suggested that another approach, passive application of anti-PrP^c antibodies, could be effective [62]. Monoclonal antibodies (mAb) were generated in non-tolerant PrP^c-knockout mice and exhibited different specificities towards PrP^c and PrP^{sc}. Mice were infected with PrP^{sc} by the intraperitoneal (i.p.) or intracerebral routes (i.c.), and mAb were injected i.p. twice per week, starting a few weeks after infection. In mice infected with prion by the i.p. route, mAb treatment produced a dose and time dependent reduction in PrP^{sc} and prion infectivity, and a significant prolongation of survival. Interestingly, the largest effects were obtained with the mAb mainly reacting with PrP^c. However, the protective effect of mAb was only observed when prions were applied by the i.p. route, whereas prion infections caused by i.c. inoculation were not influenced by mAb treatment. This suggested that the antibody cannot cross the blood-brain barrier at a sufficient concentration to exert a protective function [62].

It remains however unclear whether the above findings in cell culture and mice are transferable to humans, given the fact that the exact mode and time point of prion infection are usually unknown. In addition, in most of the mouse studies, a very high dose of mAb was applied in a continuous fashion (for review see [42]), which may produce allergic reactions and also result in inactivating antibodies in human patients.

Quinacrine and chlorpromazine

The antimalarial drug quinacrine (mepacrine), a cyclic tetrapyrrole, and the antipsychotic chlorpromazine (Figure 1) were shown to prevent the conversion of PrP^{c} to PrP^{sc} in cell culture. Doh-ura et al. (2000) reported that lysosomotropic agents (e.g. quinacrine or chloroquine) inhibited protease-resistant prion protein accumulation in scrapie-infected murine neuroblastoma cells (ScNB). The inhibition occurred without apparent effects on normal PrP^{c} biosynthesis or turnover, and without direct interactions with prion protein molecules [63]. Similar effects of quinacrine were reported later by Korth et al. (2001). These authors cultured mouse neuroblastoma cells (ScN2a) infected with PrP^{sc} to show that 6 days of treatment with quinacrine or chlorpromazine was able to reduce the conversion of PrP^{c} to PrP^{sc} [64].

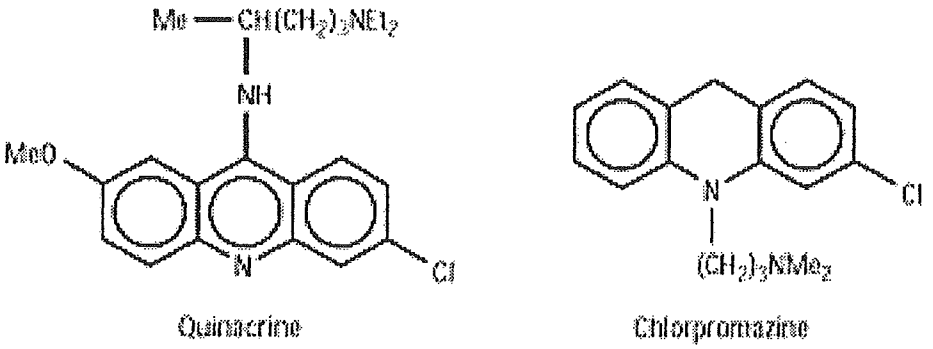


Figure 1. Chemical structure of quinacrine and chlorpromazine

Quinacrine has been used in humans for over 60 years to treat malaria, and can be administered orally at high doses on a daily basis. The currently suggested oral dose for CJD patients is however higher than the antimalarial dose and may produce significant side effects in a considerable proportion of the treated patients. Chlorpromazine, although less potent than quinacrine in cell culture, crosses the blood-brain barrier to a larger extent.

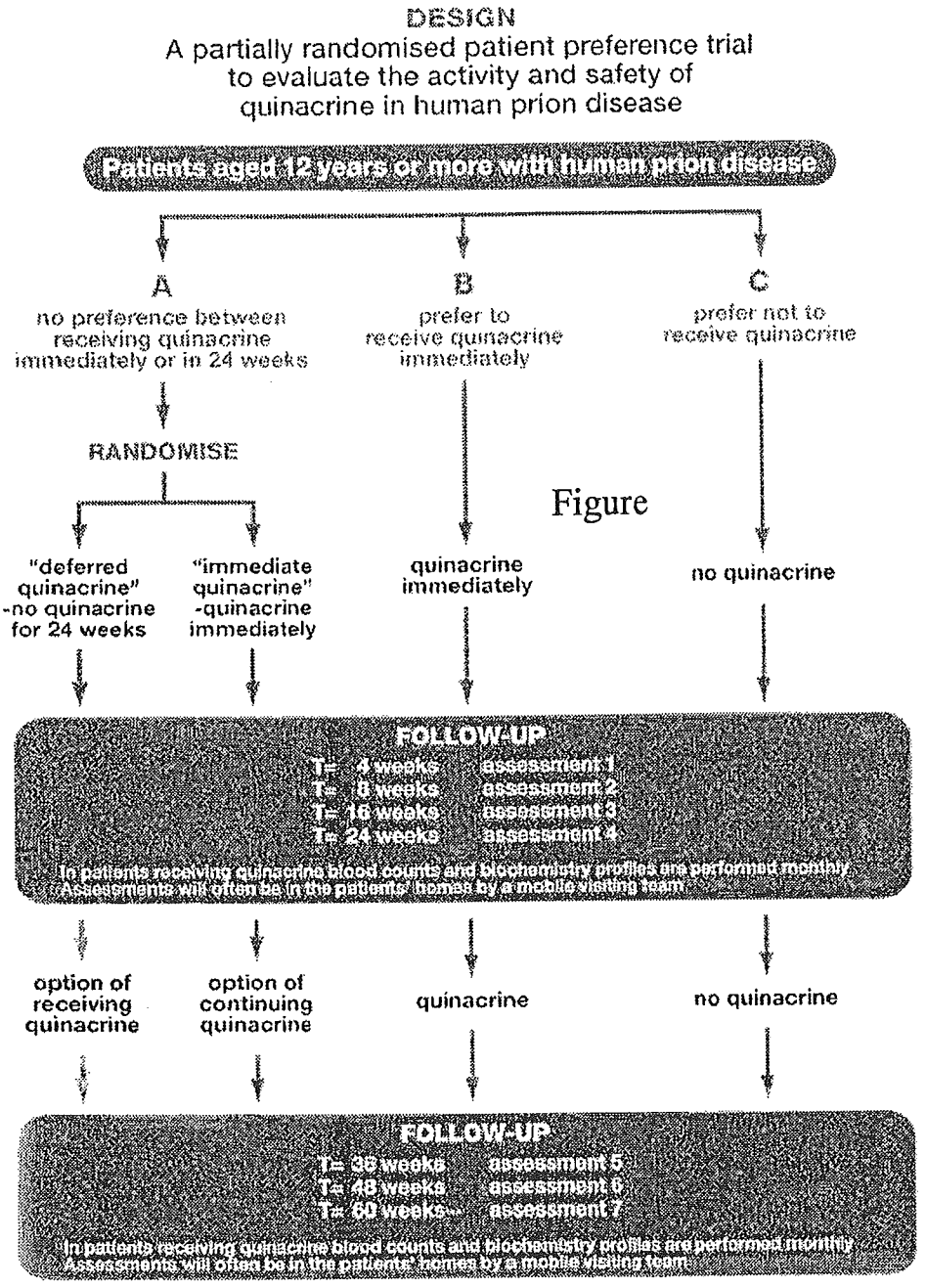
Turnbull et al. (2003) showed that quinacrine also may act as an effective antioxidant, readily scavenging hydroxyl radicals generated during incubation of toxic PrP^{sc} fragments with murine neurons [65]. Quinacrine also significantly reduced toxicity of the $PrP^{106-126}$ peptide fragment in these cells. On the other hand, Collins et al. (2002) evaluated oral quinacrine in mice infected with PrP^{sc}, but were not able to demonstrate any significant effect of the drug on overall survival of treated animals compared to controls [66]. Barret et al. (2003) also examined the efficacy of quinacrine and chlorpromazine in different *in vitro* models and in an experimental murine

model of BSE [67]. Despite the inhibition of PrP^{sc} accumulation in ScN2a cells, quinacrine was unable to produce a detectable effect in the animal model.

Japanese researchers are carrying out an ongoing clinical study of oral quinacrine in patients with sporadic and iatrogenic CJD. Results in the first 4 patients have been published recently [68]. Quinacrine (300 mg/d) has been administered for 3 months. Improved arousal level of patients with akinetic mutism, and restored eye contact or voluntary movements in response to stimuli were described. Clinical improvement was however transient, lasting 1-2 months. Quinacrine at the above dose caused liver dysfunction and skin pigmentation in all cases [68]. Further results in a larger patient population should be presented in near future.

A prospective clinical study of quinacrine in PrD, the PRION-1 study [69], is currently enrolling patients in the UK (Figure 2). Patients aged 12 years and older with all types of PrD are eligible. The study protocol features a partially randomised design, with patients who opt for quinacrine treatment split in two arms according to their preference for immediate vs. deferred (by 24 weeks) treatment. Treated patients will receive a loading dose of quinacrine (1 g on the first day), followed by 300 mg/d as a long term dose. The primary efficacy endpoints are mortality and the proportion of responders overall and at 24 weeks. Response is defined as independently rated lack of deterioration, global impression of change (based on the Clinician's Interview Based Impression of Change, CIBIC-plus), and patients score on the Brief Psychiatric Rating Scale (BPRS). Secondary efficacy endpoints are neurological and neuropsychological changes, including changes in markers of disease activity, MRI, and EEG [69].

Routine follow-up will be identical for all patients participating in the PRION-1 trial, with the exception of patients with inherited PrD, who have longer disease duration and will be followed up less frequently. Follow-up assessments are taking place monthly for the first 6 months, then every 3 months until end of study. Assessments will include medical history, physical examination, liver function and blood clotting, neurological examination recorded on video, and a series of neurological assessments. The study accrual target is 160 patients. Currently 18 patients have been enrolled [69].



Figure

Figure 2. Flow chart of the PRION-1 trial of quinacrine in patients with PrD [69].

Polysulphonated glycosides (glycans)

Several polysulphonated polysaccharides, including pentosan polysulphate (PPS) (Figure 3) and dextran sulphate (DS), have been shown to prolong the incubation period in PrP^{sc} infected rodents if given before infection [70-72], and to inhibit PrP^{sc} accumulation in neuroblastoma cells [73]. The effects of these polyanions may be due to an inhibition of the formation of PrP fibrils [74] or to reduction of the amount of PrP^{c} on the cell surface by stimulating

endocytosis of PrP^c [52]. Sulphonated polyglycosides are not known to penetrate the CNS, and hence the first attempts to demonstrate their effects were made in peripheral organs [75].

Caughey and Raymond (1993) tested various polysulphonated glycosides (PG) and found PPS, carrageenan, and dextran sulphate 500 (DS500) to be highly active in the inhibition of PrP^{sc} production [73]. PPS was most active, showing half of its maximal activity at 1 ng/ml. Shyng et al. (1995) reported PPS and related compounds to cause a decrease of PrP^c on the surface of cultured chicken and mouse neuroblastoma cells. PPS caused a redistribution of PrP^c from the surface to the interior of the cell (intracellular late endosomes). The differences in the binding strength of PrP^c to PPS and to other PG were found to parallel their *in vivo* and *in vitro* anti-PrP^{sc} formation potency [52]. Ehlers and Diringer (1984) inoculated mice i.p. or i.c. with scrapie agent and treated them systemically with DS500 [76]. None of the i.c. inoculation experiments were affected by the treatment. With i.p. inoculations, however, it was seen that DS500 did decrease (by approximately one order of magnitude) the infectivity found in the spleen at various times after single injection of the drug, and significantly prolonged incubation times. Treated mice also showed a significant increase in the mean incubation period compared with controls. It was noted that DS500 remained in the body of an inoculated mouse for up to 7 months. A maximum effect was seen when the drug was given at the same time with the infection, and no effect was seen when it was given 35 days after infection [76].

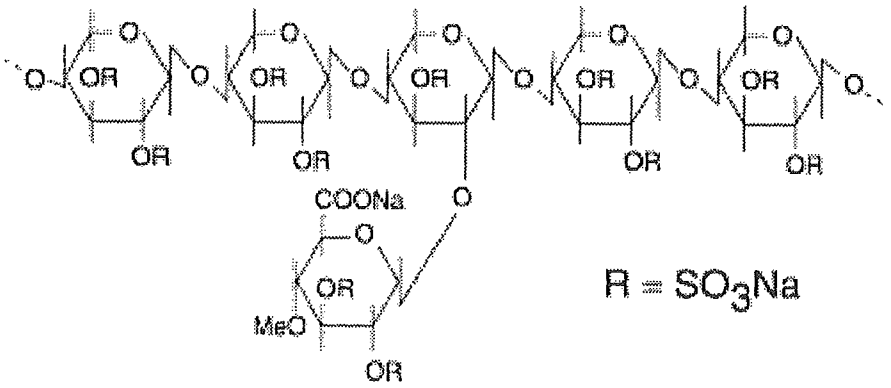


Figure 3. Chemical structure of pentosan polysulphate (PPS)

This work was followed by the study of Farquhar and Dickinson (1986), who carried out a series of murine experiments to inoculate i.p. scrapie [75]. This was associated, at various times before and after the scrapie inoculum, with various quantities of DS500 as a single i.p. injection. It was found that DS500 reliably increased the incubation period of the disease, and that this did not seem to depend on the strain of disease or the inbred strain of mouse

used. The effect seemed to be present when DS500 was given up to 4 weeks before and up to 3 weeks after the scrapie inoculum. The incubation period was extended by 5-19% at this dose, but if increased doses of DS500 were used, the incubation period could be prolonged by up to 62% [75]. Kimberlin and Walker (1986) gave various inocula of scrapie to mice either i.v. or i.p., and various drugs were tested before or after the scrapie infection [77]. DS500 proved effective in reducing the titre of the scrapie inoculum. Little effect was seen with heparin, dextran, or DEAE dextran. Diringer and Ehlers (1991) inoculated mice i.p. with scrapie and administered PPS i.p. on different days (84 to 50) before the PrP^{sc} infection. PPS increased the incubation period of mice by up to 75% [70]. Hamsters were also inoculated i.p. with various quantities of DS500 or PPS and with scrapie, separated by 2 to 24 hrs [78]. As the dose of DS500 increased, the incubation period also increased, but the maximum achieved with non-toxic doses of the drug was 21%. It was noted that a single i.p. administration of PPS increased the incubation period of i.c. inoculated scrapie by around 18% [78]. Farquhar et al. (1999) injected i.p. PPS immediately after scrapie infection of mice. Depending on mouse strain, a single PPS dose of 250 mg increased the scrapie incubation period by up to 66%. A single 1 mg i.p. dose of PPS protected mice completely from simultaneous scrapie infection. On the other hand, oral PPS was ineffective in delaying disease [79].

Doh-ura et al. (2004) recently infected transgenic mice (Tg7) expressing hamster prion protein with i.c. scrapie, and different agents were infused cerebroventricularly starting on day 10 or day 35 after infection [80]. The infusion was continued for 4 weeks. Infused drugs included lysosomotropic chemicals, such as E-64d cysteine protease inhibitor, chloroquine, quinacrine, amphotericin B, and PPS. Lysosomotropic agents demonstrated marginal effects in prolonging the incubation time when administered on day 35 after infection, and either no effect or less effect at the earlier stage (day 10 after infection). Amphotericin B and PPS demonstrated remarkable effects either early or late in the disease course. Amphotericin B resulted in around 30% prolongation of the incubation time when administered at the early stage, and 12% prolongation at the late stage. PPS showed more beneficial effects than amphotericin B, and mice which received PPS at the early stage survived 173% longer, and at the late stage 92% longer. Maximal effects of PPS at a later stage (day 42 of infection) were obtained at 230 μg/kg/day. Analysis of detailed relationship between the initiation time of the infusion of PPS and the outcome revealed that the effects of PPS were quite dependent on the timing of infusion initiation, with earlier initiation of treatment rendering a better prognosis [80]. Analysis by either immunohistochemistry or immunoblotting demonstrated that PPS potently inhibited PrP^{sc} deposition in the brain. It also showed that amount or dis-

tribution of deposited PrP^{sc} in the brain of mice treated with PPS was modified and did not return to the same level observed in the control animals, even when they were at a terminal stage. Immunohistochemical analysis demonstrated that mice treated with PPS from the early stage did not show any PrP^{sc} deposits in the brain on day 52. On the other hand, control animals demonstrated PrP^{sc} deposits in the parahippocampal white matter on day 35, and later also in the thalamus. No notable adverse effects were observed in experimental mice treated with up to 230 μg/kg/day intraventricular PPS for two months. In a separate set of experiments in normal dogs, higher doses, such as 345 μg/kg/day and 460 μg/kg/day, did show adverse effects such as partial or generalized epileptic seizures, which began within 24 hours after PPS infusion at the above high doses was initiated [80].

Both heparin and PPS are rapidly taken up into RES cells by a saturatable pathway. Low doses are cleared quickly into the RES, whereas higher doses saturate the RES and are excreted in urine [81]. PPS is metabolised by cellular non-specific desulphation in many organs and tissues, including vascular endothelium [82]. Renal excretion of desulphated PPS from plasma takes place over 6 days following a single dose, which also involves partial polyxylose chain breakdown. PPS can be administered orally, but only a low proportion (0.5-4%) of the drug is detected in the blood circulation [83, 84]. When PPS is given orally, anti-inflammatory effects are seen in the bladder after long-term administration [85]. It is considered that this is due to accumulation of the drug in cells of the RES with slow break-down and excretion. When used for anticoagulation and given s.c. or i.v., PPS may cause an early, benign, reversible thrombocytopenia and a rise in lipoprotein lipase activity [83]. Similarly to heparin, a rare, immune, severe form of thrombocytopenia has been also reported [86]. No significant neurological symptoms or signs have been reported in humans or animals treated orally or parenterally with PPS.

There has been no penetration in the CNS demonstrated with peripherally administered PPS, which is not surprising with the hydrophilic nature of the drug. On the other hand, direct intracerebral administration of PPS may afford high compartmental concentrations of the drug in the CNS, but no pharmacokinetics is available for this specific mode of administration. Direct administration of PPS to the CNS would be expected to allow PPS to concentrate inside cells, entering them via ubiquitous heparan-binding sites, and to exert biological effects on those cells infected by PrP^{sc}. In analogy to other therapeutic molecules, e.g. recombinant proteins delivered directly into the primate and human brain [87, 88], it is considered likely that cerebroventricular infusion of PPS may have the highest ratio of local versus systemic drug concentration.

Rationale for local administration of drugs to the CSF

The clinical and late preclinical phase of PrD with PrP^{sc} formation in the brain requires drugs that can cross into brain parenchyma and be present in the brain in a biologically active concentration [32, 42, 43]. However, in the early stages of PrD, with an intact blood-brain barrier (BBB), there is severe limitation of the penetration of drugs from blood into brain interstitium, and from there into glial and neuronal cells. Even at late stages of the disease, tight junctions of the brain capillaries may remain at least partially intact and therefore selectively limiting the entry of most molecules.

Compounds that are highly lipid soluble, such as alcohol, barbiturates, and some anticonvulsants, may easily pass through the endothelial cells forming the inner layer of the BBB. Lipid solubility is measured by the oil/water (octanol/water) partition coefficient, and molecules with a high coefficient usually permeate efficiently the BBB (for review see [89]). Such highly lipid soluble compounds with a high partition coefficient are phenytoin and methadone, and they cross the BBB in large quantities under normal conditions. Not all lipid soluble molecules, however, easily traverse the BBB. Compounds highly bound to plasma proteins have restricted access to the brain. For these substances, the degree of dissociation of the protein complex in transit through the capillary bed determines the degree of penetration across the BBB. Furthermore, there are special transport systems responsible for enhanced passage of certain compounds with low lipid solubility across the BBB, such as the physiologically important molecules D-glucose and phenylalanine [90]. The BBB can be subjected to pharmacological or osmotic modifications aimed at temporarily increasing its permeability to certain therapeutic molecules. These approaches are however invasive and have the potential for serious side effects [91, 92].

The CSF-brain barrier (CBB) seems to be more permeable because of its anatomical structure lacking tight junctions between the neuroependymal cells lining the cerebral ventricles. Substances administered to the CSF have been shown to penetrate into brain tissue by diffusion. The physical process of diffusion is gradient-driven, and penetration of the CBB will be enhanced by higher concentration of a molecule in one compartment [93, 94]. This fact points at an important advantage of the local application of drugs to the CSF - high local concentration in the CNS compartments, as opposed to negligible systemic concentration due to low reabsorption in the blood stream.

Continuous CSF circulation is physiologic process which lends itself to dissemination of substances throughout the CNS. CSF is continuously produced and completely replaced in the brain approximately every 8 hours.

In normal adults, the rate of CSF removal by reabsorption is equal to the rate of CSF production by filtration of blood through the intraventricular choroid plexus. CSF circulates from the sites of production, the lateral ventricles and third ventricle, into the cerebral aqueduct and into the fourth ventricle. From there CSF escapes the internal ventricular system of the brain by the foramina of Luschka and Magendie into the subarachnoid space around the brain and the spinal cord. Arachnoid granulations and dural sinuses are the route for CSF reabsorption to the blood circulation [95].

Animal models support findings in humans. In a model of cerebroventricular infusion in rats, radioactive sucrose was infused into one lateral ventricle. Within minutes after infusion, sucrose moved into the third ventricle, the aqueduct, fourth ventricle, and the subarachnoid space of the quadrigeminal, ambient and interpeduncular cisterns. About 15% of the injected sucrose entered these large cisterns. In contrast to most other CSF-brain interfaces, little sucrose moved from CSF into the medulla next to the lateral recesses and tissues adjacent to the large CSF cisterns. A thick, multilayered *glia limitans* visible on electron micrographs seemed to form a CSF-brain barrier at these interfaces [96].

Evidence exists also for the bulk flow of brain interstitial fluid via preferential pathways through the brain, which is closely related to CSF. This bulk flow of interstitial fluid has implications for drug delivery, drug distribution, and drug clearance [97].

Preliminary results with continuous long-term cerebroventricular administration of PPS in human PrD

The first objective of cerebroventricular PPS administration in PrD patients was to evaluate the short and long term safety and tolerability of escalating doses of PPS administered by continuous long term infusion. A secondary objective was to assess efficacy of PPS in delaying disease progression and improving existing neurological deficits. Patients with probable sporadic, iatrogenic, or variant CJD, or with hereditary syndromes such as GSS or FFI were eligible to receive PPS infusion. Informed consent was obtained where possible. If patients were not fit and able to consent, a legally appointed representative signed the consent forms. The primary endpoint of PPS administration studies was maximum tolerated dose of PPS as assessed by occurrence of serious toxicity resulting from PPS administration. Dose-limiting toxicity (DLT) was defined as any one of the following occurring in two or more patients:

- Any grade 4 toxicity attributed to PPS