れた。本実験ではそれらの報告を追試することに成功し、さらに遺伝子操作なしに神経系への分化誘導も可能であることを示すことが出来た。しかしながらその一方でPrPの比較的高い発現細胞であることがわかり、またプリオン病原体の感染に感受性を持つことが明らかとなった。プリオン病における細胞移植神経再生療法への応用を考えるには、感染抵抗性を付加する必要があると思われた。

今回得られたMSCをex vivoにて培養増幅することで異常型 PrP を容易に検出できるという新しい知見は、もしヒトへの応用が可能であれば、ヒトプリオン病の臨床診断においてあらたな確定診断法となる可能性がある。またさらに早期診断への応用の可能性についても検討する必要がある。

E．結論

(1)骨髄間質細胞は容易に分離継代培養可能で、神経への分化能を有していた。

(2)正常 PrP は脳内の発現と遜色のないレベルで発現していた。

(3)プリオン病原体の持続感染が可能であり、また感染個体から得た骨髄間質細胞にも感染が認められた。

(4)移植療法に用いるにはPrPの発現を抑制するなど遺伝子操作が必要と思われる。

(5)感染個体からの骨髄生検・細胞培養増幅はCJDの生前確定診断法として有効である可能性が示唆された。

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西田教行：プリオン株をめぐる謎。Antibiotics & Chemotherapy 22(1):71-78, 2006

2．学会発表

なし

H．知的財産権の出願・登録状況

なし

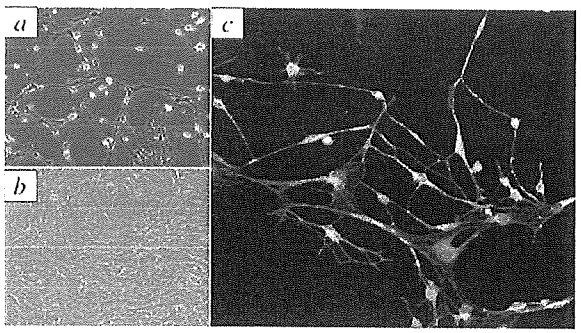

Figure 1

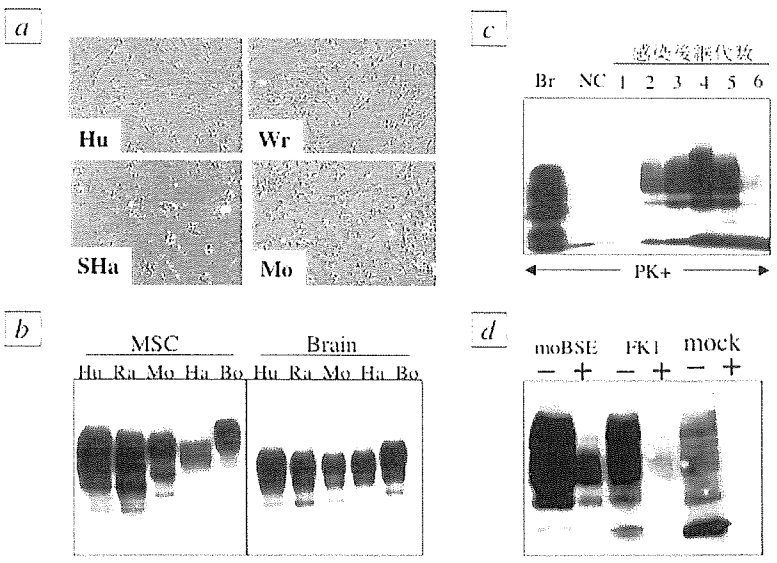

Figure 2

厚生労働科学研究費補助金（こころの健康科学研究事業）
平成１７年度　分担研究報告書

Fyn 欠損マウスにおけるプリオン病に関する研究

分担研究者：村本環　東北大学大学院医学系研究科・助教授

研究要旨
非受容体型チロシンキナーゼの１種である Fyn を欠損するマウスへのプリオン接種実験を行い、Fyn の発現レベルがプリオンの病原性に影響を与えないことを証明した。

A．研究目的

プリオン病罹患個体では海綿状変性と呼ばれる特徴的な神経変性が生じ、個体を死に至らしめるが、その発生メカニズムは不明である。分担研究者は、同メカニズムを明らかにすることが新しいプリオン病治療法の開発に繋がると考え、これを解明する研究に着手した。分担研究者のこれまでの研究から、GPI アンカーを持たないプリオン蛋白(PrP)を発現するマウスでは、PrP の異常化、プリオンの増殖が起こっても、海綿状変性が生じないことが明らかとなった。このことから、分担研究者は、PrP から GPI アンカーを介して細胞内に伝達される異常シグナルが海綿状変性の発生に重要な役割を果たしている可能性があると考え、そのようなシグナルの伝達に関与している可能性のある分子を欠損するマウスにおいて、海綿状変性が生じるか否かを検討することを計画した。これまでの研究から、Src family に属する非受容体型チロシンキナーゼである Fyn が、PrP から発せられるシグナルを感知して活性化されることが知られており、本年度は Fyn を欠損するマウスへのプリオン接種実験を行った。

B．研究方法

大阪大学大学院生命科学研究科の八木　健博士より提供して頂いた Fyn-/-、Fyn+/-マウス、および同種の野生型(Fyn+/+)である C57BL/6 マウスに福岡１株プリオンを接種して、症候の観察、潜伏期間の計測、発病マウスの脳組織の病理学的、ならびに生化学的解析を行った。また、プリオンを接種されていない上記３群のマウスの脳組織における Fyn、および PrP の発現レベルの評価を行った。

（倫理面への配慮）
動物実験は東北大学医学系研究科動物実験委員会の許可を受け、東北大学動物実験指針を遵守して行った。

C．研究結果

表１　Fyn 欠損のプリオン病潜伏期間への影響

接種* 材料	被接種マウス Fyn 遺伝子型	潜伏期間 （日）
A	+/+	161, 161, 161, 161
A	-/-	161, 161, 161, 161
B	+/-	157, 157, 157, 157、161
B	-/-	152, 152
C	+/+	146, 146, 146, 146, 146, 146
C	+/-	146, 146, 146
C	-/-	146, 146, 146, 146

*A、B、C は別々に調整された福岡１株

プリオンの接種を受けていない３群のマウスにおける Fyn の発現レベルは、Fyn-/-マウスでは発現が認められず、Fyn+/-マウスでは

Fyn+/+マウスのおよそ半分に低下していることが確認された。また、PrP の発現レベルは３群の間で有意な差がないことが確認された。

一方、プリオン接種を受けたマウスでは、３群ともに、類似の潜伏期間（表１）の後に典型的なプリオン病の症候を呈して発病した。また、病理学的検索では３群ともに、同程度の海綿状変性が認められた（図１）。生化学的検索でも、３群ともに、類似した量の異常型 PrP が検出された。

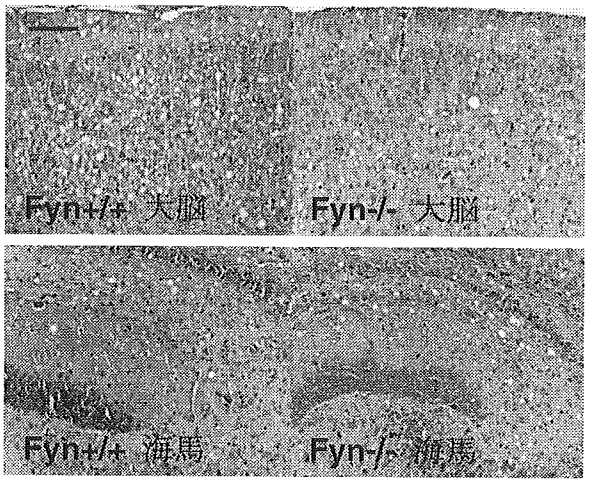

図１　福岡１株接種 Fyn 欠損マウスにおける海綿状変性
左上の Bar は 200 μm。

D．考察

臨床的、病理学的、生化学的証拠から、Fyn 欠損はプリオン病の病態形成に影響を与えないと考えられた。ただ、Src family に属する別の非受容体型チロシンキナーゼが、Fyn の機能を代償したために、Fyn 欠損の影響が検出来なかった可能性は残っている。そのような可能性を今後、マウスで検討するためには、複数の Src family チロシンキナーゼの重複欠損マウスを作成することが必要となるが、一部すでに作成された同様のマウスは、生後すぐに死亡することが知られている。これに代わる案としては、プリオン病の病理現象を培養細胞で再現する新しいモデルシステムを開発し、同モデルにおいて複数分子のノックダウンを試みることが考えられる。

E．結論

Fyn 欠損はマウスのプリオン病の病態形成に影響を与えない。

G．研究発表

なし

H．知的財産権の出願・登録状況

なし

研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

書籍

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西田教行	プリオン株をめぐる謎	Antibiotics & Chemotherapy	22(1)	71-78	2006

研究成果の刊行物・別刷

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].

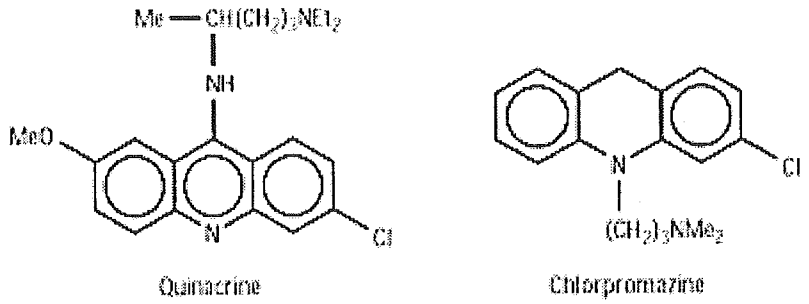


Figure 1. Chemical structure of quinacrine and chlorpromazine

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.

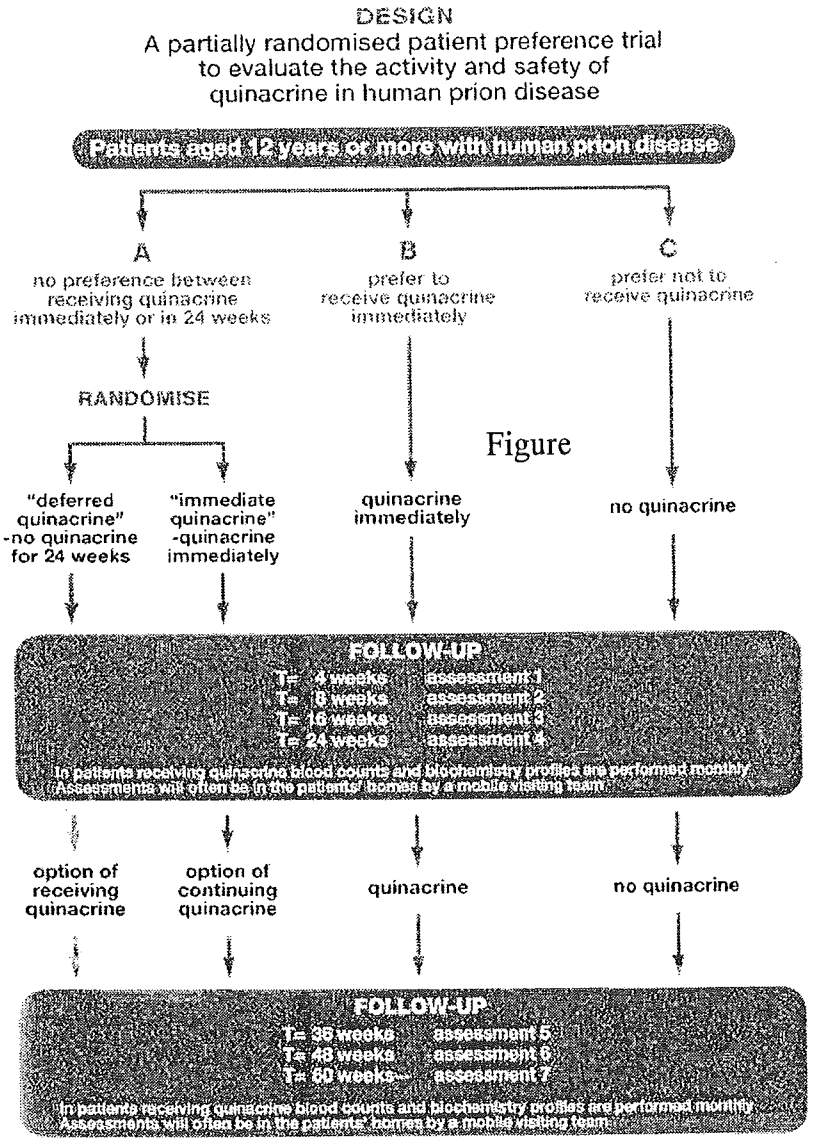
Turnbull et al. (2003) showed that quinacrine also may act as an effective antioxidant, readily scavenging hydroxyl radicals generated during incubation of toxic PrP^{sc} fragments with murine neurons [65]. Quinacrine also significantly reduced toxicity of the $PrP^{106-126}$ peptide fragment in these 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