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ORIGINAL ARTICLE

In vivo detection of prion amyloid plaques using [^{11}C]BF-227 PET

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Abstract
Purpose In vivo detection of pathological prion protein (PrP) in the brain is potentially useful for the diagnosis of transmissible spongiform encephalopathies (TSEs). However, there are no non-invasive ante-mortem means for detection of pathological PrP deposition in the brain. The purpose of this study is to evaluate the amyloid imaging tracer BF-227 with positron emission tomography (PET) for the non-invasive detection of PrP amyloid in the brain.
Methods The binding ability of BF-227 to PrP amyloid was investigated using autoradiography and fluorescence microscopy. Five patients with TSEs, including three patients with Gerstmann-Sträussler-Scheinker disease (GSS) and two patients with sporadic Creutzfeldt-Jakob disease (CJD), underwent [^{11}C]BF-227 PET scans. Results were compared with data from 10 normal controls and 17 patients with Alzheimer's disease (AD). The regional to pons standardized uptake value ratio was calculated as an index of BF-227 retention.
Results Binding of BF-227 to PrP plaques was confirmed using brain samples from autopsy-confirmed GSS cases. In clinical PET study, significantly higher retention of BF-227 was detected in the cerebellum, thalamus and lateral temporal cortex of GSS patients compared to that in the corresponding tissues of normal controls. GSS patients also showed higher retention of BF-227 in the cerebellum, thalamus and medial temporal cortex compared to AD patients. In contrast, the two CJD patients showed no obvious retention of BF-227 in the brain.
Conclusion Although [^{11}C]BF-227 is a non-specific imaging marker of cerebral amyloidosis, it is useful for in vivo detection of PrP plaques in the human brain in GSS, based on the regional distribution of the tracer. PET amyloid imaging might provide a means for both early diagnosis and non-invasive disease monitoring of certain forms of TSEs.

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Keywords Prion · PET · Amyloid · Creutzfeldt-Jakob disease

Introduction

Transmissible spongiform encephalopathies (TSEs), also known as prion diseases, are a group of fatal neurodegenerative disorders, including Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker disease (GSS) and kuru [1–3]. TSEs are characterized by progressive deposition of abnormal prion protein (PrP) in the brain. CJD is the most common type of human TSE and is classified into sporadic, genetic and infectious forms according to the aetiology of illness. GSS is a familial neurodegenerative disorder associated with mutations of the PrP gene and is clinically recognized by cerebellar ataxia combined with postural abnormalities and cognitive decline [1–3]. Two major types of abnormal PrP deposition, synaptic and plaque types, have been described in the brain of people with TSEs [1]. The synaptic type of PrP deposition, which does not have tinctorial properties of amyloid in tissue sections, is most commonly observed in sporadic CJD, whereas the plaque type, which frequently forms congophilic amyloid plaques, is a hallmark of such TSEs as GSS, variant CJD (vCJD) and iatrogenic dura CJD with plaques [1, 4]. Abnormal PrP deposition in the brain is suggested to start before the occurrence of clinical symptoms [5–7]. Thus, preclinical diagnosis and, when available, early disease-specific therapeutic interventions, can be beneficial for people predisposed to or affected by TSEs.

Several positron emission tomography (PET) imaging agents have been recently developed and used for in vivo detection of brain amyloid-β (Aβ) plaques in patients with Alzheimer's disease (AD) [8–12]. Most of these β-sheet binding agents show high binding affinity to PrP amyloid because PrP aggregates in TSEs form β-pleated sheet structures and share a common secondary structure with Aβ deposits in AD brains [13–16]. Therefore, these agents would be useful for the in vivo detection of PrP amyloid in the brain. Two clinical PET studies were performed using [^{18}F]FDDNP and/or [^{11}C]PIB in sporadic and familial CJD patients [17, 18]. The results indicated moderate retention of FDDNP and no obvious retention of PIB in the brain [17, 18]. Therefore, agents that can sensitively detect abnormal PrP deposits should be further explored for the diagnosis of TSEs. We have demonstrated in vitro and in vivo binding of benzoxazole derivatives to both Aβ and PrP amyloids [19, 20]. One of these derivatives, BF-227, was used for a clinical PET study where it successfully visualized amyloid deposits in the brain of AD patients in vivo [12, 21]. Therefore, [^{11}C]BF-227 appears to be a promising candidate for PET imaging of PrP deposits. The purpose of this study was to evaluate the clinical utility of [^{11}C]BF-227 PET for the non-invasive detection of abnormal PrP deposits in patients with TSEs.

Methods

Preparation of compounds

BF-227 and its 2-tosyloxyethoxy and *N*-desmethylated derivatives were custom synthesized by Tanabe R&D Service Co. (Osaka, Japan). [^{18}F]BF-227 was synthesized for autoradiography of brain sections, as described previously [22]. For the clinical studies, [^{11}C]BF-227 was synthesized as described previously [12]. Radiochemical yields were greater than 50% based on [^{11}C]methyl triflate, and specific radioactivities were 119–138 GBq/μmol at the end of synthesis. Radiochemical purities were greater than 95%.

Histopathological staining and in vitro autoradiography

Autopsy-diagnosed brain samples from two GSS cases with PrP plaque deposition and two sporadic CJD cases with synaptic PrP deposition were provided by Dr. Toru Iwaki of the Department of Neuropathology, Kyushu University, Japan. The brain sample from an 81-year-old man with autopsy-confirmed physiological aging was obtained from Tohoku University Hospital. The two GSS cases had a proline-to-leucine mutation at codon 102 and methionine homozygosity at codon 129 of the PrP gene, and the two sporadic CJD cases had no mutations and methionine homozygosity at codon 129; they showed type 1 abnormal PrP in immunoblotting of the brain tissues. All of the brain samples were treated with 98% formic acid for 1 h before paraffin embedding to eliminate prion infectivity. Sections from paraffin-embedded blocks of the cerebellum or frontal cortex were then dewaxed in xylene and ethanol. For staining with BF-227, tissue sections were immersed in 100 μM BF-227 solution containing 50% ethanol for 10 min. They were then dipped briefly into water and rinsed in phosphate-buffered saline for 10 min before coverslipping with FluorSave Reagent (Calbiochem, La Jolla, CA, USA). Subsequently, they were examined using an Eclipse E800 microscope (Nikon, Tokyo, Japan) equipped with a V-2A filter set (excitation, 380–420 nm; dichroic mirror, 430 nm; Longpass filter, 450 nm). For autoradiography, the section was incubated with 1.0 MBq/ml of [^{18}F]BF-227 at room temperature for 10 min and then washed briefly with water and 50% ethanol. After drying, the labelled section was exposed to a BAS-III imaging plate (Fuji Film, Tokyo, Japan) overnight. Autoradiographic images were obtained using a BAS-5000 phosphor imaging instrument (Fuji Film, Tokyo, Japan). Neighbouring sec-

tions were immunostained using 3F4 anti-PrP monoclonal antibody (Covance, Princeton, NJ, USA) as described previously [13, 20].

Subjects and patients in the clinical PET study

Five TSE patients, including two sporadic CJD patients [63-year-old woman (CJD1) and 58-year-old man (CJD2)] and three GSS patients [69-year-old woman (GSS1), 61-year-old man (GSS2) and 30-year-old woman (GSS3)], underwent PET scans with [^{11}C]BF-227 (Table 1). For comparison, [^{11}C]BF-227 PET studies were also performed in 17 AD patients [mean age ± standard deviation (SD)= 72.6±6.7; mean Mini-Mental State Examination score ± SD=19.8±4.0] and 10 aged normal controls (mean age ± SD=67.2±2.5). Some of these AD and normal subjects were included in our previous report [12].

CJD1's health was unremarkable until the manifestation of depressive symptoms at the age of 62 years. The patient then developed subacutely progressive dementia, motor disturbances and myoclonus. CJD2 showed subacutely progressive dementia and gait disturbance and then developed psychotic symptoms, dysarthria and myoclonus. Both CJD patients had no mutations and showed methionine homozygosity at codon 129 of the PrP gene. PET studies in CJD1 and CJD2 were performed when they reached grade 4 of the modified Rankin scale at 3 and 4 months after onset of symptoms, respectively. Both patients showed periodic synchronous discharges in electroencephalograms and hyperintensity in the caudate, putamen and cerebral cortex on diffusion-weighted magnetic resonance (MR) images. Diagnosis of probable CJD was made according to the WHO criteria [23].

Each GSS patient was from a different pedigree and had a family history of the same disease, carrying a proline-to-leucine mutation at codon 102 and methionine homozygosity at codon 129 of the PrP gene. GSS1 and GSS2, having a 9- and 20-month clinical duration from the onset, respectively, showed signs of moderate cerebellar ataxia, such as gait disturbance and slurred speech; however, they could walk unassisted and had slight or no cognitive impairment. GSS1 and GSS2 scored 22 and 26 points, respectively, on the Mini-Mental State Examination. GSS3, having a 27-month clinical duration, showed severe gait disturbance and slurred speech and was unable to walk unassisted; however, she had no cognitive impairment (30 points on the Mini-Mental State Examination) at the time of this study.

AD diagnosis was made according to the National Institute of Neurological and Communicative Diseases and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria [24]. CJD, GSS and AD patients were recruited from Miyagi National Hospital, Fukuoka University Hospital, Kagoshima University Hospital and Tohoku University Hospital. Normal controls were recruited from volunteers with no cognitive impairment or cerebrovascular lesions on MR images and who were not taking any centrally acting medications. No significant difference in age distribution was apparent between the groups. This study was approved by the Ethics Committee on clinical investigations of Tohoku University School of Medicine and performed in accordance with the Declaration of Helsinki. Written informed consent was obtained after complete description of the study to the patients and subjects.

Image acquisition protocols

PET scans were performed using a SET-2400W (Shimadzu Inc., Kyoto, Japan). After intravenous injection of 211–366 MBq (5.7–9.9 mCi) of [^{11}C]BF-227, dynamic PET images were obtained for 60 min with the subjects' eyes closed. Arterial blood sampling in the TSE patients was not

Table 1 Regional to pons standardized uptake value ratio (SUVRp) values in aged normal controls (Control), Alzheimer's disease patients (AD), Creutzfeldt-Jakob disease patients (CJD) and Gerstmann-Sträussler-Scheinker disease patients (GSS)

	Control (n=10) Mean ± SD	AD (n=17) Mean ± SD	CJD1	CJD2	GSS (n=3) Mean ± SD	GSS1	GSS2	GSS3
Frontal	0.60±0.03	0.64±0.04	0.57	0.61	0.67±0.08	0.74	0.69	0.57
Lateral temporal	0.59±0.03	0.69±0.04*	0.63	0.62	0.67±0.05*	0.71	0.68	0.61
Parietal	0.62±0.02	0.69±0.04*	0.62	0.62	0.67±0.06	0.72	0.68	0.61
Occipital	0.62±0.04	0.65±0.05	0.62	0.69	0.67±0.07	0.74	0.67	0.60
Medial temporal	0.64±0.04	0.62±0.03	0.57	0.65	0.67±0.02**	0.66	0.70	0.67
Striatum	0.71±0.04	0.75±0.04*	0.69	0.72	0.76±0.04	0.80	0.77	0.72
Thalamus	1.00±0.04	1.01±0.04	0.97	1.04	1.08±0.00*, **	1.08	1.07	1.08
Cerebellum	0.58±0.01	0.57±0.02	0.58	0.59	0.62±0.01*, **	0.61	0.63	0.61

*p<0.05 compared to aged normal group

**p < 0.05 compared to AD group

performed because the Committee on Clinical Investigation at Tohoku University School of Medicine did not approve blood sampling during the PET scan, from the standpoint of infection risk management. T_1-weighted MR images were obtained using a Signa 1.5-T machine (General Electric Inc., Milwaukee, WI, USA).

Image analysis

Standardized uptake value (SUV) images of [^{11}C]BF-227 were obtained by normalizing tissue concentration by injected dose and body weight. Average summations of SUV images were created from early frames (0–30 min post-injection) and late frames (40–60 min post-injection) of dynamic PET images. Early frame images were created for co-registration with individual MR images, and late frame images were used for calculation of SUV. Individual MR images were anatomically co-registered with the early frame PET images using statistical parametric mapping software (SPM2, Welcome Department of Imaging Neuroscience, London, UK) [25]. Spatial normalization was performed using an MR T_1 template of SPM2 to transfer PET images into a standard stereotactic space. Regions of interest (ROIs) were placed on a spatially normalized MR image, as described previously [12]. ROI information was then copied onto delayed PET SUV images, and regional SUV images at 40–60 min post-injection were sampled using Dr.View/LINUX software (AJS, Tokyo, Japan). Deposition of PrP plaques is reportedly frequent in the cerebellum but scarce in the pons of GSS brain [26]. Furthermore, BF-227 retention in the pons does not differ between AD patients and normal controls. Therefore, we used the pons as a reference region and calculated the regional to pons SUV ratio (SUVRp) as an index of BF-227 retention.

Statistical analysis

For statistical comparison in each group, we applied one-way analysis of variance, followed by the Bonferroni-Dunn post hoc test. Statistical comparison of age distribution was performed using the Kruskal-Wallis test, followed by Dunn's multiple comparison test. Statistical significance for each analysis was defined as $p<0.05$.

Results

Autoradiography examination indicated binding of a tracer dose of BF-227 to PrP plaque deposits. BF-227 retention was present in brain sections from GSS cases with PrP plaque deposition but not from normal control cases and sporadic CJD cases with synaptic PrP deposition (Fig. 1a–c). The regional distribution of [^{18}F]BF-227 in the autoradiograms co-localized with the immunostained PrP plaques in the cerebellar cortex of GSS cases (Fig. 1d–e). BF-227 binding to PrP plaques was additionally examined using a microscope, because BF-227 is a fluorescent compound. Core regions of the PrP plaques were intensely stained with BF-227 (Fig. 2, arrows), indicating that BF-227 preferentially binds to the fibril-rich core of PrP amyloid plaques.

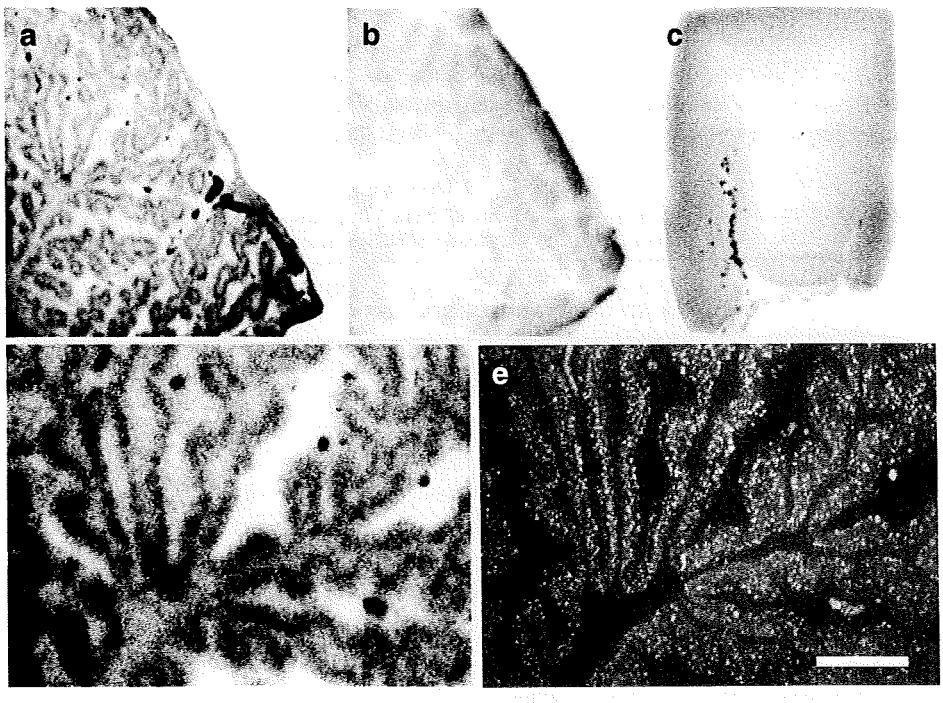

Fig. 1 [^{18}F]BF-227 autoradiograms of a cerebellar section from a Gerstmann-Sträussler-Scheinker (GSS) case (**a**), a cerebellar section from a physiological aging case (**b**) and a frontal cortex section from a sporadic Creutzfeldt-Jakob disease (CJD) case (**c**) are shown, together with a magnified view of **a** (**d**) and prion protein (PrP) immunostaining of the same field as **d** (**e**). BF-227 retention was present in the brain section from a GSS case with PrP plaque deposition, but not from a normal control case and sporadic CJD case with synaptic PrP deposition. *Bar*=200 μm

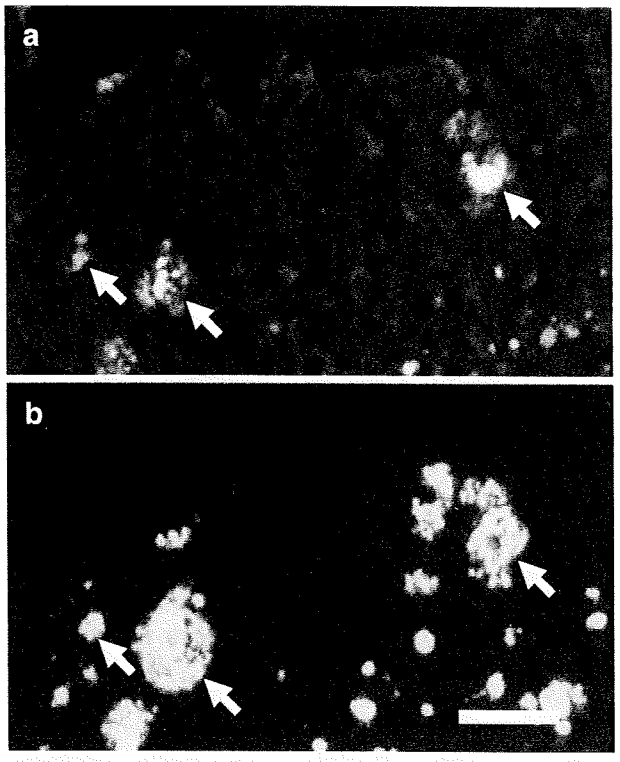

Fig. 2 Microscopic images of BF-227 staining (**a**) and PrP immunostaining (**b**) of the cerebellar cortex of a GSS case. *Arrows* indicate PrP amyloid plaques. The core regions of PrP plaques were intensely stained with BF-227. *Bar*=50 μm

Figure 3 shows the average summations of SUVRp images in an aged normal subject (64-year-old man), a sporadic CJD patient (CJD1, 63-year-old woman), a GSS patient (GSS2, 61-year-old man) and an AD patient (62-year-old woman). As reported previously, non-specific retention of [^{11}C]BF-227 was observed in the brain stem and white matter of all subjects [12]. The GSS patient showed obvious retention of [^{11}C]BF-227 in the cerebellum, and lateral and medial temporal cortices. The three GSS patients showed significantly higher SUVRp in the lateral temporal cortex, thalamus and cerebellum (Table 1, Fig. 4) when compared to aged normal controls. Furthermore, when compared to the AD group, the GSS group showed significant elevation of SUVRp in the medial temporal cortex, thalamus and cerebellum. Although two GSS patients (GSS1 and GSS2) showed retention of BF-227 in most brain regions, the youngest GSS patient (GSS3) showed BF-227 retention only in the cerebellum, thalamus and medial temporal cortex, but not in the neocortex (Table 1, Fig. 4). Furthermore, two sporadic CJD patients showed no obvious BF-227 retention in any of the brain regions examined (Table 1, Fig. 4). As previously described [12, 21], AD patients showed [^{11}C]BF-227 retention in the neocortex; however, the cerebellum and medial temporal cortex were relatively spared (Table 1).

Autopsy examination of the brain of one GSS patient (GSS1) confirmed both the presence of abundant PrP amyloid plaques in the neocortex, cerebellum, basal ganglia, thalamus, entorhinal cortex and hippocampus and the absence of Aβ amyloid plaques or other structures of misfolded protein deposition such as Lewy bodies and neurofibrillary tangles. When compared to controls, the highest SUVRp percentage difference was found in the neocortex, especially in the frontal cortex (22%), followed by the striatum (12%), thalamus (9%), cerebellum (6%) and medial temporal cortex (3%) in this case. This finding was consistent with the autopsy result showing higher density of PrP amyloid plaques in the neocortex and basal ganglia than in the cerebellum, thalamus and hippocampus. Details of clinicopathological features of this case will be published elsewhere.

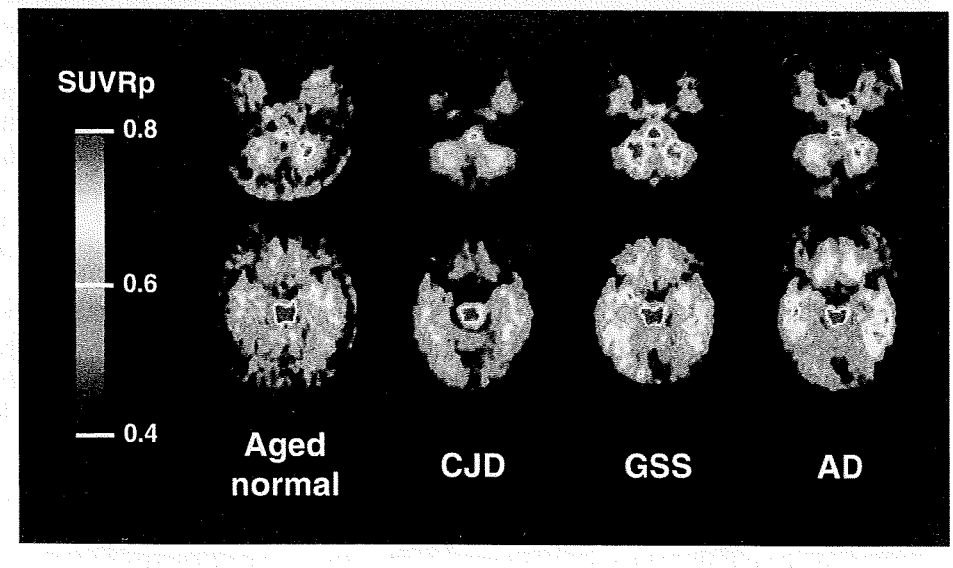

Fig. 3 Mean regional to pons standardized uptake value ratio (SUVRp) images between 40 and 60 min post-injection of [^{11}C]BF-227 in an aged normal subject (64-year-old man), a sporadic CJD patient (CJD1, 63-year-old woman), a GSS patient (GSS2, 61-year-old man) and an AD patient (62-year-old woman). Compared to the aged normal subject and CJD patient, the GSS patient showed obvious [^{11}C]BF-227 retention in the cerebellum and temporal cortex. The AD patient also showed obvious [^{11}C]BF-227 retention in the temporal cortex; however, the cerebellum was relatively spared

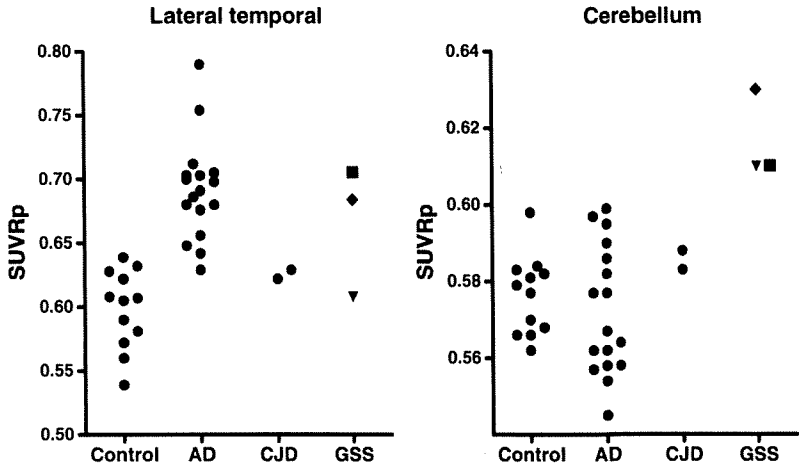

Fig. 4 SUVRp distribution in aged normal controls (*Control*), AD patients (*AD*), CJD patients (*CJD*) and GSS patients (*GSS*). GSS patients showed higher SUVRp values in the lateral temporal cortex and cerebellum. *Filled square* GSS1, *filled diamond* GSS2, *filled inverted triangle* GSS3

Discussion

This is the first study to demonstrate non-invasive detection of PrP amyloid plaques in GSS patients. GSS is neuropathologically characterized by deposits of multicentric amyloid plaques, which are especially abundant in the cerebellum, cerebral cortex and basal ganglia [3]. The present study demonstrated binding of BF-227 to PrP amyloid plaques in GSS brain sections. [^{11}C]BF-227 retention was observed in cortical and subcortical brain regions of GSS patients known for the high density of PrP plaques. Based on these findings, [^{11}C]BF-227 represents a promising candidate PET probe for the non-invasive detection of PrP amyloid plaques in the brain. However, the possibility that neocortical elevation of SUVRp in GSS patients might be caused by concomitant Aβ amyloid deposits or other misfolded protein deposits also should be considered, given that the two GSS patients showing prominent neocortical retention of [^{11}C]BF-227 were relatively older than the GSS patient showing no neocortical retention of BF-227. Although one positive GSS patient (GSS2) is still alive and was not examined neuropathologically, another positive case (GSS1) showed a high level of PrP amyloid deposits but no obvious deposits of Aβ amyloid or other misfolded proteins at autopsy. Furthermore, significant elevation of SUVRp was detected in the cerebellum, thalamus and hippocampus of all GSS cases. These brain regions are known to contain lower densities of Aβ plaques or other misfolded protein structures such as Lewy bodies. Based on these findings, it seems unlikely that concomitant deposition of Aβ amyloid or other misfolded proteins contributes to the high [^{11}C]BF-227 retention in GSS patients.

There is an increasing demand for in vivo detection of abnormal PrP deposition in the brain for the diagnosis of TSEs that might translate in early therapeutic intervention. Although GSS and other familial forms of TSEs can be diagnosed with PrP gene analysis using peripheral blood cells, it has been impossible to non-invasively measure the amount of abnormal PrP deposition in the brain. In a fashion similar to GSS, PrP amyloid deposition in the brain is commonly present in vCJD in which PrP amyloid plaques, called florid plaques, are pathognomonic [27]. Thus, [^{11}C]BF-227 PET might be a sensitive probe for the detection of PrP amyloid plaque deposition in vCJD as well as GSS, allowing longitudinal monitoring of PrP amyloid plaque deposition in the brain. Ante-mortem diagnosis of vCJD relies on the detection of abnormal PrP deposition in tonsil biopsy samples [28]. However, functional imaging using PET has an advantage over surgical biopsy tests in terms of both a non-invasive and an infection risk management point of view.

GSS is a rare form of TSE occurring in only about 3% of TSE cases in Japan. However, GSS is probably one of the TSEs most likely to benefit from early therapeutic interventions because the disease can be confirmed earlier using PrP gene analysis and progression occurs much more slowly than that in sporadic CJD, which comprises the majority of TSE cases. Recently, compounds such as pentosan polysulphate and doxycycline have been clinically used for experimental treatments for TSEs to prevent deposition of abnormal PrP in the brain, because these compounds slowed the disease progression in animal disease models when administered in an earlier stage of the disease [29–33]. Reliable surrogate markers are also required to evaluate the efficacy of these experimental interventions, and [^{11}C]BF-227 PET might be one of the best candidates to assess PrP amyloid deposition in GSS. However, it remains to be elucidated if PrP amyloid levels are a particularly relevant marker of therapeutic efficacy.

A previous PET study demonstrated moderate FDDNP retention and no remarkable PIB retention in the brain of two familial CJD patients with an octapeptide repeat insertion mutation [17]. A recent PET study has additionally demonstrated no PIB retention in two autopsy-confirmed sporadic

CJD patients [18]. In contrast with these studies, the present study successfully demonstrated prominent [^{11}C]BF-227 retention in the brain of GSS patients. Differences between the previous and present findings might mainly reside in the amount and type of PrP amyloid deposits in the brain, where histopathological studies indicate higher density of PrP amyloid plaques in GSS than in familial CJD [1]. In the present study, the findings in two sporadic CJD patients showing no obvious [^{11}C]BF-227 retention in the brain additionally support this speculation. The difference may also be attributable to higher binding affinity of BF-227 to PrP amyloid cores compared to FDDNP and PIB. To clarify this, further in vitro studies comparing the binding affinities of different amyloid tracers to PrP plaques in TSE brain homogenates are needed.

The youngest GSS patient (GSS3) showed BF-227 retention in the cerebellum and thalamus but not in the neocortex. The clinical symptoms in this patient were consistent with the brain distribution of BF-227, with the patient presenting with severe gait disturbance and slurred speech resulting from cerebellar ataxia but no signs of cognitive impairment, suggesting a close relationship between PrP plaque deposition as measured by BF-227 and regional brain dysfunction. There are variations of clinical phenotypes in GSS [1, 3]. Such variations are yet to be explained; however, the pattern of regional PrP amyloid distribution might be one of the factors affecting clinical phenotypes of GSS. In vivo PrP amyloid imaging using [^{11}C]BF-227 or other PET tracers will clarify neuropathological aspects of clinical variations in GSS.

In summary, we confirmed binding of BF-227 to PrP plaques in vitro and in vivo. A clinical PET study using [^{11}C]BF-227 demonstrated in vivo detection of PrP amyloid plaques in GSS patients. This imaging technique provides a potential means of facilitating both early diagnosis and non-invasive disease monitoring of certain forms of TSEs because, despite a lack of selectivity for PrP, brain retention of BF-227 in GSS shows a distinct pattern of regional distribution than that usually observed in sporadic AD.

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Clinical Commentary

Less protease-resistant PrP in a patient with sporadic CJD treated with intraventricular pentosan polysulphate

Terada T, Tsuboi Y, Obi T, Doh-ura K, Murayama S, Kitamoto T, Yamada T, Mizoguchi K. Less protease-resistant PrP in a patient with sporadic CJD treated with intraventricular pentosan polysulphate. Acta Neurol Scand: 2010: 121: 127–130.

Treatment with intraventricular pentosan polysulphate (PPS) might be beneficial in patients with Creutzfeldt–Jakob disease. We report a 68-year-old woman with sporadic Creutzfeldt–Jakob disease who received continuous intraventricular PPS infusion (1–120 μg/kg/day) for 17 months starting 10 months after the onset of clinical symptoms. Treatment with PPS was well tolerated but was associated with a minor, transient intraventricular hemorrhage and a non-progressive collection of subdural fluid. The patient's overall survival time was well above the mean time expected for the illness but still within the normal range. Post-mortem examination revealed that the level of abnormal protease-resistant prion protein in the brain was markedly decreased compared with levels in brains without PPS treatment. These findings suggest that intraventricular PPS infusion might modify the accumulation of abnormal prion proteins in the brains of patients with sporadic Creutzfeldt–Jakob disease.

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Key words: Creutzfeldt–Jakob disease; intraventricular infusion; pentosan polysulphate; prion protein

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Introduction

Current options for the treatment of Creutzfeldt–Jakob disease (CJD) do not slow or halt disease progression. Treatment with pentosan polysulphate (PPS), a large polyglycoside molecule with anti-thrombotic and anti-inflammatory properties, administered intraventricularly to bypass the blood brain barrier can both prolong the survival period and reduce the extent of abnormal prion protein (PrP) deposition in the brains of rodent prion disease models (1). The safety and efficacy of intraventricular PPS treatment in humans with CJD, however, remains largely unknown (2–6). We report a patient with sporadic CJD (sCJD) treated with continuous intraventricular PPS administration starting 10 months after the onset of clinical symptoms.

Case report

The patient was a 68-year-old woman with neither a family history of prion disease nor previous history of neurological disease. She had never received cadaveric growth hormone injection, a dura mater transplant, or a cornea transplant. She noticed unsteadiness of gait and forgetfulness at the age of 65 years. One month later, unsteadiness and intellectual deterioration progressed and myoclonic jerks appeared. Cerebrospinal fluid analysis was normal except for an increased concentration of neuron-specific enolase (66 ng/ml, normal < 25) and the presence of 14-3-3 protein. EEGs showed periodic spike/slow-wave complexes (spike-wave complexes). Diffusion-weighted MRI showed abnormal high-intensity signals in the head of the caudate nucleus, putamen and insular cortex.

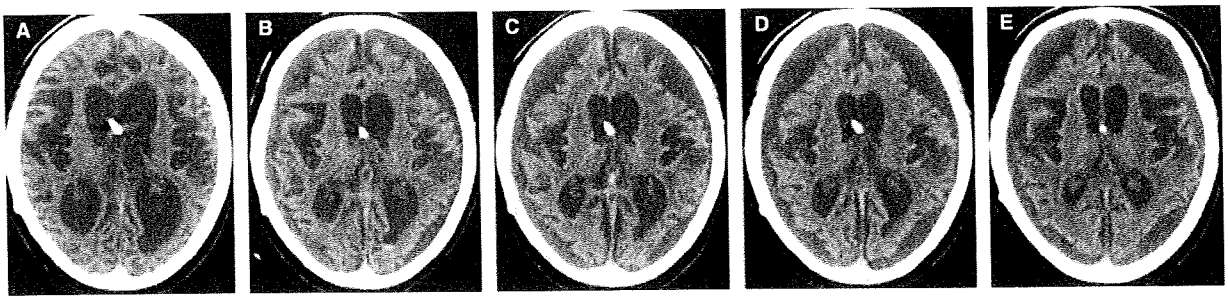

Figure 1. Sequential follow-up CT scans from 12 to 17 months after start of intraventricular PPS infusion. (A) Non-enhanced CT scan 12 months after start of intraventricular PPS infusion. Note the severe cortical and subcortical atrophy with enlargement of ventricular system. (B) Non-enhanced CT scan 13 months after start of intraventricular PPS infusion. Note the subdural fluid collection and the small sedimentation of blood in the left posterior horn. (C, D, E) Non-enhanced CT scan 14, 15, 17 months after start of intraventricular PPS infusion, respectively. The blood sedimentation in the posterior horn disappeared next month and subdural fluid collections were not progressing. No intraventricular hemorrhage was noted in scan E which was taken 7 days before death.

Genetic analysis of the *PrP* gene revealed methionine homozygosity at codon 129 and no mutations. The patient continued to deteriorate and became doubly incontinent, bed-bound and mute. Five months after the onset of symptoms, she developed akinetic mutism. Seven months after onset, the myoclonic jerks and spike–wave complexes disappeared. Ten months after onset, treatment with intraventricular PPS administration commenced under signed informed consent from her family. She received implantation of a right ventricular catheter and an epigastric subcutaneous drug infusion pump (Archimedes; 20-ml reservoir, flow rate 0.5 ml/24 h; Codman & Shurtleff Inc, Raynham, MA, USA). Using a reported protocol (5), infusion of intraventricular PPS (SP 54; bene-Arzneimittel GmbH, Munich, Germany) was started at 1 μg/kg/day, with subsequent escalation to the dose of 60 μg/kg/day 7 months later, and to the target dose of 120 μg/kg/day 15 months later, which continued until she died. However, her clinical condition did not improve and she still displayed akinetic mutism. A series of brain CT examinations demonstrated progressive brain atrophy, a transient intraventricular minor hemorrhage at the time of 13 months later, and a non-progressive collection of subdural fluid until 7 days before death (Fig. 1). Her clinical condition did not deteriorate from the time of 12 to 16 months. Monthly blood cell counts and coagulation measurements were normal. Twenty-seven months after onset, at age 68 years, the patient died of pneumonia which occurred 11 days before death and was aggravated.

Methods

Autopsy was performed within 2 h after death. The right temporal pole of the brain was dissected out and stored at −70°C. The other parts of the brain were fixed in neutral buffered formalin. Sections of representative areas of the brain were stained with hematoxylin–eosin, Klüver–Barrera and immunohistochemical methods.

Immunohistochemical staining

The following primary antibodies were used: anti-phosphorylated α-synuclein (monoclonal; Wako, Osaka, Japan), anti-phosphorylated tau (AT8, monoclonal; Fitzgerald, Concord, MA, USA), anti-amyloid β 1–42 (polyclonal; IBL, Takasaki, Japan) and anti-PrP (3F4, monoclonal; Signet, Dedham, MA, USA).

Prion protein analysis

Protease-resistant PrP was extracted from cerebral tissues of this and other sCJD patients as previously described (7). Samples were subjected to 13.5% SDS-PAGE and transferred to polyvinylidene fluoride membrane. 3F4 antibody was used as the primary antibody. Anti-mouse EnVision (Dako, Glostrup, Denmark) was used as the secondary antibody. Enhanced chemiluminescence detection (Amersham Bioscience, Little Chalfont, UK) was used to visualize Western blots. The signal intensities of the blots were quantified with Quantity One software using an imaging device, Vasa Doc 5000 (Bio-Rad Laboratories, Hercules, CA, USA) (7).

For quantitative comparison of protease-resistant PrP levels, we initially analyzed 10-fold diluted samples derived from 0.5 mg wet-weight brain tissue from the temporal pole to identify suitable dilutions. For controls, we included frontal lobe tissues from three sCJD patients (all homozygous for methionine at codon 129 of the *PrP* gene) not treated with intraventricular PPS infusion: two with a type 1 pattern of protease-resistant PrP signals in Western blot analysis (sCJD MM1) whose brains were uniformly, severely atrophied similarly to the

patient's brain, and one with cortical-type sCJD and a type 2 pattern (sCJD MM2C).

Results

Post-mortem neuropathology

The unfixed brain weighed 660 g and showed walnut-shaped severe atrophy. A massive intraventricular hematoma was present. The shape of blood cells in the hematoma was completely preserved, with no infiltration by reactive cells such as macrophages and glial cells. The PPS infusion catheter had been correctly inserted into the right lateral ventricle, and the source of hemorrhage could not be identified. There was extensive, symmetrical cortical atrophy, but the hippocampi were relatively spared.

Microscopy demonstrated extensive neuronal loss and spongiosis in most areas of the cerebral cortices, with collapsed cytoarchitecture. Axonal loss with secondary myelin loss was present in the central white matter, accompanied by a cellular reaction containing both astrocytes and microglial cells throughout the areas of myelin damage. There was widespread gliosis in the basal ganglia, thalami and cerebellar molecular layer. The cerebellar granular layer showed marked neuronal loss with gliosis and axonal loss, accompanied by secondary myelin loss in the cerebellar white matter. Lewy bodies, amyloid plaques and neurofibrillary tangles were not observed. PrP staining showed a widespread synaptic pattern in the cerebral cortices, basal ganglia and thalami. Synaptic staining was also present in the molecular layer of the cerebellum, with intense coarse deposits in the granular layer. No plaque-like PrP deposits were identified in any brain regions. The findings were consistent with the diagnosis of sCJD. There was no laterality in the extent of the neuronal loss, spongiosis, gliosis or synaptic PrP deposition.

Prion protein analysis

Western blot analysis of protease-resistant PrP showed a type 1 pattern (Fig. 2) identical to those of the two classical sCJD MM1 cases. Protease-resistant PrP levels were 1/3 to 1/8 of those in the sCJD patients with no intraventricular PPS treatment.

Discussion

Here, we present a patient with sCJD who was treated with intraventricular PPS for 17 months. The PPS dose of 120 μg/kg/day was well tolerated

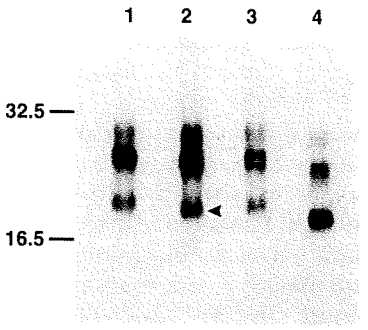

Figure 2. Comparative Western blot analysis of protease-resistant PrP. Protease-resistant PrP is categorized into three types based on the pattern of glycoform and mobility of PrP bands in Western blot analysis. Protease-resistant PrP, type 1, from the brain of this patient (threefold-diluted, lane 2) and three control subjects with sCJD: lane 1, 30-fold-diluted brain sample from an sCJD MM1 subject (65-year-old woman with a survival time of 11 months); lane 3, 20-fold-diluted brain sample from another sCJD MM1 subject (74-year-old woman with a survival time of 16 months); and lane 4, 40-fold-diluted brain sample from an sCJD MM2C subject. An unglycosylated PrP band from this patient (lane 2, arrowhead) mapped slightly lower than those in the other sCJD MM1 subjects (lanes 1 and 3). We normalized signal intensity to the band in lane 2 (100/mm^2). After dilution powers were also considered, the corrected signal intensities for lanes 1, 3 and 4 were 680/mm^2, 300/mm^2 and 770/mm^2, respectively.

but was associated with a minor, transient intraventricular hemorrhage and collection of subdural fluid. A fresh intraventricular hematoma found during autopsy probably occurred at the agonal stage, because blood cell shape was preserved and there was no inflammatory cell infiltration. Moreover, this intraventricular hematoma is unlikely to alter the patient's clinical course, because pneumonia which occurred 11 days before death was rapidly aggravated to respiratory failure responsible for her death, and no intraventricular hemorrhage was detected on CT scan 7 days before death.

Pentosan polysulphate is a candidate anti-prion compound that has shown efficacy in animal models (1, 8, 9), and has been administered by intraventricular infusion in several patients (2–6). Thrombocytopenia and abnormal coagulation can occur occasionally with PPS but did not occur in our patient. A minor, transient intraventricular hemorrhage and a non-progressive collection of subdural fluid appeared during PPS treatment but did not influence clinical progression. These findings may have resulted from a pressure imbalance within the intraventricular or subdural spaces caused by PPS infusion, although this speculation requires further proof. Overall, a PPS dose of 120 μg/kg/day seems well-tolerated and does not cause major adverse effects in CJD patients (2–6).

This patient survived for 27 months after the onset of clinical symptoms, which exceeds the mean survival period in national surveillance studies (12.7 months; range, 1–61) in Japan (10). PPS treatment did not alter the clinical course from the initial akinetic mute state. Thus, her prolonged survival might be partially attributable to both good nursing care and active medical interventions for malnutrition and pneumonia. The present study is a preliminary case study in a sCJD patient with pentosan therapy, and placebo-controlled study with PPS infusion will be needed in the future.

Prion protein deposition was not dramatically different between the hemisphere implanted with the catheter and the opposite hemisphere, unlike data reported in a rodent model (1). Here, the treatment started at an advanced clinical stage that may have already involved extensive PrP deposition, whereas treatment in the rodent model started before PrP deposition. In addition, difference of cerebrospinal fluid flow dynamics in the brain ventricular system between rodents and humans might contribute to the discrepancy. However, we found lower levels of abnormal protease-resistant PrP here than in other untreated sCJD patients, suggesting that PPS infusion might suppress the accumulation of abnormal PrP in the brain.

This speculation requires to be further evaluated, because there are possibilities that the gap of abnormal PrP levels between the patient and the control subjects might be attributable to the difference in disease durations or brain sampling regions, or to the regional variety of abnormal PrP deposition. These possibilities could not be evaluated in the present study because of limited sample availability.

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＜シンポジウム 11—4＞プリオン病の最新トピックス

プリオン病の治療予防開発

堂浦　克美

（臨床神経，49：946—948, 2009）
Key words：治療，プリオン病，多剤併用療法，発症前診断，疾患感受性

I. 現在の治療開発

プリオン病において，プリオン蛋白が病態形成で中心的な役割を果たしていることは明らかであるものの，それ以外の因子については不明である．とくに，亜急性に進行する神経変性がどのようなメカニズムによるものか未だに明らかではない．これまでに，インビトロ実験やインビボ実験で治療効果（プリオン増殖阻害や延命効果）が観察された医薬品などが臨床で試されてきた[1)2)]．現在も，ペントサンポリサルフェート脳室内投与[3)]とドキシサイクリン経口投与[4)]が実験的治療として実施されているが，病気の進行を止め症状改善をもたらすほどの効果は出ていない．インビボ実験では，感染早期に投与を開始したばあいには顕著な生命予後改善効果が観察されるものの，発症後の投与開始ではきわめて限定された効果しか観察されていないので，臨床での結果は当然ともいえる．

治療薬開発のターゲットとしては，3つ考えられる．一つは，プリオン（異常型プリオン蛋白）の増殖阻害であり，試験管内プリオン増幅法（後述の PMCA（protein misfolding cyclic amplification）法）やプリオン持続感染細胞といったアッセイ用のツールが整備されていることや，プリオン蛋白を標的とした合理的創薬の手法が応用できることから，もっとも研究が進んでいる．前述のペントサンポリサルフェートはプリオンの増殖阻害を標的にした代表的薬剤である．二つ目はプリオンの分解促進である．ドキシサイクリンの作用は，プリオンと結合することによりプリオンの構造を緩解して分解されやすくすると考えられている．最近，生体内でのプリオン分解経路についてオートファジーの関与が示唆されているものの，インビボ実験ではめだった効果はえられていない．もう一つのターゲットは神経変性の阻害である．もっとも即効的効果が期待される標的であるが，プリオン依存性神経変性をアッセイできる簡便なツールがないため研究は遅れており，めだった研究成果の報告はない．いずれにしても，単剤だけでは限界があり，3つの標的に作用する多剤併用療法が可能となれば，プリオン病の治療が現実味を帯びてくるものと思われる．

II. 今後の課題—感染キャリアーの診断

一部の患者を除いて，大半の患者では発症後は亜急性に病気が進行して数カ月以内には無動性無言状態に陥る．ミオクローヌスや脳波所見，病気の経過などからプリオン病の診断が成されるため，診断がつく時点では病気はかなり進行していることになる．プリオン病とガンはまったくことなる疾患であるものの，プリオン病の治療開発においてはガンは一つの参考となる．すなわち，プリオン病の神経症状が顕在化している時点はガンでは身体症状（体重減少，悪疫質，転移や播種による症状）が顕在化した進行期に相当しており，この時点では病気を治癒させることは不可能である．ガンの治癒が期待できるのは早期の段階であり，無症状でガン検診で異常を指摘されたり，軽微な症状を呈している早期がんに対して治療をおこなえば治癒が期待できる．プリオン病では，発症前のキャリアーの段階や発症の極早期に治療をおこなえば，治療効果が期待できることになる．したがって，感染キャリアーの診断が必要となってくるが，診断に結びつく疾患マーカーは未だに発見されていないし，疾患特異的なプリオン（異常型プリオン蛋白）を末梢から発症前に証明することもできていない．治療開発とともに，早期診断とくに発症前診断の開発がプリオン病を克服する上できわめて重要である．

III. 今後の課題—プリオン防御機構の解明

ヒトプリオン病の約 80% は，原因や感染経路が特定できない孤発性（散発性）クロイツフェルト・ヤコブ病であり，初老期から老年期でまれに発生する．一方，変異型クロイツフェルト・ヤコブ病，医原性プリオン病，kuru などの後天性プリオン病では，感染源に同程度暴露されたにもかかわらず，予想よりはるかに少人数に発病が観察されている．また，近年英国において大きな問題となっているのが変異型クロイツフェルト・ヤコブ病の未発症キャリアーの存在である．未発症キャリアーのリンパ網内系組織ではプリオンが増殖し，血液中にもプリオンが存在するためである．このような事実は，プリオン病は特定のヒトで発病がおこる可能性を示唆している．

一方，遺伝性プリオン病においては，同一の遺伝子変異で

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も，臨床像や発症時期にはバリエーションがある．端的な例は，一卵性双生児であって，同一の遺伝的背景を持つにもかかわらず，臨床像や発症時期がことなる例が報告されている[5]．また，同一の遺伝子変異を持っていても，発病するヒトと発病しないヒトがいて，この現象はペネトランス(浸透率)という言葉で説明されている．遺伝子変異が疾患をおこさせる強さと疾患がおこるのを防ごうとする体質や環境要因とのバランス状態が，ペネトランスの高低で説明されている．

動物をもちいた実験においても，感染個体が未発症キャリアー状態になるばあいがあることが知られている．きわめて低い感染力価のものを接種されたばあいや，種の壁現象がみられる宿主—プリオン株の組み合わせをもちいた実験をおこなったばあい[6]，さらにはプリオン蛋白の発現を低下させた個体に感染させたばあいなどである．また，脳内感染ではなく末梢感染の際には，B細胞欠損マウスでは未発症キャリアー状態が生じることが知られている[7]．このような動物をもちいた実験で観察されている現象が，ヒトでみられる未発症キャリアーの出現や特定の人達での発症を説明できるかもしれないが，われわれの体内に発症を防ごうとする体質(防御機構)が存在する可能性やわれわれの体に作用して発症抑制に働く環境要因が存在する可能性を否定するものではない．

さて，1982年にプルシナー博士がプリオン仮説を提唱して以来，プリオン研究でもっともホットな論争の一つはプリオンの複製増殖メカニズムであった．今日，プリオンは試験管内でPMCA法[8]で増幅させることができることについては否定する研究者はいない．この方法では，正常型プリオン蛋白と異常型プリオン蛋白を試験管の中に入れ，超音波処理を加えた後に，37℃で半時間程度振盪させる反応を数十回とくりかえすことにより，異常型プリオン蛋白量と感染力価は数百倍に増加する．驚くべきことに，異常型プリオン蛋白をふくまない反応組成物（精製した正常型プリオン蛋白とRNAの混合物[9]や，正常動物の脳乳剤のみ[10])だけでPMCA法を実施したばあいにも，感染性を有する異常型プリオン蛋白を生じたことが最近報告されるようになった．コンタミによるアーチファクトを示唆する報告も有り，現在盛んに論争されているところであるが，もしアーチファクトによる産物でなければ，「病原因子プリオンはユビキタスに存在し，健常な生体内でもプリオンが絶えず少量は産生されている」ことになる．

この仮説が正しいとすると，われわれの体内にはプリオンの増殖をおさえ発症を防ぐ疾患感受性にかかわる防御機構が備わっており，その防御機構は何らかの外的要因や内的要因の影響を受けて発病を修飾している可能性がある．プリオンに対する生体防御機構の解明は，未開拓の領域であり，治療予防開発の一つの経路として今後盛んに研究されるべき課題である．

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最新事情 プリオン病のメカニズムと治療戦略

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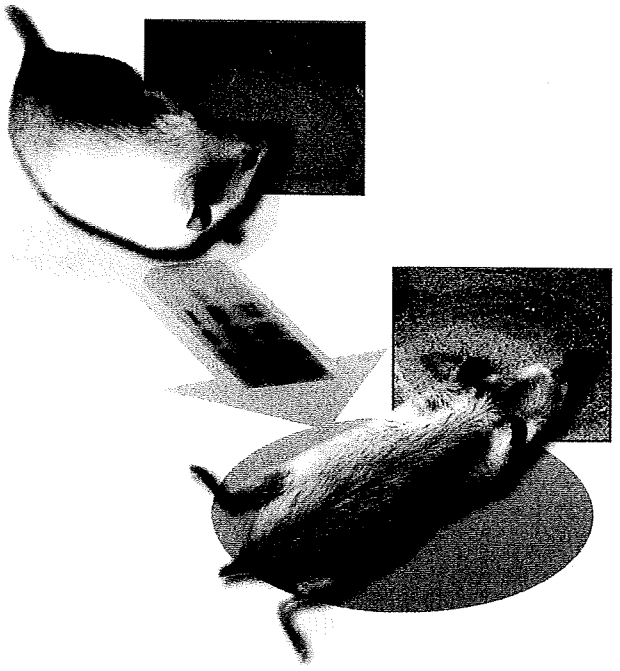
プリオンとは，ヒトおよび動物の脳に伝達性(感染性のある)海綿状変性をおこし，異常型プリオンタンパク質(異常型PrP)が蓄積する一連の疾患における「感染因子」をさす。プリオンの増殖は，タンパク質レベルでの事象と捉えられている。ここでは，その増殖メカニズムに関する知見・疾患発生の現状をはじめとして，現在進歩が注目，期待されている技術と応用，治療開発の方向性について解説する。



マウス脳における異常型プリオンタンパク質の増加

プリオン病に感染したマウスでは，病気の進行にしたがって徐々に異常型プリオンタンパク質が増加していく(電気泳動像)。
ピンク色の写真は，海馬から皮質を含む面で切断した脳切片を抗プリオンタンパク質抗体で染色したもの。病状の進行したマウス(右)では，非感染マウス(左)に比べ，プリオンタンパク質(赤く見える)が多量に沈着していることがわかる。

ヒトプリオン病の現状

(1) ヒトプリオン病の種類と診断

クロイツフェルト・ヤコブ病(Creutzfeldt－Jakob disease，CJD)[*1]をはじめとするプリオン病は，脳内における異常型のプリオンタンパク質(prion protein，PrP)の蓄積を大きな特徴とする致死性の神経変性疾患である。

わが国では1999年にCJDサーベイランス委員会が設立され，疾患発生の調査にあたっている。2008年までの調査結果[(1)]によると，新規登録罹患者数は年間100人前後であり，罹患の経緯によって，孤発型，感染性，家族性(遺伝性)に分類されている(**表**)。希少な疾患ではあるが社会的関心は高い。

診断は，① 臨床所見(急速な認知症症状，不随意運動など)，② 脳波測定(多くの症例で周期性同期性放電)，③ プリオンタンパク質遺伝子検査(遺伝的要因の判定，臨床像に影響を与える正常多型など)，④ MRIによる画像診断(脳萎縮の評価，基底核や大脳皮質での強い信号など)，⑤ 異常型PrPの検出(PrPのプロテアーゼ抵抗の生化学的評価)，⑥ 病理組織学的解析(神経細胞の脱落，沈着したPrP量と部位など)からなされる。画像診断は実施数が増加しており，画像の標準化と診断の精度向上がはかられている。また，神経変性によって脳脊髄液中に出現してくる14－3－3タンパク質測定が標準化され，判定基準の一つとして加えられた。

(2) 感染性CJDの感染リスク要因と対策

感染因子プリオンとの接触による感染性CJD(**表**)は，以下で述べる変異型CJDや医原性疾患を含んでおり，CJD全体の発生原因の7.8%を占めている[(1)]。

ウシ海綿状脳症(bovine spongiform encephalopathy，BSE)[*2]をその感染源とする変異型CJDの発生推移はイギ

FOOTNOTE

*1 クロイツフェルト・ヤコブ病
1920，1921年にそれぞれ，CreutzfeldtとJakobによって亜急性の海綿状脳症として報告された神経変性疾患。
このヒトの海綿状脳症が伝達可能，すなわち伝達性海綿状脳症であることは，1968年にGibbsとGajdusekによってチンパンジーを用いた研究から明らかとなった。これは「感染因子」の存在を示すものである。

*2 ウシ海綿状脳症
1986年，イギリスで発見・報告された。行動異常，運動失調と段階を経て，起立不能と症状が進行し，死にいたる。
2000年ごろから，迅速BSE検査キットが実用化された。
日本で最初のBSE感染牛は2001年9月に見いだされた。

表 ヒトのプリオン病の分類

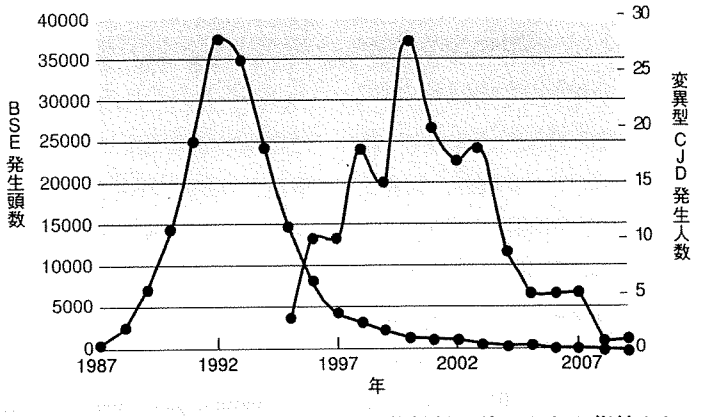
孤発性クロイツフェルト・ヤコブ病 (sporadic CJD, sCJD)	感染性プリオン病	家族性プリオン病
古典型(MM1, MV1)	クールー	家族性CJD(familial CJD)
失調型(MV2, VV2)	医原性CJD(iatrogenic CJD; 硬膜移植,下垂体製剤などによる)	ゲルストマン・シュトロイスラー・シャインカー(Gerstmann-Sträussler-Scheinker, GSS)症候群
MM2視床型	変異型CJD (vairiant CJD, vCJD)	致死性家族性不眠症
MM2皮質型		
認知症型(VV1)		

ヒトプリオン病は,罹患の経緯によって,孤発性,感染性,家族性(遺伝性)に分類されている。
孤発性クロイツフェルト・ヤコブ病(sCJD)は,異常型PrPのタンパク質分解酵素による切断部位のちがい(タイプ1と2)とPrP遺伝子の正常多型〔129番目のアミノ酸がメチオニン(M)であるかバリン(V)であるか〕,ならびに脳内における異常型PrPの蓄積部位の組合せによって,さらに五つに分けられている。

感染性プリオン病には,ニューギニア島での古い食人風習に起因するクールー,医療行為に起因する医原性CJD,牛海綿状脳症(BSE)の感染によるとみられる変異型CJDが知られている。
家族性プリオン病は,プリオンタンパク質遺伝子上のさまざまなアミノ酸変異,挿入,欠失をともなう。変異の種類によって臨床症状は多様である。変異型と臨床病理像により分類される。

図1 イギリスにおけるBSE(青)と変異型CJD(赤)の年次発生数

BSEの発生は1992～1993年,変異型CJDの発生は2000～2001年にそのピークを迎えている。
http://www.cjd.ed.ac.uk/figures.htm
http://www.oie.int/Eng/info/en_esbmonde.htm
のデータより作成。



リスの例が明瞭である(図1)。増加し続けたBSEの発生は,1992年に次段落で述べるような対策が講じられ減少に転じた。これに呼応するように,変異型CJDの発生件数は2000年にピークを迎えた後,減少に向かっている。

感染のリスク因子としては,プリオンを含む食物の摂取,感染者の血液や生体材料の使用などが指摘されている。日本では脳硬膜移植[*3]による感染例が,フランスを中心としてヨーロッパにおいては下垂体ホルモン製剤による感染が重大な問題として指摘されてきた。これらの感染性CJDに関しては,BSE感染牛の発生監視や,感染源となった乾燥硬膜や下垂体ホルモン製剤の利用の禁止措置などの対策により,それらの発生件数はピーク時に比べて有効に抑えられている。

しかしながら,各国における調査の進展によって,感染源として新たな脅威となる可能性があるものが明らかとなってきている。

その一つは,2004年に報告された,異常型PrPの蓄積部位や生化学的特徴が従来のBSEのそれとは異なる"非定型BSE(あるいは変異型BSEとよばれる)"である。症状の一部が,後に述べる孤発性CJDに類似していることもあり,これら非定型例のBSEにおける位置づけと,ヒトとの関連性を早急に明らかにすることが求められている[(2)]。なお,これら非定型BSEは従来のBSEに対する検査で見いだされてきたことを追記しておく。

いま一つの脅威は,最近ではBSEに関連した報告が減少してきているのに対して,鹿のプリオン病である慢性消耗病(chronic wasting disease, CWD)に関連した報告は増加してきて

*3 脳硬膜移植
脳外科手術の際に脳硬膜に欠損が生じた場合,その欠損部位の硬膜を補填するためにヒト屍体由来の乾燥硬膜が用いられていた。アルカリによる除染処理を施した乾燥硬膜による新規CJD患者は報告されていないものの,1987年以降,厚生省(現厚生労働省)はヒト乾燥硬膜の使用を禁止している。

図2 CWDの北米における発生分布

黄色は野生の鹿でCWDが見つかった地域，黄緑色は家畜として飼育されていた鹿でCWDが確認された州を示している。CWDは，アメリカコロラド州の野生動物研究施設内で飼育されていた鹿において，1967年に原因不明の疾患として発見された。1978年に海綿状脳症として診断された。
Chronic Wasting Disease Alliance（www.cwd-info.org）より。

いることだ。家畜鹿だけでなく野生鹿でもCWDが頻発しており，北米を中心にCWDは大きな広がりをみせている（図2）。さらに，唾液，糞便中にもプリオンが存在することが動物への伝播実験によって確認され，BSEの沈静化で成功している感染源の人為的な制御は困難であることが懸念されている。

CWDが人類にとってどれほどのリスクになるかは継続的に評価されている。現時点では，小規模のCJD患者（3名）についてはCWDとの関連が疑われたが，その後，感染との関連を強力に示唆する証拠は得られていない[3]。

このほか，プリオンは通常の滅菌操作には抵抗性であることから，輸血や手術器具を介した感染の危険性については現時点においても大きな懸念材料として存在している。実際，2004年以降，イギリスでは血液を介した変異型CJD感染を疑われるものが4例報告されている[4]。

(3) 孤発性CJDの病態とPrP分子種による発症抑制

発症年齢が高いことを特徴の一つとする孤発性CJDは，ヒトプリオン病患者の約80％を占めている[1]。孤発性CJDは，検出される異常型PrPのタンパク質分解酵素による切断部位のちがい（タイプ1と2）とPrP遺伝子の正常多型〔129番目のアミノ酸がメチオニン（M）であるかバリン（V）であるか〕と脳内における異常型PrPの蓄積部位の組合せにより，大きく5種類に分類されている（表）。このPrP分子の性質にもとづく分類は臨床像・経過・発病にいたる潜伏期間や病理と一定の対応があり，診断上の重要な情報である。

近年，同一脳においても部位によってはタイプが混在していることが示されている。Parchiらは，ヨーロッパにおける孤発性CJD例を調べ，35％の症例でタイプの混在を見いだし，症状との関連を解析し[5]，異常型PrPの状態が病態の（結果か原因かは別として）決定因子であることを強く裏づけている。

Hizumeらは，さまざまなヒト型PrP分子種のノックインマウスを用いてプリオン感染に対する応答を精査した[6]。孤発性CJDの疫学からPrPの219番目のアミノ酸の正常多型はこれまで異常化に対して抵抗性である考えられていたが，じつは容易にプリオン感染し異常型PrPを生成することを明らかにした。さらにヘテロ遺伝子型，すなわち，増殖（次項参照）の基質としてタンパク質一次配列が異なるPrP分子種が複数混在する場合には，プリオン感染に対して発症遅延効果が得られることを明らかにし，疫学的に認められていた抑制効果を説明するPrP重合モデルを提唱している。すなわちPrPの重合は，構成するPrPの分子種が互いに整合する場合に効率がよいということを意味し，ヘテロ遺伝子型の場合はこの整合がとりにくいという概念である。

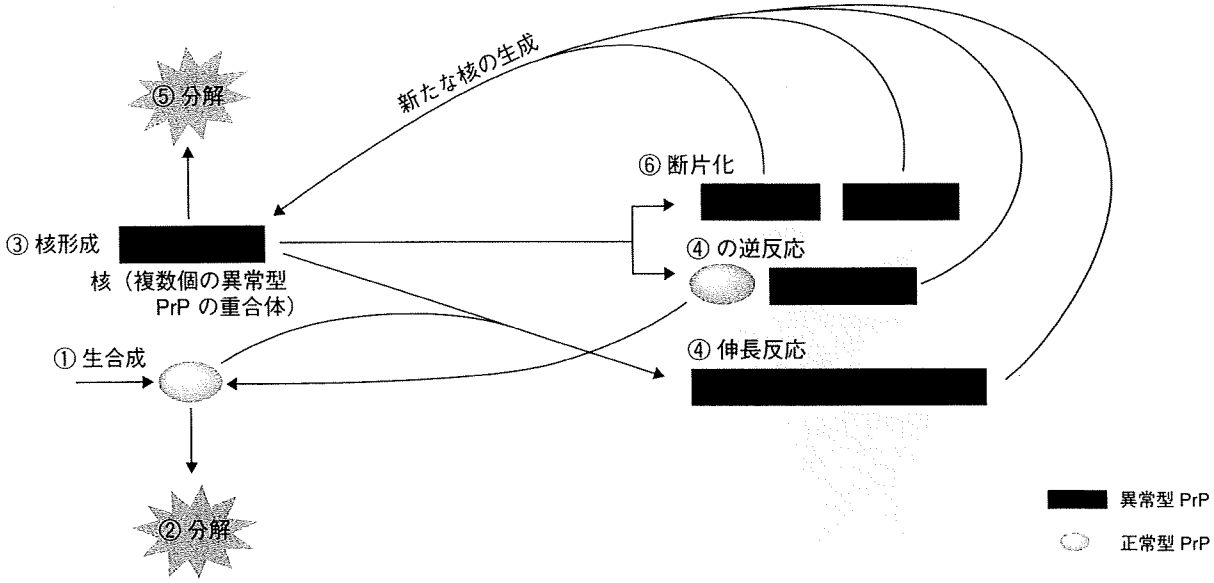
プリオン増殖メカニズム

タンパク質レベルにおけるプリオン，異常型PrP増殖メカニズムに関しては，従来，「ヘテロダイマーモデル」と「核依

FOOTNOTE

図3 Maselらの異常型PrP増殖の反応ダイアグラム[8]

正常型PrPの合成(①), 分解(②), ならびに核(異常型PrP重合体)形成(③), 重合体の伸長(④)および分解(⑤), 断片化(⑥)の6個の素過程を含む。Maselらは, 各過程に速度定数を定め速度論による立式をおこなった。感染における力価, 生存期間などの実験値から, 反応ダイアグラムの妥当性の評価と各速度定数を算出している。本文中の核依存性増殖モデルを参照。



存性増殖モデル」が提唱されていた。前者は1分子の異常型プリオンタンパク質がプリオンとしてはたらき, 異常型・正常型のプリオンタンパク質二量体の形成を通して, 異常化させるというもの。後者は複数個重合した異常型PrPが核としてはたらき, 正常型PrPを取り込んで異常化させ, 成長した異常型PrPの重合体は分断され, 新たな核を生みだすというものである。

現在では, 異常型PrPの電子顕微鏡像[7]などから幾つかの異常型PrPの構造的モデルが構築され, また, 核形成を裏づける現象の増加によって, 核依存性増殖モデルをベースにおいたモデルが主流となっている。

Maselらは, 1999年までに得られていたマウスでの感染実験の結果に対して核依存性のプリオン増殖モデルをベースに, PrPの合成, 分解, 核形成, 重合体の伸長および分解, 断片化(**図3**)の6個の素過程を含む速度論的な式をたて数理的な解析をおこない, 現象論的に速度定数を推定した[8]。**図3**のように想定されている各過程は, 治療法のターゲットとして重要である。

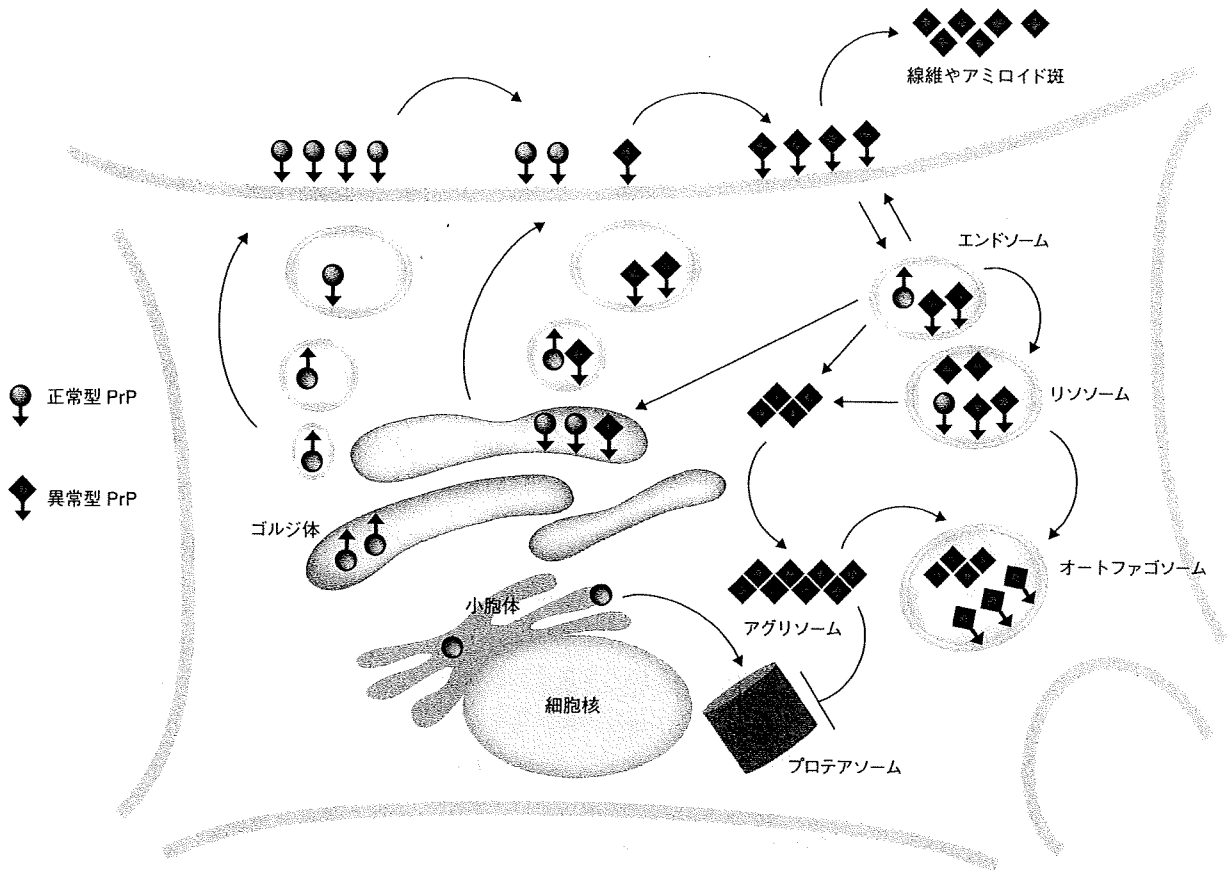
図4は, 細胞レベルでの正常型PrPと異常型PrPの代謝の概要を示している(詳細は成書[9]を参照いただきたい)。PrPは幾つかの段階で翻訳後修飾を受ける。しかしながら生理的役割についていまだ決定的なものは知られていない。

異常型PrPを実際に試験管内で増幅する技術として, protein misfolding cyclic amplification (PMCA)がある。PMCA法は2001年にSaborioらによって発表され, 研究や診断への広い応用が期待されている[10](**図5**)。この手法では, 感染因子を含む試料に基質となるPrPを共存させ, 超音波刺激により反応を促進して異常型PrPを増幅する。

この手法が開発されて以来, プリオンと基質それぞれに対してさまざまな動物種, 由来について検討され, 徐々にその有用性が確立してきている。増幅することによって微量の異常型PrPを検出することが可能であり, 迅速な感染源の診断, 感染処理効果の評価, 治療薬のスクリーニングなどへの応用が試みられている。さらに感度や特異性を改善し, 定量的解析が可能となる工夫や, あらゆる動物種・組織・プリオン株に対応できる工夫が求められている。たとえば, ヒト由来のプリオンの増幅や血液を材料とする診断を目的

図4 プリオンタンパク質の代謝・異常化の概略図

プリオンタンパク質遺伝子は翻訳後，一連の細胞内小器官において翻訳後修飾を受けて成熟する。
翻訳後，N末端のシグナルペプチドが編集を受け，糖鎖が付加され，C末端のシグナルペプチドは，グリコシルフォスファチジルイノシトールで構成されるリン脂質に置換され，細胞膜表面に繋留される。その後は小胞の動的な取り込みや排出，分解過程などの一連の工程にのる。
外来から細胞に取り込まれた異常型PrPや細胞内で異常化した異常型PrPは，同様の工程をたどって正常型PrPと接触すると考えられている。



とした効率的なPMCA法はまだ確立されていない。異常型PrPの検出や発症機序の解明におけるブレークスルーとして期待されている。

治療開発の方向性

われわれは，プリオン病治療予防薬の確立に向けてさまざまな化合物群の検索と評価研究を実施してきた。図6は本稿で紹介する化合物の構造を示している。

(1) 幾つかの候補化合物の評価

これまでのプリオン病の実験的治療研究において，抗プリオン作用*4をもつキナクリン（図6a）やキニーネ（図6b）を用いた経口投与療法は，副作用が頻発し疾患の進行を抑制する効果はみられていない。

ペントサンポリサルフェート（PPS；図6c）脳室内投与療法は，その安全性と効果について検討されている。硫酸化多糖であるPPSは血液脳関門*5を通過できないことから，プリオン病の主たる病変部位である脳実質に薬剤を到達させるために，脳室内カテーテル留置や持続注入装置の体内埋め込みのための外科的処置を必要とする[11]。イギリスと日本を中心に臨床試験が実施された。日本ではTuboiらがPPS療法を

FOOTNOTE

*4 抗プリオン作用
ここでは異常型プリオンタンパク質（PrP）の増加を抑える作用をさしている。

*5 血液脳関門
血中から脳へと流入する物質を選別する機構。
一般的にはPPSのような荷電を多くもつ水溶性高分子化合物は，血中から脳への移行が困難であり，CompBのような有機低分子化合物は比較的移行しやすい。
プリオン病ほか，脳疾患への投薬手段を講じるうえで，きわめて重要な性質の一つ。