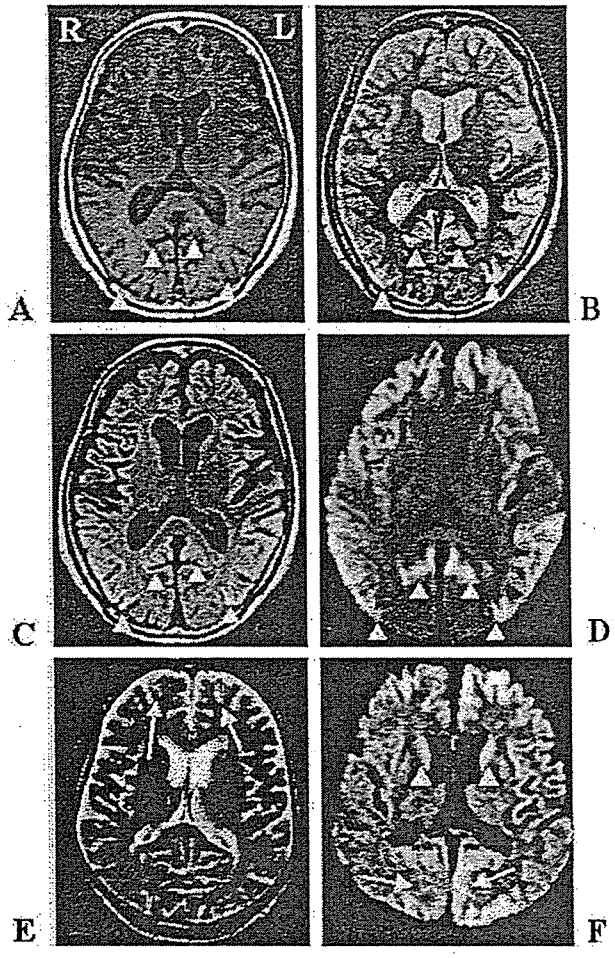

Figure. MRI of Patient 1 (A through D) and a patient with sporadic Creutzfeldt–Jakob disease (CJD) (E and F). For Patient 1 (82-year-old woman), the MRI studies were performed 4 months after the onset using 1.5 T MR unit (Magnetom Vision; Siemens, Erlangen, Germany) equipped with a conventional head coil. At this time, she demonstrated only memory disturbance and could perform her daily activities with minimal support. The wide-ranging cortical ribbon is symmetrically depicted as a low-intensity lesion by T1-weighted imaging (A) and as a high-intensity lesion by T2-weighted (B), fluid-attenuated inversion recovery (C), and diffusion-weighted (D) imaging and has a swollen appearance. The basal ganglia are not involved. Characteristically, the medial regions posterior to the parieto-occipital sulcus in the occipital lobes are not involved (arrowheads). The cerebellum was not depicted as an abnormal-intensity lesion (data not shown). For the patient with sporadic CJD with methionine homozygosity at codon 129 (70-year-old man), the MRI studies were performed 2 months after the onset using the same MR unit. At this time, he was totally bedridden and did not respond to any simple orders but opened his eyes when his name was called loudly. He showed myoclonus and startle reflex. The wide-ranging cortical ribbon including the occipital lobe (arrows) and the bilateral caudate heads (arrowheads) are depicted as a high-intensity lesion by diffusion-weighted imaging (F), although T2-weighted imaging examined at the same time demonstrates a high-intensity lesion in only the frontal lobe (arrows).

were recognized in three of four CJD180 cases with MM129 and three of five CJD180 cases with MV129 in the very early phase. On the other hand, no CJD180 patients demonstrated visual or cerebellar symptoms, which were cardinal in sCJD. Irrespective of the polymorphism at codon 129, no CJD180 patients demonstrated PSWC in repeated EEG in their disease course.

In the MRI study, the wide range of the cortical ribbon was depicted as a low-intensity area by T1I and a high-intensity area by T2I, FLAIR, and DWI and had a swollen appearance (figure, A through D). These cortical lesions were remarkable compared with the severity of the clinical symptoms. The basal ganglia lesions were less remarkable compared with the cortical lesions. Characteristically, the medial regions, posterior to the parieto-occipital sulcus in the occipital lobes (see the figure, A through D, arrowheads), and the cerebellum were never involved in the early stage. These cortical lesions were not always symmetric in the first MRI. In sequential MRI performed in six of seven patients, these cortical lesions expanded and, in one patient,[3] finally included the medial occipital regions. The swollen cortical lesions became atrophied in the advanced stage, but not as severe as compared with the brain atrophy of sCJD.

Discussion. V180I is recognized as a causative point mutation based on the result that V180I was detected only in CJD patients but not in 200 normal Japanese persons.[9] The World Health Organization also lists CJD180 as familial CJD.[1]

We clarified the clinical and laboratory characteristics of CJD180 by comparing them with those of sCJD. CJD180 showed 1) older onset age; 2) slower progression of the disease; 3) unique clinical symptoms such as frequent higher cortical dysfunction, which was less frequent in sCJD, no visual or cerebellar symptoms, which were important for sCJD, and less remarkable myoclonic jerk compared with the generalized one in sCJD; 4) a lower positive rate of brain-specific proteins such as NSE and 14-3-3 protein in CSF; and 5) no PSWC in EEG throughout the disease course. These features render it difficult to make a premortem diagnosis of CJD180 based on the clinical features without a *PRNP* analysis.

In our experience, the most useful test leading to the genetic analysis was MRI. The abnormal lesions in MRI of sCJD are varied,[10] but those of CJD180 are rather uniform. In accordance with the absence of visual or cerebellar symptoms in the early stage, the medial occipital lobes posterior to the parieto-occipital sulcus or the cerebellum were never involved until the terminal stage. A disproportionately remarkable cortical lesion compared with the severity of the clinical symptoms and less remarkable basal ganglia lesion must be recognized as characteristic MRI findings. At present, we must recognize an uncommon variant of familial CJD that might have been misdiagnosed. Therefore, we recommend MRI study including DWI for patients with progressive dementia. Then, we should perform a *PRNP* analysis

in all patients with progressive dementia and characteristic MRI abnormalities.

Parkinsonism, which was a rare symptom in sCJD, occurred in two of five CJD180 cases with MV129 in the very early stage. It is important to discriminate among neurodegenerative disorders presenting dementia with parkinsonism from CJD180. MRI can provide us useful information.

CJD180 is clearly associated with a point mutation of *PRNP* but appears as if it were a sporadic neurodegenerative disorder. We may misdiagnose such cases without a genetic analysis because of the difference in the clinical features from what we usually consider the "CJD characteristic" clinical features. Characteristic MRI findings can lead us to an accurate premortem diagnosis.

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Heidenhain Variant of Creutzfeldt-Jakob Disease: Diffusion-Weighted MRI and PET Characteristics

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ABSTRACT

Creutzfeldt-Jakob disease (CJD) is characterized by rapidly progressive dementia with a variety of neurological disorders and a fatal outcome. The authors present a case with visual disturbance as a leading symptom and rapid deterioration in global cognitive functions. The cerebrospinal fluid was positive for 14-3-3 protein, and diffusion-weighted magnetic resonance imaging (MRI) showed marked hyperintensity in the parieto-occipital cortices, where hypometabolism was clearly detected on positron emission tomography (PET). Pattern-reversal visual evoked potentials showed prolonged P100 latencies and increased N75/P100 amplitudes. All these findings supported a diagnosis of the Heidenhain variant of CJD, whereas a long clinical course, a lack of myoclonus, and an absence of periodic synchronous discharges on electroencephalography were atypical. Diffusion-weighted MRI and PET in combination with visual evoked potential recording and 14-3-3 protein detection may be useful for the early diagnosis of CJD.

Key words: Creutzfeldt-Jakob disease, visual disturbance, 14-3-3 protein, diffusion-weighted MRI, PET, visual evoked potentials.

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Creutzfeldt-Jakob disease (CJD) is a rare spongiform encephalopathy occurring sporadically in most cases. The diagnosis of CJD is based on clinical symptoms, such as rapidly progressive dementia, myoclonus, visual or cerebellar signs, pyramidal or extrapyramidal signs, and akinetic mutism, although the definite diagnosis of CJD requires pathological findings of the brain.[1] Periodic synchronous discharges (PSDs) on electroencephalography (EEG) and the detection of 14-3-3 protein in the cerebrospinal fluid (CSF) further support clinical suspicion of CJD.[1-4] Furthermore, magnetic resonance imaging (MRI), particularly diffusion-weighted imaging (DWI), has been shown to be useful in diagnosing the disease.[5-12] Herein, we report a probable case of CJD in which neuroimaging techniques proved useful in the early diagnosis of the disease. Progressive dementia, visual disturbance, 14-3-3 protein in the CSF, and neuroimaging findings supported a diagnosis of CJD, but other clinical manifestations were atypical, including a long clinical course, the absence of myoclonus, and no PSDs on EEG.

Case Presentation

A 54-year-old woman noticed blurred vision and visual metamorphosis in August 2001. Her visual disturbance worsened, and she gave up driving a car. At 2 months, her family noticed that she had memory impairment and disorientation for time and place. She often lost her way around her house. Her cognitive deterioration rapidly progressed, and she felt difficulties in

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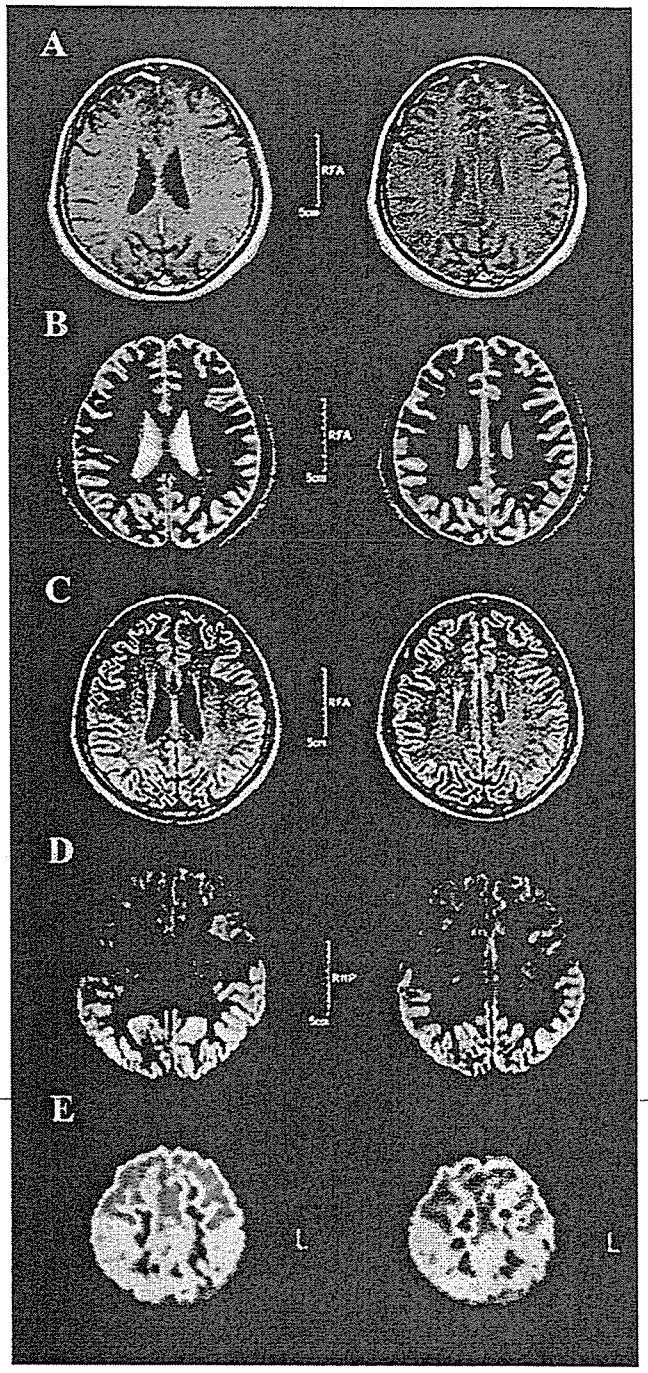
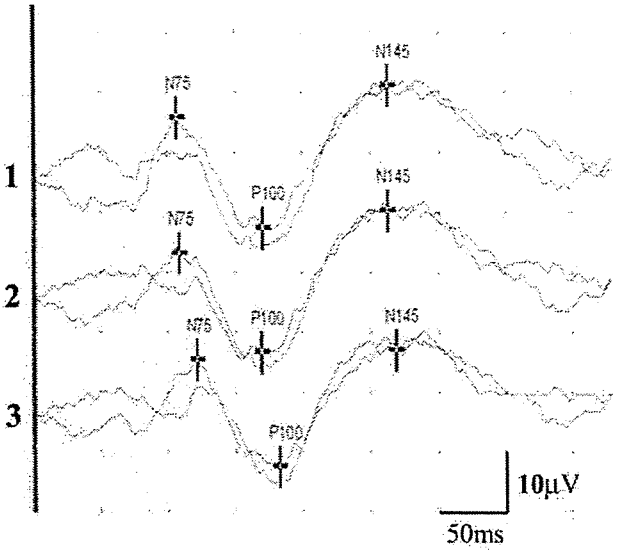


Fig 1. Magnetic resonance imaging (MRI) and positron emission tomography (PET) axial images. There was no atrophy, signs of cerebrovascular disease, or obvious signal abnormality on T1-weighted or T2-weighted MRI (A, B). High signal intensity in the parieto-occipital regions was detected on fluid-attenuated inversion recovery MRI (C), and the hyperintensity was most obvious on diffusion-weighted MRI (D). Low glucose metabolism was observed in the parieto-occipital regions as well as in the posterior cingulate cortex on PET (E). For the PET study, 5 mCi of [^{18}F]-fluorodeoxyglucose were administered intravenously, and scanning was performed using GE Advance (GE Medical Systems, Milwaukee, WI). Semiquantitative measurements were used.

calculation, reading, writing, and cooking at the beginning of 2002. She was often unable to locate the bathroom in her house by March 2002.



	Latency (ms)	Latency (ms)	Latency (ms)		Amplitude (μV)	Amplitude (μV)
1	N75 106	P100 170	N145 262		N75P100 18.75	P100N145 24.11
2	N75 108	P100 169	N145 262		N75P100 17.03	P100N145 24.27
3	N75 121	P100 182	N145 268		N75P100 18.38	P100N145 20.16

Fig 2. Pattern-reversal visual evoked potentials (VEPs). Binocular full-field pattern-reversal VEPs revealed prolonged P100 latencies and increased N75/P100 amplitudes. The active electrodes were placed on the left (1), the median (2), and the right (3) occipital scalp.

When she was admitted to Kyoto University Hospital in April 2002, she complained only of visual disturbance. Her medical history included operations for appendicitis and uterocervical cancer. There was no family history of dementia or psychiatric disease. She was not taking any regular medications. A neurological examination disclosed memory impairment, disorientation, anomia, alexia, agraphia, acalculia, dressing apraxia, color agnosia, and visual metamorphosis. A cranial nerve examination was normal. There were no pyramidal, extrapyramidal, or cerebellar signs or involuntary movements, including myoclonus. Her score on the Mini-Mental State Examination was 12 of 30, and she obtained a total IQ score of 48 on the Wechsler Adult Intelligence Scale–Revised.

The results of a blood test and a CSF examination were unremarkable except for positive 14-3-3 protein in the CSF. Lactic or pyruvic acid was not elevated in the CSF, and paraneoplastic markers, including anti-Hu and anti-Yo, were not detectable either in serum or in the CSF. Notably, an MRI examination revealed symmetric, bilateral, cortical hyperintensity in the parieto-occipital regions (Figs 1C, 1D). DWI most strikingly showed abnormalities in these areas (Fig 1D). There was no mass effect, atrophy, or signs of cerebrovascular disease (Figs 1A–1D). Moreover, positron emission tomography (PET) demonstrated metabolic disturbance in the parietal, occipital, and posterior cingulate cortices (Fig 1E). EEG showed diffuse slowing without typical PSDs. Pattern-reversal visual evoked potentials (VEPs) showed prolonged P100 latencies and increased N75/P100 amplitudes (normal P100 latency < 132 milliseconds,

normal N75/P100 amplitude < 10 μV[13]) (Fig 2). Genetic studies on the prion protein gene (PRNP) demonstrated no known mutations but disclosed homozygosity for methionine at the polymorphic codon 129. A brain biopsy could not be performed because we could not obtain permission from the patient's family.

At 16 months after the initial symptoms, limb and neck rigidity became apparent. At 20 months, she cannot recognize even her family members and has difficulty in oral communication because of the progression of agnosia and aphasia.

Discussion

Visual disturbance as a leading symptom, rapidly progressive dementia, and the detection of 14-3-3 protein in the CSF suggested a diagnosis of the Heidenhain variant of CJD.[14] Methionine homozygosity at codon 129 of the PRNP gene was consistent with this subtype.[15] However, this case did not fulfill the criteria for even possible CJD until the patient exhibited pronounced rigidity at 16 months after the initial symptoms.[1] This was due to the lack of some common clinical manifestations of CJD in this patient, including myoclonus, ataxia, and PSDs on EEG. This case not only suggests a heterogeneity of clinical presentation among patients with CJD but indicates difficulty in the early diagnosis of CJD without typical presentation. Currently used diagnostic criteria based on clinical symptoms and EEG findings may miss some CJD cases without typical sets of clinical manifestations, as in this case. Therefore, it is important to use neuroimaging and laboratory examinations for the early diagnosis of the disease.

Increased T2-weighted MRI signal has been described in the basal ganglia,[5,8] and recently, cortical hyperintensity was shown on diffusion-weighted MRI in some CJD cases with typical clinical courses.[6,7,10,11,16] Moreover, areas of signal abnormalities on diffusion-weighted MRI were well correlated with the neuropathologic findings of spongiform encephalopathy.[10] In the present case, hyperintensity in the parieto-occipital lobes was clearly shown on diffusion-weighted MRI early in the clinical course, indicating that diffusion-weighted MRI is useful for the early diagnosis of CJD.

There has been a relatively limited number of reports describing PET studies of CJD.[6,17,18] Henkel et al[18] analyzed PET studies of 8 patients with CJD and found decreased glucose metabolism in the occipital lobe, cerebellum, or basal ganglia in addition to temporal or parietal cortical region. In the preset case, metabolic disturbance was observed in the parietal, occipital, and posterior cingulate cortices. Although metabolic reductions in the parietal and posterior cingulate cortices are seen in other dementing diseases,[19,20] the clear involvement of the occipital lobes differed from the typical pattern of disturbance detected in Alzheimer's disease,[20] which is the most frequent misdiagnosis of CJD.[21] It may be more difficult to distinguish dementia with Lewy bodies (DLB) from CJD on PET, because significant metabolic reductions in the occipital cortex can be also seen in DLB.[20,22] Diffusion-weighted MRI and 14-3-3 protein detection may be useful in the differential diagnosis of the 2 diseases.[23]

VEPs may also provide a diagnostic aid for the early detection of CJD. According to previous reports, P100 latencies were increased or normal, but increased P100 amplitudes were the most frequent finding in CJD patients, particularly during the early stages of the disease.[13,24,25] Our case also showed increased P100 amplitudes at the early phase of the disease, thus indicating that VEP recording may be helpful, particularly in the early diagnosis of CJD without typical clinical presentation.

14-3-3 protein is expressed in all eukaryotic cells and participates in the regulation of diverse biological processes, including neuronal development, cell growth control, and cell cycling. There are 7 isoforms, 5 of which are present in neuronal cells and constitute nearly 1% of all soluble brain proteins.[26] The detection of 14-3-3 protein in the CSF probably reflects severe neuronal destruction.[23] 14-3-3 protein in the CSF has been shown to be a useful biochemical marker for CJD,[2,4] and Zerr et al[4] demonstrated that the specificity was even higher than that of PSDs on EEG. In the recent revised version of the French and European study criteria, positive 14-3-3 protein detection is considered as a criterion equivalent to a typical EEG.[1] According to the revised version, our patient was classified as probable CJD.

CJD may be a more heterogeneous group of disorders than has been recognized, and neuroimaging techniques, including diffusion-weighted MRI and PET, in combination with VEPs and 14-3-3 protein detection may be useful for the early diagnosis of CJD.

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＜シンポジウム 8—4＞神経感染症の克服をめざして

プリオン病：遺伝子異常と臨床像・病理像および治療薬開発の展望

堂浦　克美

（臨床神経，44：855—856, 2004）
Key words：変異型クロイツフェルト・ヤコブ病，鑑別診断，治療，抗マラリア薬，ペントサンポリサルフェート

はじめに

ウシ海綿状脳症の発生が欧州・アジア・北米に拡大し，変異型 CJD がわが国で発生しても不思議ではない状況にある．一方，わが国では多数の硬膜移植後の CJD が発生しており，これらの後天性プリオン病は，他の神経精神疾患だけでなく遺伝性プリオン病や孤発性プリオン病との鑑別も必要である．今回，とくに若年者での発生が危惧されている変異型 CJD の診断について，鑑別を要する非定型的プリオン病ついて概説する．また，これらの後天性プリオン病の発生を背景として，最近活発となっているプリオン病治療開発について臨床研究の成果を紹介する．

変異型 CJD と非定型的プリオン病

変異型 CJD は他のプリオン病とはことなる特異な臨床・病理像を呈することが知られているものの，発症早期では他の神経精神疾患との鑑別が問題となるばかりでなく，他のプリオン病との鑑別も必要である．WHO（2001 年）の変異型 CJD 診断基準によれば，〔進行性の神経精神症状〕＋〔初期の精神症状，疼痛性感覚症状，失調，ミオクローヌスなどの不随意運動，痴呆のうちの 4 症状〕＋〔PSD がみとめられない〕であれば，変異型 CJD がうたがわれることになる．このことは，精神症状，感覚症状，あるいは失調などを初期症状とする非典型的なプリオン病はすべて変異型 CJD の可能性があることになる．

遺伝性プリオン病では，挿入変異型プリオン病（オクタリピート配列の挿入変異），失調型（古典型）GSS（P102L），致死性家族性不眠症（D178N＋129M）などが鑑別にあがるが，他の変異タイプのプリオン病でも 129V や 219K の正常多型を併せ持つ際には，変異型 CJD との鑑別が必要となる可能性がある．遺伝性プリオン病は同一のプリオン蛋白遺伝子型であっても表現型は症例によってばらつきがあり，遺伝的浸透率が低いものが多いことから，診断困難な神経精神症状を呈する例や孤発性プリオン病がうたがわれる例でも，積極的にプリオン蛋白遺伝子解析をおこなう必要がある．

次に，孤発性プリオン病では，従来から古典型あるいは Heidenhain 型と呼ばれてきた MM1・MV1 型（Parchi 分類[1]）の典型的 CJD 例や MM2 大脳皮質型 CJD 例を除く他のタイプ，すなわち VV1 型，MV2 型（従来の呼称は Kuru 斑型），VV2 型（従来の呼称は失調型），および MM2 視床型は変異型 CJD との鑑別が必要である．また，医原性 CJD の中では非典型的な硬膜移植後 CJD には変異型 CJD と鑑別を要する症例がある．これらの非典型的なプリオン病では，生前に変異型 CJD と鑑別ができず，死後に脳組織から異常型プリオン蛋白を検索し MM2B 型でないことを確認してはじめて診断が確定するものもある．

治療に関する臨床研究

変異型 CJD や医原性 CJD が多発している背景のもと，プリオン持続感染細胞やプリオン病モデルマウスをもちいたプリオン病治療薬開発が活発におこなわれている．これまでに抗プリオン作用が証明されている化合物や薬剤の中で，抗マラリア薬であるキナクリン[2,3]やキニーネ[4]，および抗凝血薬であるペントサンポリサルフェート[5]は患者への応用が実現している．

キナクリン治療，キニーネ治療は，それぞれ本邦のプリオン病患者 31 例，6 例で実施された．キナクリン治療では 39% の症例に，キニーネ治療では 33% の症例に，投与開始後 1～2 週で認知機能などに部分的改善が短期間（1～4 週間）観察され，早い病期の患者で効果発現率が高かった．明らかな生命予後改善効果は観察されなかった．肝機能障害などの副作用による投薬中止はキナクリン治療では 68% の症例に，キニーネ治療では 50% の症例にみられた．血中濃度解析がおこなわれたキナクリンでは，肝機能障害発生と血中キナクリン濃度に関連がみとめられた．いずれの副作用も可逆的な障害であったが，全身状態が不安定な進行例でキナクリン投与中に死亡した 2 例がみとめられた．注意深い経過観察と血中濃度モニターにより重篤な副作用は十分に防げると考えられるが，今後のキナクリン・キニーネ治療では，適応を早い病期の症例に絞込む必要がある．また，今回みとめられた効果は，キナクリンの血中濃度が比較的低い治療早期であったことから，低用量投与と肝臓への取り込みを下げるような薬剤との併用を検討する必要がある．

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（受付日：2004 年 5 月 13 日）

一方，脳室内ペントサンポリサルフェート持続投与療法は，動物実験での成果を踏まえ，英国の変異型CJD患者1例で臨床試験がおこなわれた．進行期での治療開始であったが，ある程度の効果が観察された．これまでにペントサンポリサルフェートによる副作用はまったく出現していないが，患者で最大効果が期待できる安全投与量を如何に見つけるかが課題である．英国では，この症例の成功を踏まえ，存命中の変異型CJDや遺伝性プリオン病患者（とくに発症早期例）の6症例にも同治療法が実施された．また，ドイツと米国でも各1症例に実施され，フランスでも1症例に実施予定である．複数の患者で同治療法の効果と安全性が確認されることになる．英国と同様に“man-made disease”と呼ばれる後天性プリオン病が多発しているわが国でも，この日本発の治療法を早急に臨床で検討する必要がある．

まとめ

変異型CJDとの鑑別が必要となる非定型的なプリオン病について概説した．また，最近活発になっている治療開発について臨床研究の最新成果を紹介した．

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Abstract

Prion diseases : disease diversity and therapeutics

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More than one hundred victims of iatrogenic CJD with cadaveric dura mater grafting have been recognized in Japan, and the people have been also exposed to a risk of outbreaks of variant CJD. These diseases are distinct from other forms of prion diseases as well as other neuropsychiatric disorders, but on an early clinical stage, their differential diagnoses from other atypical forms of prion diseases are not necessarily easy. Thus, atypical forms of prion diseases were overviewed and discussed here. In addition, data on recent clinical trials of enteral antimalarial drug (quinacrine or quinine) treatment or intracerebroventricular pentosan polysulfate treatment were presented and discussed, because research progress in the therapeutics for prion diseases has been remarkably made on the basis of the prevalence of those acquired forms of prion diseases.

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Key words : variant CJD, differential diagnosis, therapeutics, antimalarial, pentosan polysulfate

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Results of Quinacrine Administration to Patients with Creutzfeldt-Jakob Disease

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Key Words
Creutzfeldt-Jakob disease · Prion · Quinacrine

Abstract
Several chemicals inhibit the accumulation of abnormal prion proteins in vitro. We administered one, the antimalarial agent quinacrine, to three patients with sporadic Creutzfeldt-Jakob disease (CJD) and to one with iatrogenic CJD. Quinacrine at 300 mg/day was given enterally for 3 months. Within 2 weeks of administration, the arousal level of the patient with akinetic mutism improved. The other 3 patients, insensible before treatment, had integrative responses such as eye contact or voluntary movement in response to verbal and/or visual stimuli restored. Clinical improvement was transient, lasting 1–2 months during treatment. Quinacrine was well tolerated, except for liver dysfunction and yellowish pigmentation. Although its antiprion activity in the human brain has yet to be proved, these modest effects of quinacrine suggest the possibility of using chemical intervention against prion diseases.

Introduction

Creutzfeldt-Jakob disease (CJD), a prion-mediated disease in humans, is invariably fatal. Accumulation of the abnormal protease-resistant prion protein (PrP^{Sc}), formed posttranslationally from the normal endogenous protease-sensitive isoform (PrP^{c}), is a central event in CJD pathogenesis [1]. Recent outbreaks of a new variant of CJD in young people [2], and of iatrogenic CJD after cadaveric dura grafting [3], require that treatment be immediately available for dying humans. The antimalarial agent quinacrine has long been used to treat patients with malaria and giardiasis. Two recent reports found that quinacrine inhibits and eradicates PrP^{Sc} in scrapie-infected neuroblastoma cells [4, 5]. Korth et al. [5] found that of the acridine and phenothiazine derivatives they tested, quinacrine and chlorpromazine inhibited PrP^{Sc} accumulation, and they noted the importance of the aliphatic side chain on the middle ring moiety of tricyclic compounds. Quinacrine was 10 times more potent than chlorpromazine, its effective concentration for half-maximal inhibition (EC_{50}) of PrP^{Sc} formation being 300 n*M* [5] (400 n*M* in the report of Doh-Ura et al. [4]). After chronic oral administration of quinacrine to humans, its serum concentration exceeded 450 n*M* for a total dose of 4.5 g given over 6 days [6]. Quinacrine is also deposited in the brain [7], and the tissue to plasma concentration ratio

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Table 1. Clinical findings before and after quinacrine treatment

Patient No./ age, years/ gender/Dx	Duration of illness	Before quinacrine administration		Feeding	After quinacrine administration		Duration of changes
		cognitive state	motility		cognitive state	motility	
1/46/M/ sCJD	11 months	akinetic mutism; roving eye movement; cortical blindness	eyes open to noxious stimuli; reflex myoclonus	NG tube	fixation of eyes	gaze oriented to the direction of a voice; decreased reflex myoclonus	from the 2nd to 5th week
2/58/M/ sCJD	2 months	alert; eye tracking for the object; startle response to visual, auditory and tactile stimuli; ignorance of object presented in the right visual field	withdrawal and purposeless movement; action myoclonus; paraplegia in flexion	NG tube	smiles at family members; eye tracking and startle response to an object presented in the right visual field	decreased action myoclonus	from the 6th day to the 6th week
3/61/F/ sCID	2 months	alert; fearful, startle response; response to visual and auditory stimuli; right hemi-anopsia	withdrawal movement;	fed orally, or NG tube	increased eye contact with the examiner; laughter at visual and auditory stimuli	voluntary left arm movement and side-to-side head movement	from the 8th day to the 3rd week
4/58/F/ possibly iatrogenic CJD	6 years	alert; grimacing and moaning to noxious stimuli; listless to visual and auditory stimuli	palilalia; stereo-typed limbs and orolingual movement; impossible to sit or stand up even with assistance	fed orally	apparent eye contact with people; laughter at visual and auditory stimuli; appropriate 'yes or no' to questions	able to sit up on a reclining chair	from the 2nd to 8th week

Dx = Diagnosis; NG = nasogastric.

is very high [8]. Its pharmacokinetics suggests that a concentration of quinacrine can be obtained in the human brain sufficient to inhibit abnormal prion accumulation, as shown in an in vitro experiment [4, 5].

Patients and Methods

Patients

Three patients with clinically probable sporadic CJD (sCJD; patients 1–3) and one with possible iatrogenic CJD which may have been transmitted by dura mater grafts (patient 4) were studied. Their ages, sex, duration of illness and status at the start of the study are given in table 1. These patients were admitted to Fukuoka University Hospital between October 2001 and February 2002. The three sCJD patients fulfilled the Masters', French and European criteria for probable CJD [9] and showed progressive dementia, myoclonus, visual or cerebellar signs, extrapyramidal signs, typical periodic sharp and slow wave complexes (PSWCs) on EEGs, and positive detection of CSF 14-3-3 proteins.

Patient 4 had undergone removal of a right cerebellopontine angle tumor and had had dura mater grafts in July 1991. She received a single brand of dura mater graft, LYODURA®, processed by B. Braun Melsungen AG before 1987, which brand was found to be responsible for a Japanese outbreak of iatrogenic CJD [3]. She developed progressive dementia in January 1996, became listless within 2 years, and was bedridden within 4 years of onset. Stereotyped repetitive limb movement (palikinesia) and a few patterns of simple sound repetition (palilalia) characterized her status. She moaned emotionally on manipulation of her limbs and had dysphasia, but swallowing was possible when fed. She had extrapyramidal rigidity and exaggerated tendon reflexes, but no ataxia, myoclonus, PSWCs or CSF 14-3-3 proteins. MRI showed diffuse cerebral atrophy. Nondegenerative dementias caused by anoxic brain damage or normal pressure hydrocephalus, and dementias of infectious, neoplastic, metabolic, nutritional or endocrine origin were excluded.

Methods

The four patients were administered 300 mg/day quinacrine enterally for 3 months. The study had been approved by our institution's ethics committee, and the patients' relatives had consented to the procedure. Quinacrine was given as 100 mg of powder in capsule form. It was administered orally 3 times a day after each meal, or through a nasogastric tube after being dissolved in water at 37°C. The patients' behavior and neurological examinations were videotaped every 2 weeks. Routine hematological and blood chemistry studies were done weekly, and EEGs were obtained every 2 weeks. Brain MRI that included diffusion-weighted (DW) images was done in the 4th and 12th weeks after treatment began. Quinacrine was withdrawn if major side effects such as convulsion, bone marrow suppression (white blood cell count <2,000/μl) or significant liver dysfunction (>5 times the normal upper limits for aspartate aminotransferase or alanine aminotransferase) occurred. In addition, if the patient's condition was complicated by infection, metabolic irregu-

larities or gastrointestinal problems, quinacrine was withdrawn and readministered only when the condition had returned to normal. No other medicines were given during the period of quinacrine administration. The plasma concentration of quinacrine in the patients' blood samples was measured by high-performance liquid chromatography.

Results

Quinacrine was well tolerated by all the patients. Yellowish skin pigmentation invariably appeared 10–14 days after treatment began. Transaminase values were elevated in 3 of the 4 patients, but never reached 5 times the normal upper limits. Patients 1 and 2 had quinacrine withdrawn temporarily because of aspiration pneumonia, urinary tract infection or diarrhea, but both finally completed the 3-month treatment course.

Clinical Course

A change in cognitive state appeared during the first 2 weeks of treatment (table 1). Patient 1's unfocused, occasionally roving eyes (fig. 1a) became fixed (fig. 1b) and sometimes were oriented to the side of auditory stimuli (fig. 1c). When stimulated, patients 2 and 3 showed mitigation of irritable mood and the return of smiles or laughter. Patient 3, who had been apathetic (fig. 1d), made apparent eye contact with people (fig. 1e), turned her head from side to side in response to the examiner's position, and had purposeful, voluntary movement of the left arm (fig. 1f). Patient 4 also made eye contact with people and nonpathological laughter in response to stimuli was restored. She also nodded or shook her head, apparently indicating 'yes' or 'no', in response to simple verbal questions such as those about pain or thirst.

These changes in mood or cognitive function were invariably transient, lasting 2–8 weeks during the period of quinacrine administration, after which cognitive function gradually decreased to baseline levels. Due to the associated conditions described previously, quinacrine was temporarily withdrawn from patient 1 in the 5th and patient 2 in the 6th week. Both patients' conditions deteriorated to akinetic mutism that remained even after quinacrine was restarted. After 3–8 weeks of treatment, cognitive function in patients 3 and 4 had regressed with no predisposing factors.

EEG and MRI Findings

Patient 2 showed changes in both EEG and MRI findings, which may be associated with the clinical changes that occurred. Typical PSWCs in the severely suppressed background before treatment (fig. 2a) had become irregular slow activities with less periodicity and no suppression by day 16 of treatment (fig. 2b). Before treatment, DW-MRI detected high signals bilaterally in the corpus striatum and insular and cingulate gyri, as well as in the temporal and parietal lobes. The signal intensities in the temporal and parietal lobe lesions were higher on the left than the right (fig. 3a), at which time the patient did not respond to visual stimuli presented in the right visual field, but did respond to stimuli in the left visual field. Startle responses to threatening stimuli in the right visual field, which appeared on day 19 of treatment, lasted 2 weeks. DW-MRI on day 23 showed decreased high signal intensities in the left temporal and parietal lobes, whereas intensity remained high in the other regions (fig. 3b).

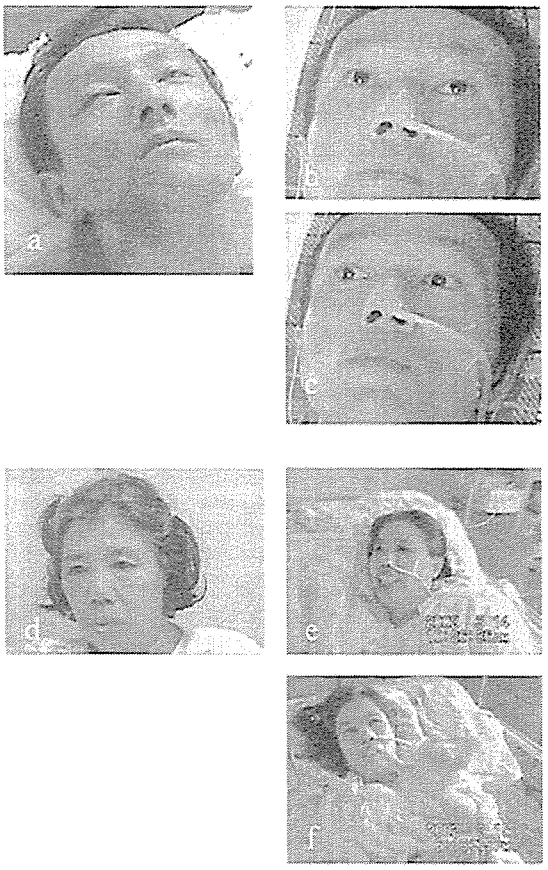

Fig. 1. Patients' appearances before (left) and after (right) quinacrine treatment. **a–c** Patient 1 had an apathetic appearance and unfocused, roving eye movements before treatment (**a**). After treatment, there was eye fixation (**b**) and gaze oriented to auditory stimulus (**c**). **d–f** Patient 3 was listless and apathetic before treatment (**d**). After treatment, she had a well-oriented facial expression (**e**) and purposeful limb movement (**f**).

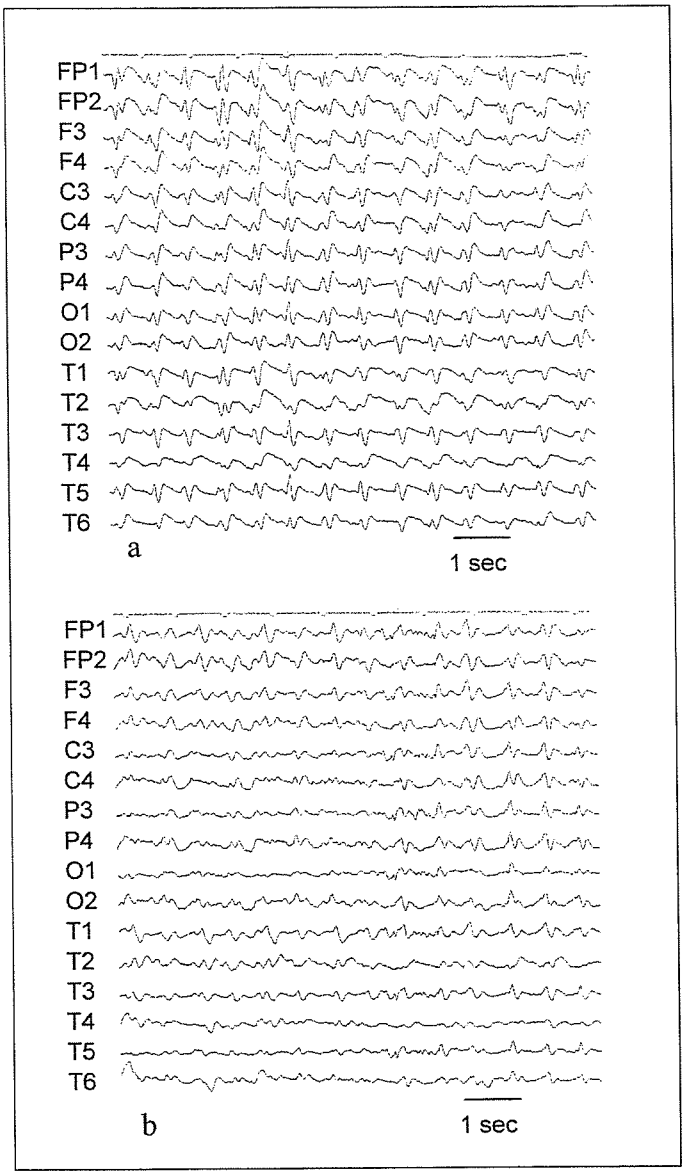

Fig. 2. Electroencephalograms for patient 2 before (**a**) and after (**b**) quinacrine treatment. PSWCs with totally suppressed background activities (**a**) were replaced by fewer periodic patterns and restored background activities after treatment (**b**).

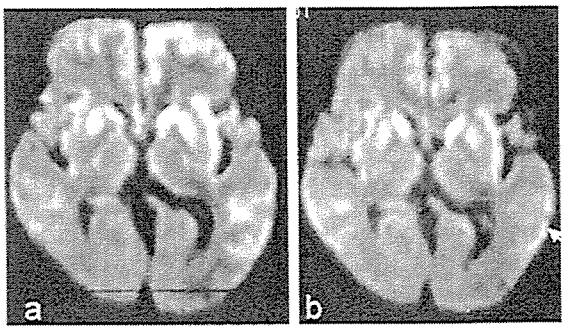

Fig. 3. DW-MRI of patient 2. The right side of each image corresponds to the left side of the brain. High signal intensities were present in the corpus striatum and insular and cingulate gyri on both sides, and in the parietal and temporal lobes before (**a**) and after (**b**) quinacrine treatment. In **b**, the high signal in the left temporoparietal lobes (arrow) is attenuated, whereas signals in the other regions remain unchanged.

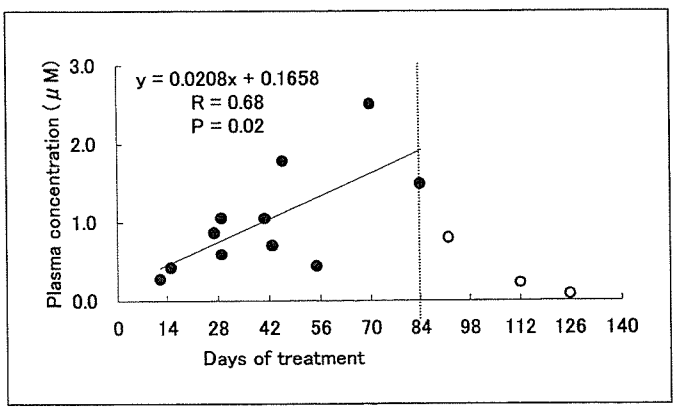

Fig. 4. Plasma concentrations of quinacrine during and after its administration. The concentration increased with the number of days of treatment. Quinacrine was still detectable in the plasma after drug discontinuation on day 84, indicating that it had accumulated in tissues.

Before treatment, patient 3 had high DW-MRI signals in the corpus striatum, cingulate gyrus and left parietal and temporal cortices. These had not changed by the 4th week of treatment, but they disappeared during the 12th week. The other two patients showed diffuse cerebral atrophy without high signals on DW-MRI, which findings did not change after treatment. On the EEGs, patient 1 had PSWCs in the suppressed background before treatment. These disappeared and were replaced by diffuse slow activities of 3–7 Hz during the 2nd week of treatment, but they returned during the 4th week and disappeared thereafter. Patient 3 had PSWCs superimposed on slow background activities, and patient 4 showed diffuse slowing with occasional alpha activities. These features remained unchanged after treatment.

Plasma Concentration of Quinacrine

The plasma concentration of quinacrine increased with the length of administration (fig. 4). It reached 300 n*M* within 14 days and was as high as 2,500 n*M* near the end of the treatment period. Moreover, quinacrine was detectable in the patients' plasma 6 weeks after its discontinuation.

Discussion

In their search for potent agents to treat prion diseases, Doh-ura et al. [4] reported inhibition of PrP^{Sc} accumulation in scrapie-infected neuroblastoma cells by lysosomotropic agents, including quinacrine and cysteine protease inhibitors. These agents may interfere with the conversion of PrP^{c} to PrP^{Sc} at the plasma membrane or along an endocytotic pathway to the lysosomes. Recently, Collins et al. [10] reported that quinacrine did not prolong survival in a murine CJD model. Results of animal experiments, however, depend on the animal species and prion strain. Subcutaneous quinacrine administration prolonged the survival of transgenic mice inoculated with 263K scrapie agents into the brain [Doh-ura, pers. commun.].

We studied four patients, of whom three had clinically probable sCJD and one possibly iatrogenic CJD. All four had improved arousal levels after quinacrine treatment. Other changes in global brain function included decreased frequencies of reflex or action myoclonus (patients 1 and 2) and startle response (patients 2 and 3), and mitigation of the hyperkinetic state (patient 4). Focal brain functions restored were nonpathological laughter (patients 2–4), visual field (patient 2) and voluntary movement (patient 3). These changes might have been due to factors other than quinacrine, e.g. encouragement of contact by family members or caregivers, but this could be excluded for two reasons. Firstly, in Japan, intensive care is customarily given to severely disabled patients who have definitely poor prognoses. As in the case of patient 1, tube feeding is usually initiated and continued for irreversibly disabled patients because family members will not accept the cessation of feeding. The contact provided by family members and caregivers did not change after quinacrine treatment was begun in the present study. Secondly, the changes seen after treatment were transient, lasting 2–8 weeks. Thereafter, the patients' conditions regressed, even during quinacrine administration.

Of the three patients with clinically probable sCJD, transient improvement occurred not only in patients 2 and 3, who were in the early stage, but also in patient 1, who was in the terminal, akinetic mutism stage. This last patient had an improved arousal level associated with directed fixation of the eyes (fig. 1a–c) attributable to the function of the brainstem reticular formation, a structure relatively preserved in the classic, Heidenhain variant of sCJD [11]. The most marked change, seen in patient 2, was the restored response to objects presented in the right visual field. This change was accompanied by decreased DW-MRI signal intensities in the left temporal and parietal lobes. DW-MRI is a new technique that noninvasively images molecular water proton diffusion processes that occur on a micrometer scale [12]. Mittal et al. [13] reported that in CJD, the high-intensity signal areas seen in DW-MRI are correlated with a high degree of spongiform change. They speculated that these changes are the result of the microvacuolation of neuritic processes, heralding spongiform degeneration. Of our four patients, the two who were in the early stage of illness had high signals on DW-MRI that lasted for 2–3 months but which had disappeared 5 months into the illness. Although the exact mechanism is unknown, the immature attenuation of the high signals in the temporal and parietal lobes, which was correlated with clinical changes in patient 2, suggests that the high DW-MRI signals in CJD may represent reversible changes. Decreases in the action myoclonus and startle response in patient 2 were accompanied by decreased PSWCs and increased EEG background activity. These EEG findings suggest that mitigation of the irritable state, which produced a calm appearance, was due to improved cortical function and not to deterioration.

Cognitive function was restored temporarily in patient 4, who had an unusually prolonged course. She may have received contaminated cadaveric dura mater before onset, and her incubation period of 66 months compares with the incubation periods of other Japanese patients (mean incubation period 89 ± 44 months, range 16–193 months) during the CJD outbreak [3]. She had rapidly progressive dementia during the first 2 years of her total, prolonged 6-year course. Some Japanese patients with dura mater-associated CJD may have a clinically variant, longer duration of illness, characterized pathologically by florid-type plaques [14]. The diagnosis in that patient's case has had to be postponed, but quinacrine treatment appears to be beneficial for patients with rapidly progressive dementia and prolonged survival.

Whether the changes found are due to the antiprion effect of quinacrine, as reported in in vitro experiments, is unknown. Therapeutic doses of quinacrine are known to cause psychomotor hyperactivity. The incidence of quinacrine psychosis is reported to be 0.9–4 per 1,000 persons

[15]. Engel et al. [16] administered 2.1–2.8 g of quinacrine to 5 healthy individuals over a 10-day period, doses sufficient to obtain plasma levels exceeding 250 n*M*. Their subjects had various degrees of psychomotor hyperactivity and increases in EEG frequencies. The EEG changes occurred at plasma quinacrine levels of 75–100 n*M* and continued for up to 8 days after discontinuation of the drug. The increased arousal levels in our patients, therefore, may be attributable to the cortical stimulation action of quinacrine, but the mechanism of its direct effect on the central nervous system has yet to be determined.

The plasma concentrations of quinacrine in our patients suggest that a therapeutic dose of 300 mg/day quinacrine may reach the EC_{50} of PrP^{Sc} formation, i.e. 300–400 n*M*, in brain tissues. Quinacrine accumulates progressively in tissues when administered chronically [7]. The lowest concentrations are in the brain, heart and skeletal muscle [7], but tissue to plasma concentration ratios may be very high, as in dog skeletal muscle [8]. The clinical changes in our patients occurred from the 2nd to 8th week of administration, and the plasma concentrations ranged from 300 to 1,000 n*M*. Those concentrations would be sufficient for the drug's accumulation in brain tissues as well as for its action either as a direct cortical stimulant or through its antiprion activity. Cognitive state regression during quinacrine treatment may be due to its toxicity on the brain [6]. We believe that the quinacrine dose should be decreased after the initial loading dose and the plasma concentration of the drug monitored. Although its effectiveness is limited in terms of extent and duration, our findings support undertaking a clinical trial of quinacrine and the search for other chemicals that prevent the accumulation of, or conformational changes in, prion proteins.

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Sporadic Creutzfeldt-Jakob disease with MM1-type prion protein and plaques

To the Editor: Ishida et al.[1] report a case of sporadic Creutzfeldt-Jakob disease (sCJD) characterized by homozygosity for methionine at codon 129 of the prion protein gene (PRNP) and type 1 PrPres associated with focal plaque-like deposits in the brain. They report that these deposits were mainly in the cerebellar cortex, while the cerebral cortex showed mostly diffuse, synaptic type PrP immunoreactivity. The immunoblot analysis was performed only on homogenates from the frontal cortex.

We previously found the co-occurrence of focal and synaptic PrP immunoreactivity in the same subject, both in different brain regions and within the same region.[2] In each, we demonstrated the presence of both type 1 and type 2 PrPres in the brain, with a strict relation between PrPres type and pattern of PrP immunoreactivity. In particular, the synaptic pattern was associated with type 1 PrPres, while the focal (plaque-like perivacuolar, and perineuronal) deposits were linked to type 2 PrPres. Most of these cases were homozygous for methionine at codon 129 of PRNP. Sample selection for immunoblot analysis was based on the microdissection of discreet areas of cerebral cortex, subcortical gray structures, and cerebellum from frozen brain slices adjacent to the paraffin-embedded section. In this section, immunohistochemistry revealed either diffuse or focal PrP deposits or both. Immunoblot analysis should be extended to other brain regions, particularly to the cerebellar cortex, where focal PrP immunoreactivity was prevalent.

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Reply from the Authors: Dr. Puoti et al.[1] raise the possibility of co-occurrence of type 1 and type 2 protease-resistant prion protein (PrP^{Sc}) in the brain of our patient with CJD. However, the report of the distribution of synaptic (granular) and plaque-like prion protein (PrP) deposits in our patient was clear. We found not only the PrP granular deposits of the synaptic type but also plaque-type PrP deposits in the cerebral cortex.[1] In their study, Dr. Puoti et al. concluded that the synaptic PrP deposits were associated with type 1 PrP^{Sc} and that the plaque-like PrP deposits and a perivacuolar PrP immunoreactivity were related to type 2 PrP^{Sc} in the patients with a homozygosity for methionine at codon 129.[2] According to their results, our patient should reveal co-occurrence of type 1 and type 2 PrP^{Sc} in the frontal cortex yet only type 1 PrP^{Sc} was demonstrated. Dr. Puoti et al. reported two patients (Patients 3 and 4) of MM homozygote with focal perivacuolar and plaque-like PrP deposits and found the coexistence of both type 1 and type 2 PrP^{Sc} in those patients. However, the plaque-like deposits detected in our patient were unicentric plaque type as shown in our report[1] and appear morphologically different from those in Patient 4 shown in their study.[2] Our patient had different molecular and neuropathologic features of PrP than those reported by Dr. Puoti et al.

Recently, we reported that a C-terminal PrP fragment of 11–12 kDa (fPrP11–12) is related to subtypes of dural graft-associated CJD (dCJD) and other prion diseases.[6] There are two subtypes of dCJD—dCJD with plaque-type PrP deposits (p-dCJD) and dCJD without PrP plaques (np-dCJD), and both the subtypes have type 1 PrPSc.[6] Interestingly, all of the p-dCJD cases show no fPrP11–12, while the np-dCJD cases reveal fPrP11–12.[6] Our patient showed absence of fPrP11–12 as found in p-dCJD cases (data not shown). In addition, our patient and p-dCJD patients shared common neuropathologic features as described in our report.[1]

Taken together, we consider that a prion strain in our patient may be similar to that in p-dCJD rather than that in the sporadic CJD patients of MM homozygote with co-occurrence of type 1 and type 2 PrP^{Sc}.

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Toxicity of Quinacrine Can Be Reduced By Co-Administration of P-Glycoprotein Inhibitor in Sporadic Creutzfeldt-Jakob Disease

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SUMMARY

1. Recent publication has suggested that quinacrine may be a candidate for treatment of Creutzfeldt-Jakob disease (CJD). But serious toxicity of quinacrine to liver and hematological system has been reported.

2. We disclosed the permeability of quinacrine can be enhanced by presence of p-glycoprotein inhibitor at blood–brain barrier in vitro. Therefore, we tried the protocol of combination of quinacrine and p-glycoprotein inhibitor, verapamil for patients with CJD.

3. When compared clinical effects by quinacrine and the combination therapy, improvement of clinical findings was observed at the same level without any adverse effects. Low-dose quinacrine with verapamil can be used as safe treatment of CJD.

KEY WORDS: quinacrine; sporadic Creutzfeldt-Jakob disease; p-glycoprotein inhibitor.

INTRODUCTION

Although there are number of promising agents to control prion protein in vitro or in vivo, no sufficiently safe agent has yet been discovered for patients with Creutzfeldt-Jakob disease (CJD) (Doh-ura *et al.*, 2000).

Quinacrine, originally used as an anti-malaria agent, was reported as a possible agent useful for treatment of CJD (Korth *et al.*, 2001). Recent report found that quinacine might present serious toxicity to the liver and hematological system (Scoazec *et al.*, 2003).

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Quinacrine inhibited the accumulation of PrP^{Sc} in cultured infected cells, but did not have an apparent effect on PrP^{C} biosynthesis or turnover.

To develop some method of suppression of the adverse effects of quinacrine, we investigated the mechanism of quinacrine transport across the blood–brain barrier (BBB), and found that the permeability of quinacrine could be enhanced at the BBB by the presence of a p-glycoprotein inhibitor such as verapamil or cyclosporine (Dohgu *et al.*, 2003).

Therefore, we administrated a therapy regimen of combination of 200 mg/day of quinacrine and 120 mg/day of oral verapamil and compared it to one of 300–600 mg/day of quinacrine only.

We administrated quinacrine without verapamil for one patient, 64-year-old female who developed dementia and gait disturbance within two months. She was given 300 mg/day for the first two weeks, then the quantity was increased to 600 mg/day without p-glycoprotein inhibitor. Frequency of myoclonus, gaze, and smile were markedly improved. We stopped quinacrine administration due to liver dysfunction after four weeks. Two other sporadic CJD cases were treated by combination of quinacrine (200 mg/day) and verapamil (120 mg/day). The first case treated with combination therapy was a 71-year-old male, who had developed unstable gait, disorientation, and myoclonus. After two weeks administration of quinacrine and verapamil, frequency of myoclonus was dramatically decreased. Before starting medication, his eyes had rolled aimlessly. He began to gaze at his family and his doctor after the combination of quinacrine and verapamil. However, his symptoms returned to the non-medicated state after eight weeks, although he has been receiving medication.

The second case treated with combination therapy was a 65-year-old male. He was bedridden as a result of cerebellar ataxia and progressive dementia. Action myoclonus was observed. We started combination treatment of quinacrine and verapamil on him. After two weeks, his eye movement and myoclonus had improved markedly though the improvement was temporal. These three patients were diagnosed as possible CJD by based on clinical criteria of World Health Organization, diffusion-weighted MRI, and 14-3-3 protein in cerebrospinal fluid (CSF).

To determine whether quinacrine could be sufficiently transported to the brain, we measured the concentration of quinacrine in CSF at 4 weeks after administration of case in 2 and case 3 (Table I). Concentrations of quinacrine in CSF were measured by high-performance liquid chromatography method as described previously (Björkman and Elisson, 1987). The concentration of quinacrine in CSF, supposed to

Table I. Effects and Adverse Effects of Quinacrine in Patients with CJD

						Clinical effects	
Case	Co-administration	Concentration of quinacrine in CSF	AST level[a]	Hematological dysfunction	Skin color change	Frequency of myoclonus	Improvement of gaze and smile
1	None	ND	158	—	+	Decreased	+
2	Verapamil	392 nM	24	—	+	Decreased	+
3	Verapamil	226 nM	53	—	+	Decreased	+

Note. Plus symbol shows that each patient has the indicated findings.
[a]ALT, peak data under quinacrine administration.

be approximately equal to the concentration of quinacrine in experimental treatment in vitro approx. 200–400 nM (Korth *et al.*, 2001).

When the clinical effects on the first patient were compared with the other two patients (combination of 200 mg/day of quinacrine and 120 mg/day of verapamil), improvement of the clinical findings in patients receiving a combination of low dose quinacrine and verapamil was observed to be approximately equal to the level improvement seen in the patient receiving quinacrine only. In two patients treated with the combination of low-dose quinacrine and verapamil, no liver dysfunction and hematological toxicity was observed. Although French National Surveillance Network of Prion Diseases recommended to use quinacrine 1000 mg the first day, then 300 mg each day, we conclude that low-dose quinacrine can be used as a safe and effective treatment of CJD when given in combination with a p-glycoprotein inhibitor such as verapamil.

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Tacrolimus-Induced Neurotoxicity and Nephrotoxicity Is Ameliorated by Administration in the Dark Phase in Rats

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SUMMARY

1. Tacrolimus, a potent immunosuppressant, induces impaired renal function and neurological complications. We investigated the influence of dosing time on the neurotoxicity, nephrotoxicity, and immunosuppressive effect of tacrolimus in rats.

2. The repeated injection of tacrolimus in the light phase (8:00) produced a significantly greater increase than that in the dark phase (20:00) in the duration of harmine-induced tremors and in the blood urea nitrogen (BUN) concentration in rats. An immunosuppressive effect of tacrolimus on the xenotransplantation of mouse-to-rat skin grafts was apparent in the dark phase but not in the light phase.

3. The dosing time-dependent pharmacokinetic results were not observed when tacrolimus concentrations in rat whole blood were measured after a single or repeated injection in the light or dark phase.

4. These findings suggest that treatment in the active phase of the diurnal cycle ameliorates neurotoxicity and nephrotoxicity while maintaining the immunosuppressive effect of tacrolimus. The present findings have important implications for therapeutic approaches to avoid tacrolimus-induced neurotoxicity and nephrotoxicity.

KEY WORDS: tacrolimus; neurotoxicity; nephrotoxicity; circadian rhythm; rats.

INTRODUCTION

Tacrolimus is a potent immunosuppressant that blocks calcineurin-mediated T cell activation by binding to immunophilin (FKBP12). This compound is used to prevent allograft rejection in solid organ transplantation and in fatal graft-versus-host diseses after bone marrow transplantation. Multicenter, randomized trials in the USA and Europe demonstrated that tacrolimus induced impaired renal function and neurological complications with a relatively high frequency (20–40%) (European FK506 Multicentre Liver Study Group, 1994; The U.S. Multicenter FK506 Liver Study

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Group, 1994). Neurotoxicity including tremors, convulsions, and encephalopathy occurred frequently in patients with high blood concentrations of tacrolimus, although these concentrations were within the therapeutic range. The efficacy or toxicity of drugs such as anticancer drugs has been shown to depend on the dosing time (Levi *et al.*, 1997; Ohdo *et al.*, 2001). Various physiological rhythms in living organisms are considered to be responsible for such chronopharmacological reactions to drugs. Chronotherapy including an optimization of the dosing time is capable of producing more efficient and safer prescriptions for patients than conventional therapy.

The mechanisms of neurotoxicity and nephrotoxicity of tacrolimus are not fully understood. On the basis of our findings plus several reports concerning cyclosporine, an immunosuppressive agents, we present the notion that the immunophilin ligands exert neurotoxicity because of an inhibition of γ-aminobutyric acid neurotransmission and an activation of serotonergic neural activity and nitric oxide production (Fujisaki *et al.*, 2002; Ikesue *et al.*, 2000; Shuto *et al.*, 1998, 1999; Snyder *et al.*, 1998; Steiner *et al.*, 1996; Tominaga *et al.*, 2001;). Several bioactive substances including renine, endothelins, transforming growth factor-β, and nitric oxide are involved in the nephrotoxicity induced by cyclosporin and tacrolimus (Bobadilla *et al.*, 1998; Isram *et al.*, 2001; Kupferman *et al.*, 1994; Lanese *et al.*, 1994; Lanese and Conger, 1993). The physiological activities of these various substances in the brain or kidney are known to indicate circadian rhythms (Brandenberger *et al.*, 1994; Cardinali *et al.*, 1998; Hutson *et al.*, 1984; Hwang *et al.*, 1998). It is, therefore, likely that the incidence of adverse reactions to tacrolimus shows dosing time-dependent variations.

In the present study, we investigated the influence of subchronic treatment with tacrolimus in the light or dark phase of a day for 1–2 weeks on the occurrence of harmine-induced tremors and renal dysfunction in rats. The dosing time-dependent immunosuppressive effect of tacrolimus was also examined using a mouse-to-rat xenotransplantation model. To test whether pharmacokinetic factors are involved in the chronopharmacological action of tacrolimus, the amounts of tacrolimus in rat whole blood were assessed at various periods after a single or subchronic injection in the light or dark phase.

MATERIALS AND METHODS

Animals

Male Wistar rats (7 weeks old) were purchased from Kyudo (Saga, Japan). The animals were maintained on a 12 h light/dark schedule (lights on 7:00 A.M.) at a temperature of 23 ± 2°C with free access to food and water. They were adapted to the light/dark cycle for 2 weeks before the experiments. All the procedures involving experimental animals adhered to the law (No. 105) and notification (No. 6) of the Japanese Government, and were approved by the Laboratory Animal Care and Use Committee of Fukuoka University.