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Quinoline Derivatives Are Therapeutic Candidates for Transmissible Spongiform Encephalopathies

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We previously reported that quinacrine inhibited the formation of an abnormal prion protein (PrPres), a key molecule in the pathogenesis of transmissible spongiform encephalopathy, or prion disease, in scrapie-infected neuroblastoma cells. To elucidate the structural aspects of its inhibiting action, various chemicals with a quinoline ring were screened in the present study. Assays of the scrapie-infected neuroblastoma cells revealed that chemicals with a side chain containing a quinuclidine ring at the 4 position of a quinoline ring (represented by quinine) inhibited the PrPres formation at a 50% inhibitory dose ranging from 10^{-1} to 10^{1} μM. On the other hand, chemicals with a side chain at the 2 position of a quinoline ring (represented by 2,2′-biquinoline) more effectively inhibited the PrPres formation at a 50% inhibitory dose ranging from 10^{-3} to 10^{-1} μM. A metabolic labeling study revealed that the action of quinine or biquinoline was not due to any alteration in the biosynthesis or turnover of normal prion protein, whereas surface plasmon resonance analysis showed a strong binding affinity of biquinoline with a recombinant prion protein. In vivo studies revealed that 4-week intraventricular infusion of quinine or biquinoline was effective in prolonging the incubation period in experimental mouse models of intracerebral infection. The findings suggest that quinoline derivatives with a nitrogen-containing side chain have the potential of both inhibiting PrPres formation in vitro and prolonging the incubation period of infected animals. These chemicals are new candidates for therapeutic drugs for use in the treatment of transmissible spongiform encephalopathies.

Transmissible spongiform encephalopathies (TSEs), or prion diseases, are a group of fatal neurodegenerative disorders that include Creutzfeldt-Jakob disease and Gerstmann-Sträussler-Scheinker disease (GSS) in humans and scrapie, bovine spongiform encephalopathy, and chronic wasting disease in animals. These disorders are characterized by the accumulation of an abnormal isoform of prion protein (PrPres), which is high in beta-sheet content and resistant to digestion with proteases (15). Recent outbreaks in younger people of acquired forms of human TSEs, such as variant Creutzfeldt-Jakob disease (19) and iatrogenic Creutzfeldt-Jakob disease with cadaveric growth hormone or dura graft (4), are prompting the development of therapeutic interventions as well as early diagnostics.

One possible therapeutic strategy is to inhibit PrPres formation in the infected host. Doh-ura et al. first reported that cysteine protease inhibitors and lysomotropic agents inhibited PrPres formation in scrapie-infected neuroblastoma (ScNB) cells and that among them, quinacrine was one of the most potent inhibitors (8). Another research group has also reported that quinacrine and its related tricyclic compounds are effective in inhibiting PrPres formation (11). Quinacrine is a synthesized chemical which has a quinoline ring in its structure. It is used as a substitute for quinine in the treatment of malaria. Accordingly, in this study we chose to focus on the quinoline derivatives to examine the structure-activity relationship involved in inhibiting PrPres formation as well as in prolonging the incubation time of infected animals.

MATERIALS AND METHODS

Chemicals and ScNB cells. Chemicals were purchased from Sigma, Maybridge (Cornwall, United Kingdom), Peakdale (Derbyshire, United Kingdom), Specs (Rijswijk, The Netherlands), and Bionet (Cornwall, United Kingdom) and were dissolved in 100% dimethyl sulfoxide (DMSO) or 96% ethanol just before use. ScNB cells (16) were grown in six-well culture plates in Opti-MEM (Invitrogen) supplemented with 10% fetal bovine serum. Chemicals at various concentrations were added to the medium when 1/20 of the confluent cells were passed. The final concentration of either DMSO or ethanol in the medium was less than 0.2%. The cultures were allowed to grow to confluence for 4 days.

Western blot analysis. PrPres was analyzed as described previously (5) with slight modification. Briefly, the cells in confluency were rinsed with phosphate-buffered saline (PBS) and lysed with lysis buffer (0.5% sodium deoxycholate, 0.5% Nonidet P-40, PBS). After low-speed centrifugation, the supernatant was treated with 10 μg of proteinase K/ml for 30 min at 37°C. Digestion was stopped with 0.5 mM phenylmethylsulfonyl fluoride, and the supernatant was centrifuged at 100,000 × g for 30 min at 4°C. Pellets were resuspended in 30 μl of the sample buffer by sonication. After being boiled, the sample was separated by electrophoresis on a Tris-glycine–sodium dodecyl sulfate–15% polyacrylamide gel electrophoresis (SDS-PAGE) and then electroblotted onto a polyvinylidene difluoride membrane (Millipore). The membrane was incubated with PrP-2B, an anti-PrP polyclonal antibody, against a mouse-hamster PrP fragment (amino acids 89 to 103) and then with an alkaline phosphatase-conjugated goat anti-

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rabbit antibody (Promega). Signals were visualized with CDP-Star detection reagent (Amersham) and were densitometrically analyzed. Either the concentration of a chemical giving 50% inhibition of PrPres formation relative to the control 50% inhibitory concentration (IC_{50}) or the maximal concentration of a chemical that does not affect the rate of cell growth to confluence (TC) was estimated from more than three independent experiments.

Metabolic labeling study. Metabolic labeling of prion protein was performed as described previously (5). Briefly, subconfluent ScNB cells in 25-cm^2 flasks were rinsed three times with PBS and preincubated at 37°C in 1.5 ml of methionine-free minimal essential medium with 1% dialyzed fetal bovine serum and 1 μM quinine or 2,2′-biquinoline. After 60 min of preincubation, 125 μCi of ^{35}S-labeled methionine (Amersham) was added to each flask and incubated for 60 min. Then 10 ml of chase medium with 1 μM quinine or biquinoline was added, and the incubation was continued for 18 min, 2 h, or 8 h. Cells were rinsed three times with PBS and lysed with lysis buffer. After low-speed centrifugation, an aliquot of the supernatant was electrophoresed for total protein analysis; the remainder was used for immunoprecipitation of total prion protein. For the detection of cell surface phosphatidylinositol-anchored prion protein, cells were incubated for 30 min in the chase medium with 1 μM quinine or biquinoline after pulse labeling, rinsed three times with PBS, and then incubated with 1.33 U of phosphatidylinositol-specific phospholipase C (PIPLC)/ml in PBS at 37°C for 60 min. The soup was used for immunoprecipitation of cell-surface prion protein. Immunoprecipitation was performed with a PrP-2B antibody after whole proteins in the soup were precipitated with methanol and resuspended in detergent-lipid-protein complex solution.

Surface plasmon resonance sensorgram study. Interaction between prion protein and a chemical was analyzed using a BIAcore X systems. A recombinant murine prion protein fragment (amino acids 121 to 231) (PrP121-231) was immobilized on a sensor chip (CM5) according to the manufacturer's instructions. Each chemical was injected at a 100 μM concentration in running buffer (2.5% DMSO in PBS) for 1 min at a flow rate of 20 μl/min; then running buffer without a chemical was injected for 1 min at the same flow rate. Data were corrected by using a blank sensor chip as a control.

In vivo study. In vivo evaluation of the effectiveness of a chemical at prolonging the incubation times in infected animals was performed by using a mouse model of Tg7 (14, 17) or Tg20 (10), both of which have substantially shorter incubation periods than wild mice. Briefly, a 20-μl aliquot of 1% 263K pathogen homogenate for Tg7 mice, or the same amount of aliquot of 1% Rocky Mountain Laboratory (RML) pathogen homogenate or Fukuoka-1 pathogen homogenate for Tg20 mice, was inoculated into the right parietal portion of the brain. A 4-week continuous intraventricular infusion of vehicle alone (25% DMSO) or of a chemical dissolved in 25% DMSO was initiated at day 10 or 35 in Tg7 mice or at day 14 or 49 in Tg20 mice by using an Alzet osmotic pump equipped with a brain infusion kit (Durect, Cupertino, Calif.). An intraventricular infusion cannula from the brain infusion kit was fitted into the left frontal portion of the brain.

The infusion initiation date was selected at an early stage of the infection (day 10 or 14), or at a late stage (day 35 or 49), when abnormal PrP deposition in the brain definitely appeared in the 263K-infected Tg7 or RML-infected Tg20 mice. However, day 49 postinoculation in the Fukuoka-1-infected Tg20 mice was not exactly at a late stage of the infection, and no information on when abnormal PrP deposition appeared in this model was available.

In some experiments, intraperitoneal administration of a chemical was provided by a single injection once a day for 5 days per week from day 10 or day 35 postintracerebral inoculation until death. The incubation period during which the animals were observed every day lasted from the time of intracerebral infection until the time of death. Five male mice (each weighing about 30 g) per group were used in the experiments. Animal handling and killing were in accordance with national prescribed guidelines, with ethical approval for the study granted by the Animal Experiment Committee of Kyushu University.

Mice which died within a few days due to operational procedures were excepted from the statistical analysis after pathological confirmation. Doses of less than 8 nmol of quinine/day were examined, because toxicity shortened life span at doses beyond 8 nmol/day. Biquinoline was examined at doses of less than 16 nmol/day, which provided no toxicity yet solubility in 25% DMSO.

Immunohistochemistry. An indirect immunoperoxidase method was applied as described previously (9) with slight modification. Briefly, brains were obtained postmortem and fixed in 10% buffered formalin for several weeks. The tissue was immersed in 98% formic acid for 1 h to reduce infectivity and then embedded in paraffin. The samples were cut into 5-μm-thick sections, and then the sections were deparaffinized in xylene and hydrated using an ethanol gradient. The endogenous peroxidase activity was blocked with 0.3% H_2O_2 in absolute methanol for 30 min at room temperature. After being rinsed with tap water, the sections were treated with a hydrolytic autoclave (1 mM or 1.5 mM HCl, 121°C, 10 min) and washed in 50 mM Tris-HCl, pH 7.6, before being incubated with PrP-C polyclonal antibody (Immuno-Biological Laboratories, Gunma, Japan) (1:200) at 4°C overnight. The sections were then incubated with a horseradish peroxidase-conjugated secondary antibody (Vector Laboratories, Burlingame, Calif.) (1:200). The color reaction product was developed with 3,3′-diaminobenzidine tetrahydrochloride solution, and the sections were then counterstained with hematoxylin.

RESULTS

Screening of chemicals in vitro. Clinically available drugs with a quinoline ring and their related chemicals were first screened for the inhibition of PrPres formation in ScNB cells. The antimalarial drug quinine and its related chemicals (such as quinidine, hydroquinine, cinchonine, cinchonidine, and hydroquinidine 4-methyl-2-quinolyl ether) were found to be effective (Table 1, left column). The IC_{50} doses of these chemicals ranged from 3 to 18 μM, and the effective dose range between the IC_{50} and the TC was relatively narrow. Hydroquinidine 4-methyl-2-quinolyl ether, which has two quinoline rings, was slightly more effective than the chemicals with only one quinoline ring. Quinine-related chemicals with a carbonyl base located between a quinoline ring and a quinuclidine ring, such as MQAC (cinchonan-9-ol, 6′-methoxy-ethylcarbonate), MQAA (cinchonan-9-ol, 6′-methoxy-acetate), and MAM [(6-ethynyl-1-azabicyclo[2.2.2.]oct-2yl) (6-methoxy-4-quinolinyl) methanone], were more effective, and their IC_{50} dose ranges were 0.45 to 0.9 μM (Table 1, middle column). Chemicals with either the motif of quinine or that of quinidine on each lateral side of anthraquinone, $(DHQ)_2AQN$ (hydroquinine anthraquinone-1,4-diyl diether) and $(DHQD)_2AQN$ (hydroquinidine anthraquinone-1,4-diyl diether), were much more effective than those with only one motif, and their IC_{50} doses were 0.04 and 0.01 μM, respectively. A chemical with a 4-dimethylaminostyryl moiety was also very effective; and its IC_{50} was 0.012 μM. Except for this chemical, all of the effective chemicals shared a common structure composed of a quinoline ring plus a relative large side chain containing a quinuclidine ring at the 4 position of the quinoline ring. The chemicals listed in the right column of Table 1 also had this common structure, but they showed toxicity at a lower dose and were not effective within a nontoxic dose range.

Other quinoline chemicals unrelated to quinine were also screened. Chemicals with a side chain at the 2 position of a quinoline ring, such as 2,2′-biquinoline, inhibited PrPres formation at 0.003 μM, the minimum IC_{50} dose (Fig. 1A), and this effectiveness was reduced by the replacement of the quinoline ring by a pyridine ring or a naphthyridine ring (Table 2, left column). The addition of a carboxyl moiety to both the 4 position and the 4′ position of the quinoline ring abolished the inhibiting activity of biquinoline (Table 2, right column, top). QCQH (8-hydroxy-8-quinolinylhydrazone-2-quinolinecarboxaldehyde) and PCQH (2-quinolinylhydrazone-2-pyridinecarboxaldehyde) were also very effective in inhibiting PrPres formation at an IC_{50} dose of 0.0075 and 0.004 μM, respectively. They shared a common structure with biquinoline respecting the arrangement of nitrogen atoms. DMEDAPQ (*N,N*-dimethyl-N′-[2-{4-pyridinyl}-4-quinolinyl]-1,2-ethanediamine) a chemical with a nitrogen-containing side chain at both the 2 position and the 4 position of a quinoline ring (thereby resem-

TABLE 1. Structure-activity relationship of quinine analogues on PrPres inhibition

Effective								Ineffective			
Chemical	Structure	IC_{50} (μM)[a]	TC (μM)[b]	Chemical	Structure	IC_{50} (μM)[a]	TC (μM)[b]	Chemical	Structure	IC_{50} (μM)[a]	TC (μM)[b]
Quinine		6	50	MQAC		0.45	25	Hydroquinine 4-chlorobenzoate		-	5
Quinidine		3	>50	MQAA		0.5	>50	Hydroquinidine 4-chlorobenzoate		-	>5
Hydroquinine		12.5	50	MAM		0.9	>50	Hydroquinidine		-	2.5
Cinchonine		6	25	$(DHQ)_2AQN$		0.04	5	Hydrocinchonine		-	25
Cinchonidine		18	50	$(DHQD)_2AQN$		0.01	5				
Hydroquinidine 4-methyl-2-quinolyl ether		3.5	>5	4,(4-Dimethylaminostyryl) quinoline		0.012	10				

[a] IC_{50}, approximate concentration of a chemical giving 50% inhibition of PrPres formation relative to the control.
[b] TC, approximate maximal concentration of a chemical that does not affect the rate of cell growth to confluence.

bling quinine rather than biquinoline in terms of the arrangement of nitrogen atoms), was less effective than biquinoline, and its IC_{50} dose was 0.5 μM.

Chemicals containing a quinoline ring without a large side chain were also examined. They included quinoline hydrochloride, 8-hydroxyquinoline, 2,8-quinolinediol, 8-acetoxyquinoline, and CHIQ (5-chloro-7-iodo-8-quinolinol). All of them, with the exception of 2,8-quinolinediol, were ineffective at inhibiting PrPres within a nontoxic dose range (Table 2, right column). Quinolinediol showed an IC_{50} dose of 8 μM, which was much higher than those of other chemicals with a side chain at the 2 position of a quinoline ring (Table 2, left column, bottom).

Mechanism of inhibition of PrPres formation. Because quinine and biquinoline represented the effective chemicals found here, we focused on these chemicals and studied the mechanism behind their action. After ScNB cells had been treated with different concentrations of quinine or biquinoline for 4 days and then left without treatment for an additional 10 or 17 days, PrPres signals did not reappear even 17 days after discontinuation of the chemical treatment (Fig. 1B [for biquinoline] and data not shown [for quinine]). Thus, treatment with the chemicals permanently cured the cells of the accumulation of PrPres.

Because phospholipase-sensitive cell surface PrP (PrPsen) is

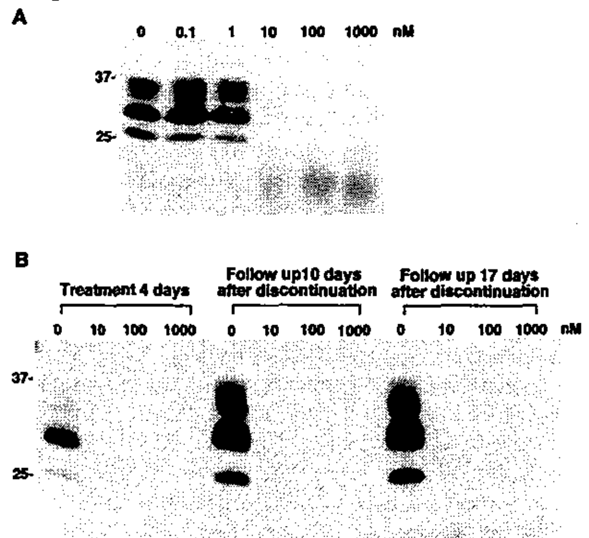

FIG. 1. Inhibition of PrPres accumulation in ScNB cells grown with 2,2′-biquinoline (A) and lack of restoration of PrPres formation in ScNB cells treated once with biquinoline (B). (A) Biquinoline was added at designated concentrations to the medium when the cells were passed, and the culture was allowed to grow to confluence. Then, PrPres in the cells was analyzed by immunoblotting. (B) ScNB cells were treated with 10, 100, or 1,000 nM biquinoline for 4 days. The medium was replaced by fresh medium, and the cells were left without treatment for an additional 10 or 17 days. Then PrPres levels were assayed. Molecular size markers (in kilodaltons) are indicated.

TABLE 2. Structure-activity relationship of biquinoline analogues on PrPres inhibition

Effective				Ineffective			
Chemical	Structure	IC_{50} (μM)[a]	TC (μM)[b]	Chemical	Structure	IC_{50} (μM)[a]	TC (μM)[b]
2,2'-Biquinoline		0.003	>10	BQDA		-	>100
2-(2-Pyridinyl)quinoline		0.11	50	Quinoline hydrochloride		-	>25
2,2'-Bi(1,8-naphthyridine)		38	>100	8-Hydroxyquinoline		-	1
2-(2-Pyridinyl)-1,8-naphthyridine		12	200	8-Acetoxyquinoline		-	2.5
QCQH		0.0075	2.5	CHIQ		-	>5
PCQH		0.004	1				
DMEDAPQ		0.5	50				
2,8-Quinolinediol		8	>50				

[a] IC_{50}, approximate concentration of a chemical giving 50% inhibition of PrPres formation relative to the control.
[b] TC, approximate maximal concentration of a chemical that does not affect the rate of cell growth to confluence.

the precursor of PrPres, it is possible that the inhibition of PrPres accumulation by these chemicals was due to an indirect effect on PrPsen metabolism or turnover. However, biquinoline showed no effects on the metabolic labeling of cellular proteins or on the biosynthesis and turnover of PrPsen (Fig. 2A, B, and C).

Surface plasmon resonance analysis showed that the interaction of biquinoline with recombinant PrP121-231 occurred very slowly and failed to reach saturation even after 1 min. During the dissociation phase, furthermore, complete dissociation did not occur (Fig. 2D). On the other hand, the interaction of quinine or quinacrine occurred very quickly, reaching saturation within several seconds, and dissociation was completely over within seconds.

From observations of the structure of the effective chemicals, it was predicted that they might exert their inhibiting action through some mechanism which involved chelating metals. Thus, quinine and biquinoline were preincubated (before being added to the ScNB culture medium) with an equivalent dose of, a 10-times-higher dose of, or a 100-times-higher dose of various metal ions, including copper, zinc, manganese, iron, cobalt, and aluminum ions. The results showed no change in the inhibiting activities of the chemicals (data not shown).

In vivo study. To examine whether these chemicals could be effective in improving the prognosis in vivo, quinine or biquinoline was continuously administered intraventricularly in animal models which had been intracerebrally infected with three different TSE pathogen strains, comprising 263K scrapie agent, RML scrapie agent, and Fukuoka-1 GSS agent. Quinine administration from an early stage of infection prolonged the incubation period by 13.6% (days 47 to 53.4) at 0.64 nmol/day in 263K-infected mice (Fig. 3A), by 10.8% (days 68.6 to 76) at 1.6 nmol/day in RML-infected mice (Fig. 3B), and by 12.8% (days 104.2 to 117.5) at 0.64 nmol/day in Fukuoka-1-infected mice (Fig. 3C). The effect of quinine administration from a late stage of infection was clearly demonstrated in 263K-infected mice, resulting in 36% (days 47 to 63) prolongation of the incubation period at 1.6 nmol/day (Fig. 3A), with some of the RML-infected mice displaying a tendency to survive much longer than the control at 0.64 nmol/day (Fig. 3B). On the other hand, the effect of biquinoline administration was examined only in 263K-infected mice; it demonstrated 10.8% (days 49 to 54.3) prolongation of the incubation period in the group receiving 1.6 nmol/day at an early stage of infection, but no significant effects were observed in the groups which received it at a late stage (Fig. 3D). Intraperitoneal administration of biquinoline was also performed in 263K-infected mice, and this resulted in 7.7% (days 49 to 52.8) prolongation of the incubation period in the group receiving 0.39 mmol/day from an early stage of infection.

Postmortem histopathological examination of the brains treated with quinine or biquinoline was performed to see

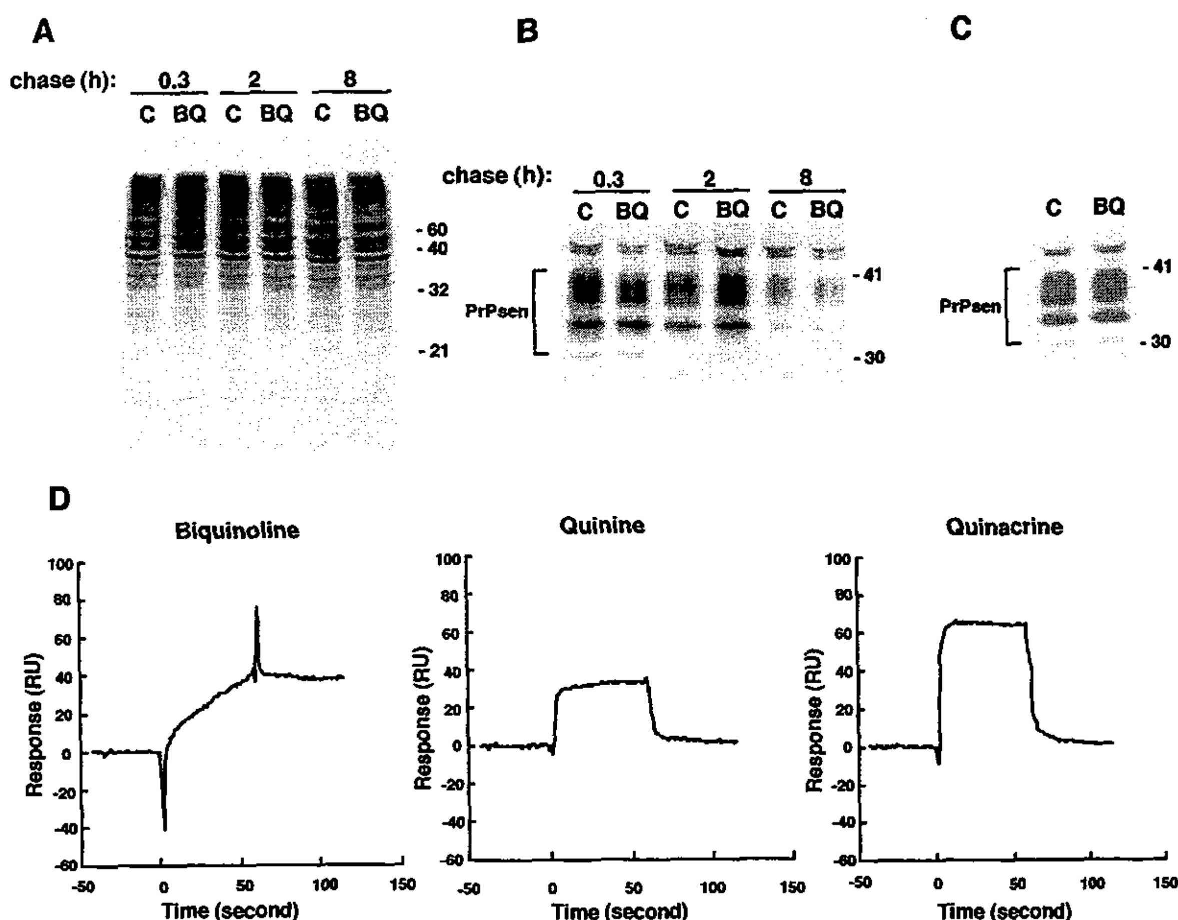

FIG. 2. Lack of effect of the presence of biquinoline on the metabolic labeling of total protein (A), total PrPsen (B), and PIPLC-sensitive, cell surface PrPsen (C). (D) Direct interaction of biquinoline with recombinant PrP121-231 analyzed using a surface plasmon resonance sensorgram. (A) Control ScNB cells (lanes C) and biquinoline-treated cells (lanes BQ) were pulse labeled and then incubated in chase medium for the indicated chase time. The total lysate proteins were methanol precipitated from the detergent lysates of the cells and analyzed by SDS-PAGE. Equal flask equivalents were loaded onto all lanes in each panel. Molecular size markers (in kilodaltons) are indicated. (B) PrPsen was isolated from the total lysate proteins by immunoprecipitation and analyzed by SDS-PAGE. (C) PrPsen was immunoprecipitated from the cell soup treated with PIPLC. Biquinoline at 1 μM was included in all media, starting with the preincubation, except in the case of the control cells. (D) Interaction between a PrP121-231 fragment and a chemical was analyzed using a BIAcore system. A recombinant murine PrP121-231 fragment was immobilized on a CM5 sensor chip; biquinoline, quinine, or quinacrine (at 100 μM in buffer solution) was injected for 1 min at a flow rate of 20 μl/min for the association, and then the buffer solution without a chemical was injected at the same flow rate for the dissociation.

whether there was any modification in abnormal PrP deposition patterns following treatment. Those mice with prolonged incubation periods had a tendency to show less PrP deposition in the white matter between the cerebral cortex and the hippocampus of the brain hemisphere implanted with an intraventricular cannula, although they showed no apparent alteration in PrP deposition patterns in the bilateral thalamus or hypothalamus (Fig. 4).

DISCUSSION

In the studies reported here, we were able to identify quinoline derivatives that inhibited PrPres accumulation in ScNB cells. The commonly shared structure in these chemicals was a quinoline ring bound at its 2 or 4 position with a side chain containing a nitrogen atom, which was located at a particular distance from a nitrogen atom in the ring. Chemicals with a side chain at the 2 position of a quinoline ring were more effective than those with a side chain at the 4 position. Replacement of a quinoline ring with a pyridine ring or a naphthyridine ring resulted in a weaker inhibiting activity, while modification of biquinoline by a moiety that caused less flexibility in the hinge portion between the quinoline rings completely suppressed the inhibiting activity. These findings suggest that a certain proper alignment of two nitrogens, one in a quinoline ring and the other in a side chain, might be important with regard to inhibiting activity.

As for the inhibiting mechanism of these chemicals, the representative chemicals, quinine and biquinoline, demonstrated no alteration either in the protein biosynthesis in general or in the metabolic labeling and turnover of PrPsen in particular. However, biquinoline showed a very strong binding affinity with recombinant PrP121-231 in the Biacore study. Thus, some of the chemicals, including biquinoline, may inhibit

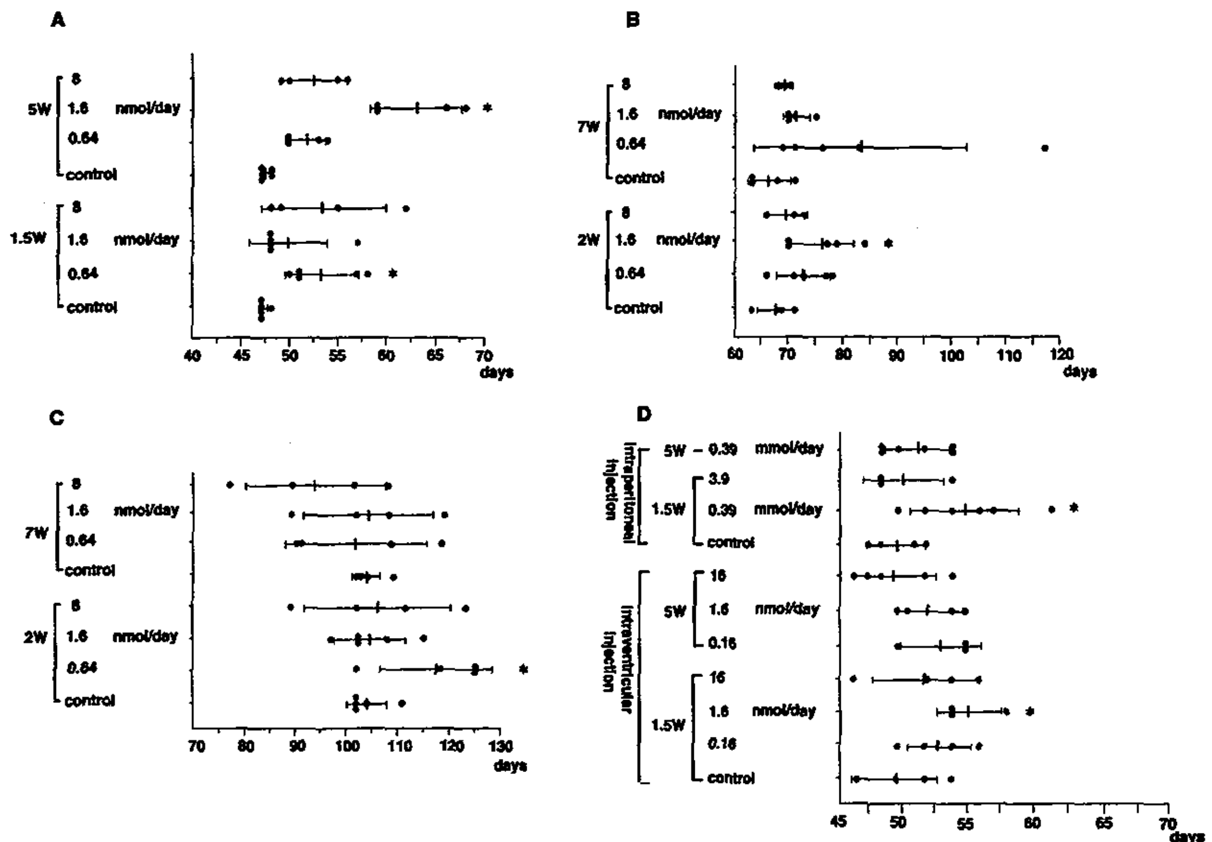

FIG. 3. Prolongation of incubation times in intracerebrally TSE-infected mice treated with quinine or biquinoline. (A) Tg7 mice infected with 263K agent strain and intraventricularly treated with quinine; (B) Tg20 mice infected with RML agent strain and intraventricularly treated with quinine; (C) Tg20 mice infected with Fukuoka-1 agent strain and intraventricularly treated with quinine; (D) Tg7 mice infected with 263K agent strain and intraperitoneally or intraventricularly treated with biquinoline. A 4-week continuous intraventricular infusion of a chemical was initiated by using an osmotic pump at day 10 (1.5W) or day 35 (5W) post-intracerebral inoculation in Tg7 mice or at day 14 (2W) or day 49 (7W) in Tg20 mice. For intraperitoneal treatment, injection of a chemical in Tg7 mice was performed intraperitoneally once a day for 5 days per week from day 10 (1.5W) or day 35 (5W) until the death of the mouse. Each closed circle represents the incubation time of an individual mouse. Each solid line and bar represent the average and standard deviation of the incubation times of each group. The star indicates groups with results with $P < 0.05$ compared to the results seen with the vehicle control. Each of the experiments was performed independently using different lots of the pathogen homogenate; thus, there was some variation in the data shown in panels A and D even for the same vehicle control.

the conversion of PrPsen to PrPres through direct interaction with PrPsen molecules. Since biquinoline (IC_{50} dose, 0.003 μM) was much more effective than quinine (3 μM) or quinacrine (at a concentration of 0.4 μM [8] or 0.3 μM [11]) in ScNB cells, the binding affinity of the PrP fragment (which was much stronger with biquinoline than with quinine or quinacrine) would appear to be clearly correlated with the inhibiting activity of PrPres formation in vitro. The potential binding site(s) of these chemicals in PrPsen molecules remains to be determined.

On the other hand, the involvement of chelating metal(s) in their inhibiting activity (as determined on the basis of the structure of the chemicals which were found to be effective in this study) was predicted. PrPsen is known to bind copper at its N-terminal octameric repeat region (3, 13, 18), and it is suggested that interaction between PrPres and copper stabilizes PrPres conformation (12). Manganese also binds PrP molecules instead of copper and increases proteinase K resistance and beta-sheet content (2). However, our observation suggests that the chelating mechanism seems unlikely to be involved in the inhibiting action of the chemicals found here.

Among the chemicals tested here, CHIQ is an antibiotic (called clioquinol) and a Cu/Zn-selective chelator known to be effective in decreasing beta-amyloid deposits in Alzheimer's disease (6). However, in this study, CHIQ and its related compounds, quinoline hydrochloride, 8-hydroxyquinoline, and 8-acetoxyquinoline, did not inhibit PrPres formation in ScNB cells. These findings also suggest that chelating drugs which are effective in inhibiting beta-amyloid formation are not necessarily effective at inhibiting PrPres formation.

The in vivo study revealed that the chemicals with a quinoline ring were effective not only in inhibiting PrPres formation in vitro but also in prolonging incubation times of intracerebrally infected animals. The greatest effectiveness was obtained by intraventricular administration of quinine at 1.6 nmol/day, which prolonged the incubation time by 36% in 263K-infected mice (compared to the results seen with the control) when initiated at a late stage of infection. Quinine was also effective

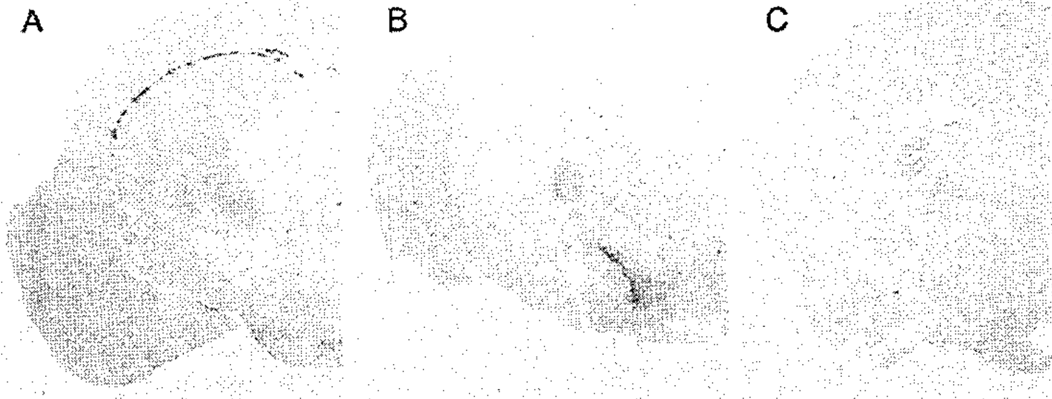

FIG. 4. Effects of intraventricular treatment with quinine or biquinoline on abnormal PrP deposition in the brain of intracerebrally 263K-infected Tg7 mice. The results for brain treated from day 10 postinfection for 4 weeks with vehicle (25% DMSO) alone (A), 0.64 nmol of quinine/day (B), or 1.6 nmol of biquinoline/day (C) are shown. Immunohistochemistry for abnormal PrP deposition was performed in the brains obtained postmortem from the longest-surviving members in each group, and representative examples of the brain hemisphere at the chemical injection side are shown.

in prolonging incubation times of the mice inoculated with different pathogen strains such as RML scrapie agent and Fukuoka-1 GSS agent. These findings indicate that application of quinine, an antimalarial drug, to humans infected with other TSE agents could be judicious.

Recently two research groups have reported that quinacrine is not effective in prolonging incubation times of intracerebrally infected TSE animals (1, 7). Our findings regarding quinine, which is a quinacrine-related chemical, appear to be inconsistent with their findings about quinacrine. However, differences in the structures of the chemicals and in the administration routes, doses, and durations as well as experimental models might have caused this gap but it remains to be elucidated.

Biquinoline was 1,000 times more effective than quinine in inhibiting PrPres formation in vitro with respect to the IC_{50} value, but when initiated from an early stage with intraventricular injections of 1.6 nmol/day or intraperitoneal injections of 0.39 mmol/day, its effectiveness in prolonging incubation times in vivo was clear, albeit marginal. The stability of chemicals and accessibility to targets in vivo might be different between these chemicals, and the reason for the gap between inhibiting activity in vitro and therapeutic activity in vivo remains to be found.

In investigations of the immunohistochemistry of the postmortem materials, abnormal PrP deposition in the white matter adjacent to the ventricle (where a chemical was injected continuously) was less evident in the mice treated with quinine or biquinoline from a early stage than in the control, although abnormal PrP deposition in the thalamus and hypothalamus was demonstrated in a fashion similar to that seen in the control. This would seem to imply that following treatment with a chemical, prolongation of incubation times in mice treated with the chemical might be associated with a reduction in abnormal PrP deposition in the brain.

In conclusion, we have demonstrated that quinoline derivatives with a relatively large side chain with a nitrogen are able to inhibit PrPres accumulation in ScNB cells and can prolong the incubation periods of infected mice. The inhibition was not caused by interference in the biosynthesis or turnover of PrPsen or by the chelation of metals. Some of the chemicals, including quinine, are already in clinical use and are known to pass the blood-brain barrier. Thus, these drugs might be immediately available for clinical trials in investigations of the treatment of human TSEs.

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Clinical features of Creutzfeldt–Jakob disease with V180I mutation

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Abstract—The authors describe the clinical features of Creutzfeldt–Jakob disease (CJD) with the causative point mutation at codon 180. The symptoms never started with visual or cerebellar involvement. The patients showed slower progression of the disease compared with sporadic CJD. They never showed periodic sharp and wave complexes in EEG. MRI demonstrated remarkable high-intensity areas with swelling in the cerebral cortex except for the medial occipital and cerebellar cortices. These characteristic MRI findings are an important clue for an accurate premortem diagnosis.

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Approximately 10 to 15% of all Creutzfeldt–Jakob disease (CJD) cases are estimated to be familial.[1] Some of them are sporadic cases with no relevant family history because of incomplete genetic penetrance and the misdiagnosis of other affected family members. The clinical features depend on the genetic mutations. However, most patients demonstrate periodic sharp and wave complexes (PSWC) in EEG, an accepted diagnostic marker for CJD.

CJD with a causative point mutation of valine to isoleucine at codon 180 (V180I)[2-5] is a type of familial CJD with no relevant family history. In case reports, the clinical features of CJD with V180I (CJD180) were different from those of sporadic CJD (sCJD). Therefore, the premortem clinical diagnosis was difficult, and the cases had been misdiagnosed as neurodegenerative disorders with dementia. We herein report the clinical features and characteristic MRI findings of five original cases of CJD180 together with a review of four reported cases.

Patients and methods. *Patients.* Nine patients including our five original patients and four other previously reported pathologically verified patients[2-6] were studied retrospectively. The clinical features of these patients are shown in table 1. The previously reported case with a double mutation at codon 180 and codon 232 of the *PRNP* was excluded from this study because the codon 232 mutation might influence the clinical course.[6] All nine had neither family history of dementia nor obvious iatrogenic exposure. Their PRNP analyses at codon 129 revealed that four had methionine homozygosity (MM129) and five had methionine/valine heterozygosity (MV129), in which the V180I mutation and valine at codon 129 were on different alleles.

The common histopathologic findings in the five pathologically verified patients were evident spongiform changes in all layers of the cerebral cortex with less prominent neuronal loss and gliosis without Kuru plaque. Immunohistochemical analysis showed weak prion protein staining of the synaptic type in three of three patients examined.[2,4,5]

Methods. We compared the clinical features, laboratory findings, and MRI findings of the 9 patients with those of 123 patients (25 were pathologically verified) with genetically verified sCJD, which were reported to the Japanese CJD Surveillance Committee.[7] The *PRNP* analysis revealed that 116 of the 123 had MM129, 5 had MV129, and 2 had valine homozygosity at codon 129. We then compared the features between CJD180 and sCJD by dividing them into two groups: patients with MM129 and patients with MV129.

CSF was examined within 6 months from the onset for the differential diagnosis. The neuron-specific enolase (NSE) value in CSF was measured commercially using an ELISA method (SRL Laboratory, Tokyo, Japan), and a value of >35 ng/mL was considered positive.[8] The 14-3-3 protein immunoassay was performed by western blot using polyclonal antibody SC-629 (Santa Cruz Biotechnology, Santa Cruz, CA). EEG using the International 10–20 method was examined repeatedly during the disease course.

In the MRI study, T1-weighted (T1I), T2-weighted (T2I), fluid-attenuated inversion recovery (FLAIR), and diffusion-weighted (DWI) imaging was performed for Patients 1, 3, 4, and 5. T1I and T2I were performed for Patients 2, B,[3] and D.[5]

The Mann–Whitney *U* test was used for a comparison of the clinical findings and NSE values between CJD180 and sCJD. The Fisher exact probability test was used for a comparison of the positive rates of clinical symptoms, NSE, 14-3-3 protein, and PSWC.

Results. The results of the comparison between CJD180 and sCJD in each group, the MM129 group and MV129 group, are listed in table 2. The two groups had similar results, even though some were not statistically significant. CJD180 had an older onset age, longer duration from the onset to the appearance of myoclonic jerk that was less prominent compared with that of sCJD, longer duration from the onset to becoming akinetic and mute, lower value of NSE in CSF, and lower positive rate of NSE and 14-3-3 protein in CSF compared with those of sCJD. As cardinal symptoms, higher cortical dysfunctions such as aphasia and apraxia, which were not frequent symptoms in sCJD,

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Table 1 Patients' profiles in this study: five original and an additional four reported patients

Clinical features	Original patients					Previously reported patients			
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient A[2]	Patient B[3]	Patient C[4]	Patient D[5]
Age at onset, y/sex	81/F	74/M	78/M	58/M	72/M	77/F	65/F	70/F	80/M
Duration until appearance of each clinical symptom, mo									
Myoclonic jerk	−*	5	8	10	4	6	14	9	+†
Visual or cerebellar symptom	−*	−‡	−‡	−§	−‡	−‡	−‡	−‡	−‡
Akinetic mutism	−*	12	18	−§	10	18	14	9	>18
Higher cortical dysfunction‖	−	−	+	+	+	−	+	+	+
Parkinsonism‖	−	−	+	−	−	+	−	−	−
NSE value in CSF, ng/mL	32.1	13.0	19.5	22.0	60.4	NE	NE	NE	29.9
14-3-3 protein in CSF	+	−	+	−	NE	NE	NE	NE	NE
Duration from onset to CSF study, mo	3	6	1	4	3	5	Uncertain	2	1
PSWC in EEG	−	−	−	−	−	−	−	−	−
Codon 129 in *PRNP*	M/V	M/M	M/V	M/V	M/M	M/V	M/M	M/M	M/V
PrP staining	NE	NE	NE	NE	NE	+	NE	+	±

Patient A was previously reported by Matsumura et al.[2] Patient B was previously reported by Ishida et al.[3] Patient C was previously reported by Kobayashi et al.[4] Patient D was previously reported by Iwasaki et al.[5]

* We could not detect referring symptoms during our observation period in Patient 1 (until 15 mo after the onset). † The duration until the appearance of myoclonic jerk in Patient D is uncertain. ‡ We or the authors could not detect visual or cerebellar symptoms during the observation period. The patients' severe dementia or consciousness disturbance prevented us from making a close neurologic examination in the advanced stage.

§ We could not detect the referring symptoms during our observation period in Patient 4 (until 16 mo after the onset).

‖ The presence or lack of presence as initial symptoms.

NSE = neuron-specific enolase; NE = not examined; PSWC = periodic sharp and wave complexes; *PRNP* = prion protein gene; M/V = methionine/valine heterozygosity at codon 129 in *PRNP*; M/M = methionine homozygosity at codon 129 in *PRNP*; PrP = prion protein.

Table 2 Comparison of clinical features between CJD180 and sCJD

Features	CJD with MM129			CJD with MV129		
	CJD180	sCJD	*p* value	CJD180	sCJD	*p* value
Age at onset, y	70.3 ± 3.9 (n = 4)	65.3 ± 11.6 (n = 116)	0.32	74.8 ± 9.5 (n = 5)	62.6 ± 10.5 (n = 5)	<0.05
Myoclonic jerk, mo*†	8.0 ± 4.5 (n = 4)	2.7 ± 2.4 (n = 113)	<0.01	9.8 ± 3.9 (n = 4)	8.2 ± 5.1 (n = 5)	0.71
Akinetic mutism, mo*‡	11.3 ± 2.2 (n = 4)	3.5 ± 2.8 (n = 113)	<0.005	17.0 ± 1.4 (n = 5)	10.4 ± 5.4 (n = 5)	<0.05
Visual symptom, %§	0.0 (n = 4)	24.0 (n = 96)	0.26	0.0 (n = 5)	20.0 (n = 5)	0.29
Cerebellar symptom, %§	0.0 (n = 4)	12.5 (n = 96)	0.45	0.0 (n = 5)	40.0 (n = 5)	0.11
Higher cortical dysfunction, %§	75.0 (n = 4)	5.2 (n = 96)	<0.0001	60.0 (n = 5)	0.0 (n = 5)	<0.05
NSE value, ng/mL‖	36.7 ± 33.5 (n = 2)	75.9 ± 65.7 (n = 66)		25.9 ± 6.1 (n = 4)	50.6 ± 7.9 (n = 2)	
Positive rate of NSE, %‖¶	50.0 (n = 2)	72.7 (n = 66)		0.0 (n = 4)	100.0 (n = 2)	
Positive rate of 14-3-3 protein, %‖	0.0 (n = 1)	87.7 (n = 65)		66.7 (n = 3)	100.0 (n = 2)	
PSWC in EEG, %	0.0 (n = 4)	94.0 (n = 116)	<0.0001	0.0 (n = 5)	60.0 (n = 5)	<0.05

Values are means ± SD where applicable.

* The duration until the appearance of referring symptoms.

† Patient 1 had not demonstrated myoclonic jerk 15 mo after the onset, and we accepted 15 mo for the statistical comparison. Patient D[5] demonstrated myoclonic jerk, but we could not identify the duration until the appearance. Therefore, we eliminated Patient D from the statistical comparison.

‡ Patients 1 and 4 had not become akinetic and mute, and we could not identify the date when Patient D[5] become akinetic and mute. Therefore, for the statistical comparison, we accepted the date when they were last confirmed not to be akinetic and mute, i.e., 15 mo for Patient 1, 16 mo for Patient 4, and 18 mo for Patient D.

§ The rate of referring symptoms as initial symptoms.

‖ NSE or 14-3-3 protein in CSF.

¶ The cut-off value was 35.0 ng/mL.

CJD = Creutzfeldt–Jakob disease; sCJD = sporadic CJD; NSE = neuron-specific enolase; PSWC = periodic sharp and wave complex.

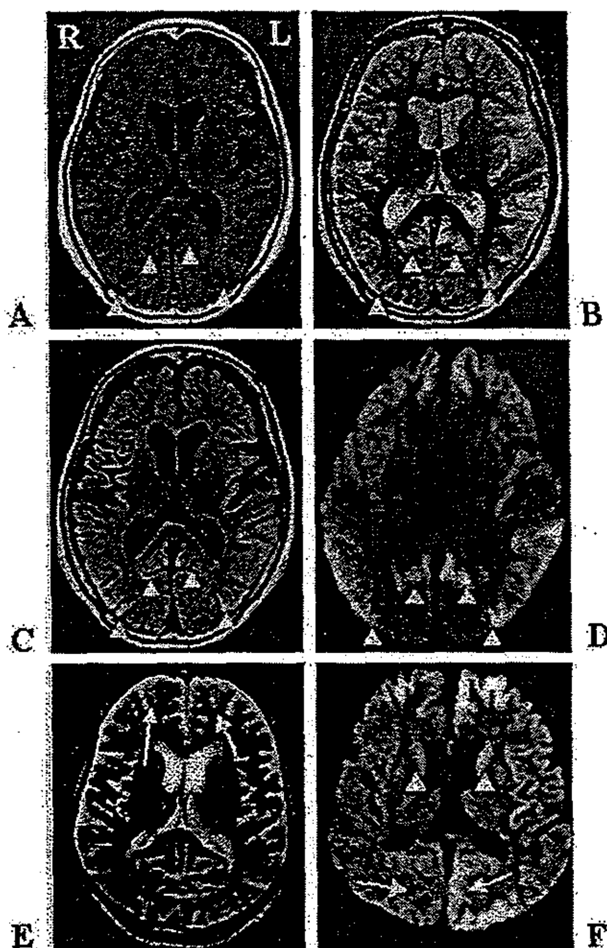

Figure. MRI of Patient 1 (A through D) and a patient with sporadic Creutzfeldt–Jakob disease (CJD) (E and F). For Patient 1 (82-year-old woman), the MRI studies were performed 4 months after the onset using 1.5 T MR unit (Magnetom Vision; Siemens, Erlangen, Germany) equipped with a conventional head 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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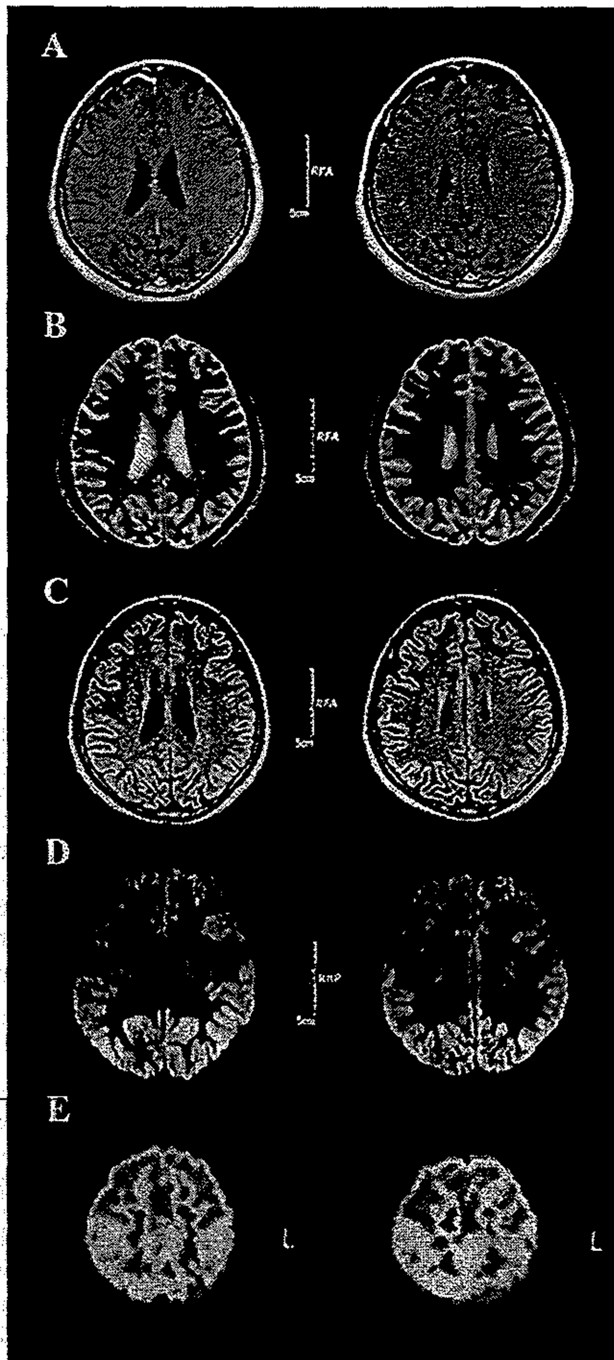
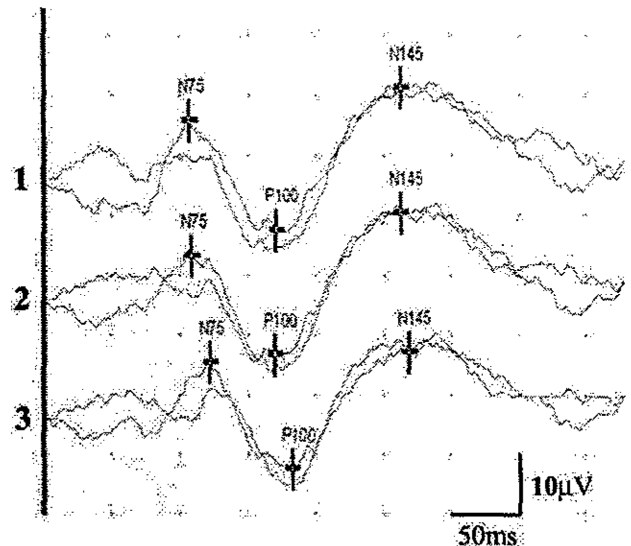


Fig 1. Magnetic resonance imaging (MRI) and positron emission tomography (PET) axial images. There was no atrophy, signs of cerebrovascular disease, or obvious signal abnormality on T1-weighted or T2-weighted MRI (A, B). High signal intensity in the parieto-occipital regions was detected on fluid-attenuated 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 utero-cervical 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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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.

(Clin Neurol, 44 : 855—856, 2004)
Key words : variant CJD, differential diagnosis, therapeutics, antimalarial, pentosan polysulfate

Original Research Article

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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.

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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-