Organ Distribution of Proteinase-resistant Prion Protein in Humans and Mice with Creutzfeldt-Jakob Disease

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SUMMARY

We attempted to clarify the organ distribution of human and murine proteinase-resistant prion protein (PrP^CJD) in Creutzfeldt-Jakob disease (CJD), and to measure the concentration of PrP^CJD, using a semi-quantitative Western blot analysis. Human PrP^CJD was restricted to the central nervous system, whereas murine PrP^CJD was present in the central nervous system and in the lymphoreticular system at the end stage of CJD. PrP^CJD concentration in the central nervous system of mice was almost identical to that of humans. The minimum wet weight of an organ with a positive reaction was 0.3 mg for brain, 1 to 3 mg for spleen, 3 mg for spinal cord, 3 mg for lymph node, 10 mg for thymus and 10 to 30 mg for intestine of the CJD-infected mice. There were no immunoreactions in purified PrP^CJD fractions from 300 mg of spleen, lymph node, liver or peripheral nervous systems of humans, nor in 300 mg of liver, lung or kidney of CJD-infected mice. Within the limits of our method, the distribution of murine PrP^CJD differed from that of human PrP^CJD. Antibodies on the Western blot membrane from murine spleen PrP^CJD fractions stained the kuru plaques in the CJD-infected mouse brain. Therefore, PrP^CJD in the murine spleen probably shares the epitopes of the antigen in the murine kuru plaques. Although the immunological detection of PrP^CJD does have limits of sensitivity, PrP^CJD concentrations did correlate with infectivity titres in scrapie-infected or CJD-infected mice.

INTRODUCTION

Creutzfeldt-Jakob disease (CJD), kuru and Gerstmann-Sträussler syndrome are human neurodegenerative diseases that are transmissible to laboratory animals (Gajdusek et al., 1966; Gibbs et al., 1968; Masters et al., 1981; Tateishi et al., 1979, 1984b). Scrapie is a similar disease that occurs in sheep. The causative agents of these diseases have not been conclusively identified. Attempts to purify the scrapie agent from hamster brain led to the discovery of a particular protein, named prion protein (PrP) (Prusiner, 1982). The scrapie form of PrP (PrP^Sc) is the major protein component of the fraction containing the scrapie agent (Prusiner, 1982); it aggregates to form amyloid (Prusiner et al., 1983), and makes up the kuru (amyloid) plaque seen in the brains of subjects with scrapie, CJD or Gerstmann-Sträussler syndrome (Bendheim et al., 1984; Kitamoto et al., 1986b). PrP was found to be encoded by a cellular gene (Cheesebro et al., 1985; Oesch et al., 1985). Antiserum against PrP detected proteins with an apparent Mr of 33K to 35K, in both normal and scrapie-infected hamster brains (Cho, 1986; Oesch et al., 1985). In normal (scrapie-uninfected) brains, the product of the PrP gene is a protein designated PrP^C (cellular form), which is susceptible to digestion by proteinase K. In contrast, PrP^Sc is resistant to proteinase K digestion (McKinley et al., 1983). At present, proteinase K digestion is the only method available to differentiate PrP^Sc from PrP^C.

It is a matter of controversy whether prions are scrapie agents (Prusiner, 1982) or by-products of scrapie infection that co-purify with scrapie agents (Braig & Diringer, 1985). One of the arguments raised is whether PrP mRNA and PrP^Sc are present in spleen tissue. The infectivity...
title is high in the spleen of mice with scrapie (Fraser & Dickinson, 1970, 1978); thus, it would be necessary to identify PrPSc in the spleen if PrPSc is indeed an essential component of the scrapie agent. Some investigators did not detect either PrP mRNA or PrPSc (Cheesbro et al., 1985; Czub et al., 1986), but others have shown that PrP mRNA or PrPSc is present in spleen tissue from subjects with scrapie (Doi et al., 1988; Oesch et al., 1985; Race et al., 1988; Rubenstein et al., 1986; Shinagawa et al., 1986).

In our initial study with spleens from humans and mice with CJD, we obtained evidence for protease-resistant PrP (PrP(CJD)) in murine, but not in human spleen. Therefore, we examined various organs from these two species with CJD, and noted a different pattern of distribution between human and murine PrP(CJD). We also measured PrP(CJD) concentrations, using a semi-quantitative Western blot analysis.

METHODS

Materials. The cerebral cortex, spinal cord, peripheral nerve, dorsal root ganglion, spleen, liver and lymph node were obtained at autopsy on three Japanese patients with CJD. All tissues were kept at -35 °C. The diagnosis of CJD was made by their clinical course, histopathological examination, and detection of PrP(CJD) in the brain, using a Western blot technique. Transmission of CJD from these three patients to small rodents is now in progress in our laboratory.

New Zealand white (NSW) mice were inoculated intracerebrally (i.c.) with the Fukuoka-1 CJD strain (Tateishi et al., 1979). The breeding and the experiments were all carried out under specific pathogen-free conditions, using a barrier system set up in the Animal Center in Kyushu University. After about 4 months, the mice were sacrificed by cervical dislocation at the end stage of the illness. Brain stem portions of the mice were examined pathologically, and the organs of mice that exhibited spongiform encephalopathy were studied. The brain, spinal cord, spleen, liver, lymph node, kidney, lung and intestine of mice were all preserved at -35 °C and examined as pooled samples in subsequent experiments.

Purification of PrP(CJD). PrP(CJD) was prepared as described (Kitamoto et al., 1986a; Kitamoto & Tateishi, 1988), with modifications. Various organs from each patient with CJD, and from pooled samples of CJD-infected mice were used. The tissue (wet weight 300 mg) was homogenized in 1 ml of 40 mM-Tris-HCl pH 7.9, 10 mM-NaCl and 6 mM-MgCl₂ with a glass homogenizer. DNase I (150 μg; BDH) was added to the homogenate, and the preparation was incubated at 37 °C for 60 min. One ml 10% N-lauroylsarcosine (Sarkosyl) was added to the DNase-treated sample and the preparation was rehomogenized. The homogenate was centrifuged at 22000 g for 30 min. The supernatant was then centrifuged at 41000 g for 60 min (CP100H model, RP100AT4, Hitachi Koki Co.). The pellet was sonicated in 7 ml of 50 mM-Tris-HCl pH 7.6 and 10% NaCl. The sample was centrifuged at 22000 g for 30 min. The pellet was then sonicated in 1 ml of 50 mM-Tris-HCl pH 7.6 and 10% NaCl containing 0.6 μg of proteinase K (E. Merck), incubated at 37 °C for 60 min, and then centrifuged at 15000 g for 30 min. The final pellet was used as the PrP(CJD) fraction. Major modifications are the DNase treatment and the ultracentrifugation step. The DNase treatment was added to avoid DNA and Sarkosyl interaction (Doi et al., 1988). A micro-ultracentrifuge was used for this small-scale purification.

Non-purified tissue preparations. The tissue (wet weight 250 mg) was homogenized in 900 μl of 40 mM-Tris-HCl pH 7.9, 10 mM-NaCl and 6 mM-MgCl₂ with a glass homogenizer. DNase I (125 μg) was added to the homogenate and the preparation was incubated at 37 °C for 60 min. Then 100 μl 10% Sarkosyl was added to the DNase-treated sample, which was then rehomogenized. The homogenate, to which 125 μg of proteinase K had been added, was incubated at 37 °C for 60 min. At the termination of the proteinase digestion, the sample was boiled with 1 volume of Laemmli's sample buffer (62.5 mM-Tris-HCl pH 6.8, 2% SDS, 5% 2-mercaptoethanol and 5% glycerol) for 5 min.

Western blot analysis. Electrophoresis was performed according to the method of Laemmli (1970) on 15% polyacrylamide gels containing 0.1% SDS. According to the method of Towbin et al. (1979), proteins were electrophoretically transferred from the gel to Durapore membrane GVHT (Millipore). The blotted membrane was washed with TTBS (25 mM-Tris-HCl pH 7.6, 0.05% Tween 20 and 0.5 mM-NaCl) for 10 min, and blocked with TTBS containing 5% low fat milk at 37 °C for 30 min. PrP antisemur against kuru plaque core proteins had been raised in a rabbit (Kitamoto & Tateishi, 1988). The membrane was then incubated overnight with diluted PrP antisemur (1:1000) in TTBS containing 5% low fat milk at 37 °C. After washing with TTBS, the membrane was reacted with diluted anti-rabbit IgG–alkaline phosphatase conjugate (1:7500; Promega Biotech) in TTBS containing 5% low fat milk at 37 °C for 30 min. After a rinse with TTBS, the colour was developed with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (Promega Biotech) for 90 s. Molecular standard proteins were purchased from Pharmacia.

At first, Western blot analysis was performed with PrP(CJD) fractions from 300 mg of various human and murine organs in each lane. Next, we measured PrP(CJD) concentrations in organs, using serial dilutions (fractions from 100,
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For non-purified tissue preparations, serially diluted samples (1, 0-3, 0-1, 0-03, 0-01, 0-003 and 0-001 mg wet weight tissue in each lane) were applied. As a measure of the amount of PrPCJD, we determined the minimum amount (wet weight) of tissue necessary for visualization of the immunolabelled bands.

Affinity-purified antibodies and immunostaining. To check the specificity of immunolabelled bands, we obtained antibodies on the Durapore membrane, according to Talian et al. (1983). Portions of the blotted membrane strip corresponding 18K to 32K were cut off and incubated overnight at 37 °C with diluted PrP antiserum (1:1000) in TTBS containing 5% low fat milk. After rinsing with TTBS, the bound antibodies were eluted with 1 ml of 0-2 M-glycine–HCl pH 2-5 and immediately neutralized with 100 p.1 of 1-5 M-Tris base. With formic acid enhancement on tissue sections, as described (Kitamoto et al., 1987), we examined formalin-fixed, paraffin-embedded serial sections of CJD-infected mouse tissues containing numerous kuru plaques (Tateishi et al., 1984a, 1987). The affinity-purified antibodies were used as primary antibodies and the following steps were performed with unlabelled antibodies using the biotin–streptavidin method (Stravigen, BioGenix Laboratories).

RESULTS

PrPCJD recovery

To estimate the PrPCJD recovery, we compared the data on PrPCJD purification with that from the non-purified tissue preparations. In the human CJD brain, the minimum wet weight for a positive immunolabelling of PrPCJD was 0-3 to 1 mg in the purified PrPCJD fractions, and was 0-03 to 0-1 mg in the non-purified preparations (Fig. 1). The bands revealed major proteins of 27K to 31K, 23K to 25K and 19K to 21K, and a minor one of 16K to 18K. In the spleens of humans with CJD, we found no positive immunoreactions, either in the purified PrPCJD fractions or in the non-purified tissue preparations. With murine CJD brains, positive labelling was seen in 0-3 mg of the PrPCJD fractions or in 0-03 mg of the non-purified preparations. In the CJD-infected mice, the spleen had a relatively high PrPCJD concentration (see below). With spleen tissue from the CJD-infected mice, a positive labelling was seen in 1 to 3 mg of the purified PrPCJD fractions and in 0-1 to 0-3 mg of the non-purified preparations. Therefore, we estimated the PrPCJD recovery to be about 10%. The non-purified preparations (1 mg wet weight) from thymus and intestine in the CJD-infected mice showed no or only weakly immunolabelled bands.

With a Western blot analysis, there is the disadvantage of volume limitation for each well in the polyacrylamide gels, a problem especially in the case of non-purified tissue preparations (maximum 1 mg wet weight in a well). In the purified PrPCJD fractions, we applied the final pellet from more than 300 mg tissue to each well. The examination with the purified PrPCJD fractions covers a wider range of 0-3 to 300 mg wet weight than did that with the non-purified preparations (0-03 to 1 mg). Therefore, the organ distribution of PrPCJD was examined using the purified PrPCJD fractions.

To find the sensitivity of our Western blot analysis, we obtained gel-eluted PrPCJD. To purify the 23K to 25K band of human PrPCJD, SDS-PAGE and electroelution were performed according to the methods of Hope et al. (1988). Proteins were eluted for 5 h from a gel slice with an electroelutor (Bio-Rad) and purified by high-performance liquid chromatography (TSK 3000SW, 0-75 × 60 cm). Then, we examined this eluted PrPCJD using Western blot analysis. The minimum PrPCJD content of a positive immunolabelling was 0-3 ng of gel-eluted PrPCJD (Fig. 2).

PrPCJD distribution in humans

The organ distribution of human PrPCJD is summarized in Table 1. Positive reactions were found in PrPCJD fractions of the central nervous system (cerebral cortex and spinal cord). Minimum wet weight of positive labelling was 0-3 mg in the PrPCJD fraction of brain tissues from two of the patients and 1 mg from the other. In spinal cord tissue, a positive reaction was seen in 3 mg of the PrPCJD fraction from two of the patients, and in 10 mg from the other. In each case, the PrPCJD concentration in the spinal cord was 10% of that in the cerebral cortex. There were no immunolabelled bands in purified PrPCJD fractions from 300 mg of non-neuronal organs.
Fig. 1. Semi-quantitative Western blot analysis of the purified PrP<sub>CJD</sub> fraction (a) and the non-purified tissue preparations (b) from cerebral cortex of a patient with CJD. In the purified PrP<sub>CJD</sub> fraction (a), we applied the fraction from 10 mg wet weight in lane 2, 3 mg in lane 3, 1 mg in lane 4, 0.3 mg in lane 5 and 0.1 mg in lane 6. The minimum wet weight giving a positive reaction was 1 mg (lane 4). In the non-purified tissue preparation (b), we applied the fraction from 1 mg wet weight in lane 2, 0.3 mg in lane 3, 0.1 mg in lane 4, 0.03 mg in lane 5 and 0.01 mg in lane 6. The minimum wet weight giving positive labelling was 0.1 mg (lane 4). Lanes 1 contain Mr markers.

Fig. 2. Semi-quantitative Western blot analysis of the gel-eluted PrP<sub>CJD</sub> (23 to 25K) from human brain tissue with CJD. We applied 3 ng of the gel-eluted PrP<sub>CJD</sub> in lane 1, 1 ng in lane 2, 0.3 ng in lane 3, 0.1 ng in lane 4, 0.03 ng in lane 5 and 0.01 ng in lane 6. The minimum content of PrP<sub>CJD</sub> giving a positive reaction was 0.3 ng (lane 3).

(spleen, liver and lymph node) and peripheral nervous systems (dorsal root ganglion and peripheral nerves). In addition to these three CJD cases, we examined two other spleen tissues from patients with CJD, and from whom the disease has been transmitted to mice (Tateishi et al., 1987). However, there was no positive immunolabelling of the purified PrP<sub>CJD</sub> fractions or of the non-purified tissue preparations from various organs of non-CJD control cases.

**PrP<sub>CJD</sub> distribution in mice**

The organ distribution of murine PrP<sub>CJD</sub> in CJD-infected mice is summarized in Table 2. Positive data in the Western blot analysis were obtained from the PrP<sub>CJD</sub> fractions from the central nervous system, spleen, lymph node, thymus and intestine. Minimum wet weight for positive labelling was 0.3 mg of brain, 1 to 3 mg of spleen, 3 mg of spinal cord, 3 mg of lymph node, 10 mg of thymus, and 10 to 30 mg of intestine (Fig. 3). The pattern of immunolabelling showed three major proteins of 27K to 31K, 23K to 25K and 20K to 22K. There were no immunolabelled bands in purified PrP<sub>CJD</sub> fractions from 300 mg of liver, lung and kidney of the CJD-infected mice. There was also no positive immunolabelling of the purified PrP<sub>CJD</sub> fractions and the non-purified tissue preparations from control (non-CJD) mice.
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Fig. 3. Semi-quantitative Western blot analysis of the purified PrP<sub>CJD</sub> fraction from pooled intestines of CJD-infected mice. We applied the fraction from 30 mg wet weight intestine in lane 2, 10 mg in lane 3, 3 mg in lane 4 and 1 mg in lane 5. The minimum wet weight giving positive labelling was 10 mg (lane 3). Lane 1 contains Mr markers.

Table 1. Distribution of PrP<sub>CJD</sub> in patients with CJD

<table>
<thead>
<tr>
<th>Organ</th>
<th>No. of organs examined</th>
<th>Amount of tissue required for PrP&lt;sub&gt;CJD&lt;/sub&gt; detection (mg)</th>
<th>Estimated PrP&lt;sub&gt;CJD&lt;/sub&gt; concentration (μg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex</td>
<td>3</td>
<td>0.3 to 1</td>
<td>12 to 40</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>3</td>
<td>3 to 10</td>
<td>1.2 to 4</td>
</tr>
<tr>
<td>Dorsal root ganglion</td>
<td>2</td>
<td>ND*</td>
<td>-</td>
</tr>
<tr>
<td>Peripheral nerve</td>
<td>2</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>Spleen</td>
<td>5</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>Lymph node</td>
<td>3</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>Liver</td>
<td>3</td>
<td>ND</td>
<td>-</td>
</tr>
</tbody>
</table>

* ND, Not detected.

Table 2. Distribution of PrP<sub>CJD</sub> in CJD-infected mice

<table>
<thead>
<tr>
<th>Organ</th>
<th>Amount of tissue required for PrP&lt;sub&gt;CJD&lt;/sub&gt; detection (mg)</th>
<th>Estimated PrP&lt;sub&gt;CJD&lt;/sub&gt; concentration (μg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>0.3</td>
<td>30</td>
</tr>
<tr>
<td>Spleen</td>
<td>1 to 3</td>
<td>3 to 10</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Lymph node</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Thymus</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Intestine</td>
<td>10 to 30</td>
<td>0.3 to 1</td>
</tr>
<tr>
<td>Liver</td>
<td>ND*</td>
<td>-</td>
</tr>
<tr>
<td>Lung</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>Kidney</td>
<td>ND</td>
<td>-</td>
</tr>
</tbody>
</table>

* ND, Not detected.

PrP<sub>CJD</sub> fractions from non-neuronal organs may share the epitopes of antigens in kuru plaques

To check the specificity of the immunolabelled bands on the blotted membrane, we carried out affinity purification of antibodies bound on the membrane. Durapore membrane strips containing the purified PrP<sub>CJD</sub> fractions from 30 mg of brain, 120 mg of spleen and 120 mg of liver in CJD-infected mice were used as solid-phase antigens for the purification. The PrP<sub>CJD</sub> fraction from the brain was used as a positive control, and the fraction from the liver as a negative one, because we detected PrP immunoreactions from brain, but not from liver. We examined the fraction from the spleen. The antibodies eluted from the brain fraction stained typical birefringent kuru plaques in CJD-infected murine brain (Fig. 4a). However, antibodies eluting from the liver fraction did not immunolabel murine kuru plaques in the adjacent section (Fig. 4b). On the other hand, antibodies eluted from the spleen fraction stained murine kuru...
Fig. 4. Immunostaining of kuru plaques in CJD-infected murine brains with affinity-purified antibodies. (a) The antibodies eluted from the brain fraction stain murine kuru plaques. (b) Antibodies eluting from the liver fraction do not stain the murine kuru plaque. Arrows show unlabelled kuru plaques. (c) Antibodies eluting from the spleen fraction also stain the murine kuru plaques in serial section. Bar markers represent 25 μm.

plagues in the serial sections (Fig. 4c). The staining with the spleen fraction eluted antibodies was of a lesser intensity than that with the brain fraction eluted antibodies.

DISCUSSION

We compared the PrP<sub>CJD</sub> concentration in CJD-infected murine brains with the infectivity titre. The Fukuoka-1 strain, which was isolated as a mouse-adapted CJD strain (Tateishi et al., 1979), has an infectivity titre of 10<sup>7.4</sup> LD<sub>50</sub>/g in the NZW mouse (Mohri & Tateishi, 1989). Concerning the concentration of PrP<sub>CJD</sub> in NZW murine brains, a positive immunoreaction in Western blot analysis was obtained with over 0.03 mg (10<sup>-4.52</sup> g) of the non-purified brain preparation, and with more than 0.3 mg (10<sup>-3.52</sup> g) of the purified PrP<sub>CJD</sub> fraction. The infectivity titration was approximately 10<sup>3</sup> to 10<sup>4</sup> times as sensitive as our Western blot analysis. When comparing the purified PrP<sub>CJD</sub> fraction with the non-purified tissue preparation, the former has the advantage of use for determining a wide range of PrP<sub>CJD</sub> measurements. In our Western blot analysis, we needed more than 0.3 ng of gel-eluted PrP<sub>CJD</sub> to obtain a single immunoreactive band; we estimated PrP<sub>CJD</sub> concentration in human brain with CJD to be 30 to 40 μg/g wet tissue.
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For a comparison with tissues from autopsied humans with CJD, murine PrP\text{CJD} distribution was examined at the end stage of the illness. Human PrP\text{CJD} was restricted to the central nervous system. PrP\text{CJD} concentration in the central nervous system of mice was almost identical to that in humans. In addition, murine PrP\text{CJD} was detected in the lymphoreticular organs. PrP\text{CJD} in murine spleen had the same epitopes as in murine kuru plaques. The intestine also is rich in lymphoid tissue, i.e. Peyer's patches. The failure to detect PrP\text{CJD} in human lymphoreticular organs may have been due to a low recovery of PrP\text{CJD} in the purification processes, a different sensitivity to proteinase \( K \) digestion, a low affinity of our PrP antiserum, or a low PrP\text{CJD} concentration. There were no positive labellings in the non-purified human spleen preparations, this result differing from the findings in the murine spleen. Concerning the sensitivity to proteinase \( K \) digestion in the human spleen, the abnormal form of PrP cannot at present be differentiated from the normal cellular form without proteinase \( K \) digestion. Our PrP antiserum was raised against kuru plaque core protein purified from a patient with Gerstmann-Sträussler syndrome. This antiserum had the highest affinity of all our PrP antisera (Kitamoto \textit{et al.}, 1986b; Kitamoto & Tateishi, 1988), and cross-reacts with murine PrP\text{CJD} (Kitamoto \textit{et al.}, 1989). Murine PrP\text{CJD} in the spleen was readily recognized by its interspecies cross-reactivity, but human PrP\text{CJD} in the spleen was not immunolabelled. As it is unlikely that the negative result is due to a low affinity of PrP antiserum for human PrP\text{CJD}, it seems that the human spleen has a lower concentration of PrP\text{CJD} than does the murine spleen. Within the limits of our method, it was demonstrated that the distribution pattern of murine PrP\text{CJD} differs from that in humans.

Intensive studies of the infectivity titres from various organs have been done on scrapie-infected mice (Eklund \textit{et al.}, 1967), scrapie-infected goats (Hadlow \textit{et al.}, 1974) and CJD-infected mice (Kuroda \textit{et al.}, 1983). Murine PrP\text{CJD}-positive organs at the end stage of CJD were the brain, spinal cord, spleen, lymph node, thymus and intestine, and PrP\text{CJD}-negative organs were the liver, kidney and lung. In 1967, Eklund \textit{et al.} reported that the LD\(_{50}\) in scrapie-infected mice at 42 weeks after subcutaneous inoculation was 0.03 ml of a 10\(^{7.5}\) dilution in brain, 10\(^{6.6}\) in spinal cord, 10\(^{6.5}\) in spleen, 10\(^{4.6}\) in lymph node, 10\(^{4.5}\) in thymus, 10\(^{4.5}\) in intestine, 10\(^{5.8}\) in lung and 10\(^{4}\) in kidney and liver. We detected PrP\text{CJD} in organs which had a higher titre than 10\(^{4.5}\) LD\(_{50}/0.03\) ml, but could not detect it in organs below 10\(^{3.8}\) LD\(_{50}/0.03\) ml infectivity. The PrP\text{CJD} concentration correlates well with the infectivity titre in various organs.

It is impossible to evaluate the infectivity titre of human CJD because human to human transmission is not feasible. However, our experiment involving transmission from humans to mice revealed that the transmissibility of CJD from brains was higher than from the spleen (Tateishi \textit{et al.}, 1987). There are reports that the infectivity in the spleen differed with the species (Hadlow \textit{et al.}, 1974; Kimberlin & Walker, 1977). Mouse spleen had a titre of 10\(^{9}\) to 10\(^{9}\) LD\(_{50}\) i.c. units/0.05 g, while hamster spleen had a lower titre ranged from 10\(^{2.3}\) to 10\(^{4.3}\) LD\(_{50}\) i.c. units/0.05 g in 'Chandler' scrapie (Kimberlin & Walker, 1977). The spleen tissue of the scrapie-infected goat is also less infective (Hadlow \textit{et al.}, 1974). Therefore, the PrP concentration and infectivity titre in non-neuronal organs may differ with the species; however, PrP\text{CJD} concentrations and the infectivity titre in various murine organs seem to correlate.

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