J. Comp. Path. 2011, Vol. 144, 269–276

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Predominant Involvement of the Cerebellum in Guinea Pigs Infected with Bovine Spongiform Encephalopathy (BSE)

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Summary

This study reports the experimental transmission of bovine spongiform encephalopathy (BSE) to guinea pigs and describes the cerebellar lesions in these animals. Guinea pigs were inoculated intracerebrally with 10% brain homogenates from BSE-affected cattle. These animals were designated as the first passage. Second and third passages were subsequently performed. All guinea pigs developed infection at each passage. The mean incubation period of the first passage was 370 days post-infection (dpi) and this decreased to 307 dpi and 309 dpi for the second and third passages, respectively. Mild to severe spongiform degeneration and gliosis were observed in the cerebral cortex, thalamus and brainstem. In addition, the affected animals had marked pathological changes in the cerebellum characterized by severe cortical atrophy associated with Bergmann radial gliosis of the molecular layer and reduction in the width of the granular cell layer. Immunohistochemically, intense PrP^{Sc} deposition and scattered plaque-like deposits were observed in the molecular and granular cell layers. Cerebellar lesions associated with severe atrophy of the cortex have not been reported in animal prion diseases, including in the experimental transmission of PrP^{Sc} to small rodents. These lesions were similar to the lesions of human kuru or the VV2 variant of sporadic Creutzfeldt–Jakob disease, although typical kuru plaques or florid plaques were not observed in the affected animals.

Keywords: BSE; cerebellum; experimental transmission; guinea pig

Introduction

Scrapie in sheep and goats, bovine spongiform encephalopathy (BSE), chronic wasting disease (CWD) in deer and Creutzfeldt–Jakob disease (CJD) (sporadic, familial and transmitted or acquired forms), Gerstmann–Sträussler–Sheinker disease or syndrome (GSS) and kuru in man are neurodegenerative disorders belonging to a group of prion diseases or transmissible spongiform encephalopathies (TSEs). They are characterized by the accumulation of abnormal protease-resistant prion protein (PrP^{Sc}), which is an isoform of the cellular protease-sensitive prion protein (PrP^{C}). These accumulations are the result of post-translational modifications resulting in an increase in the presence of the β-sheet conformation of the protein in the brain (Prusiner, 1998).

The most characteristic finding of TSEs in animals is widespread spongiform degeneration of the central nervous system (CNS) accompanied by reactive astrocytic gliosis and microglial activation. In human prion diseases, including kuru and the VV2 type of sporadic Creutzfeldt–Jakob disease (sCJD-VV2), severe pathological changes are observed in the cerebellum with the loss of granular and Purkinje cells, fusiform swelling of the proximal portion of Purkinje cell axons (torpedoes) and intense Bergmann radial gliosis (DeArmond and Prusiner, 1997; DeArmond *et al.*, 2004). These cerebellar lesions have not been reported in animal prion diseases, including in the

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0021-9975/$ - see front matter
doi:10.1016/j.jcpa.2010.10.004

experimental transmission of PrP^{Sc} to small rodents, although PrP^{Sc} immunolabelling of varying intensity or deposition patterns was reported in the cerebellar cortex in BSE (Wells *et al.*, 1991; Orge *et al.*, 2000; Debeer *et al.*, 2003), scrapie (Wood *et al.*, 1997; González *et al.*, 2002) and CWD (Spraker *et al.*, 2002; Williams, 2005).

In the study of the transmission of BSE to various species of small rodents for development of an animal model, guinea pigs showed high susceptibility for the BSE prion. In addition, the cerebellar lesions in infected guinea pigs resembled the lesions observed in kuru or sCJD-VV2, although there were no typical amyloid plaques or florid plaques. The aim of this study was to describe the neuropathology of the cerebellar lesions in guinea pigs inoculated intracerebrally with BSE.

Materials and Methods

BSE Sample, Animals and Experimental Design

BSE cattle (BSE/JP4) used in this study were slaughtered at an abattoir in Japan for meat consumption and diagnosed by means of a rapid enzyme-linked immunosorbent assay (ELISA) for BSE (Grassi *et al.*, 2001). A sample of brain from the pons to the medulla oblongata, including the obex, was sectioned sagittally along the midline. One half was sampled for the rapid ELISA and confirmatory western blotting, while the other half was fixed in 15% neutral buffered formalin. That sample was submitted to our laboratories and re-examined for confirmation by histological and immunohistochemical analyses and western blotting.

Guinea pigs (3–4-week-old female Hartley strain; SLC, Shizuoka, Japan) and mice (Jcl:ICR strain; Clea, Tokyo, Japan) were inoculated intracerebrally with 10% brain homogenates in phosphate-buffered saline (PBS) from BSE-affected cattle (BSE/JP4); these animals were designated as first passage. For the second passage, guinea pigs and mice were inoculated with 10% brain homogenate in PBS from the first passage animals that developed clinical signs. Guinea pigs and mice for the third passage were inoculated with 10% brain homogenate in PBS from the second passage animals that developed clinical signs. Five guinea pigs and five mice were used in each passage. Animals inoculated with 10% normal mouse or guinea pig brain homogenate in PBS were used as controls. When animals showed clinical signs of the terminal stage of the disease, they were sacrificed under anaesthesia and the brain was removed and used for histopathological, immunohistochemical and immunoblot examinations. The experiments were conducted in accordance with the guidelines of the University Animal Care and Use Committee.

Neuropathology

Brains were fixed in 15% neutral buffered formalin for 1 week and then immersed in 98% formic acid for 1 h to reduce the risk of prion infectivity. Samples were embedded in paraffin wax and sections (4 μm) were stained with haematoxylin and eosin (HE), luxol fast blue-HE (LFB-HE) or the Bielschowsky silver method.

Immunohistochemistry (IHC) was performed with anti-PrP monoclonal antibody (mAb) 110 (recognizing mouse prion protein residues 56–90; Furuoka *et al.*, 2007; diluted 1 in 500) and anti-glial fibrillary acidic protein (GFAP; DAKO, Carpinteria, California; diluted 1 in 1,000) as the primary antibodies, and a secondary antibody conjugated to horseradish peroxidase-labelled polymer (DAKO Envision Kit; DAKO). The pretreatment method to retrieve PrP^{Sc} immunoreactivity was the 135DWHA method as previously reported (Furuoka *et al.*, 2004, 2007). Endogenous peroxidase activity was blocked by incubation in H_2O_2 3% for 5 min at room temperature. The sections were exposed to each primary antibody for 1 h at room temperature and then incubated with the second antibody for 30 min at room temperature. The signals were detected using diaminobenzidine (Simple stain DAB; Nichirei, Japan) followed by counterstaining with Mayer's haematoxylin.

Immunoblotting

Frozen brain samples from cattle (BSE/JP4), Jcl:ICR mice and guinea pigs infected with BSE prions were stored at −80°C and investigated for the presence of PrP^{Sc} by immunoblotting. PrP-enriched fractions were prepared from affected brain tissues by sarcosyl extraction, differential centrifugation and proteinase K (PK) digestion as previously described (Horiuchi *et al.*, 2002). For western blotting, the purified PrP^{Sc} was separated by 12% sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride membranes (Immobilon-P, Millipore, Massachusetts). Membranes were blocked with 5% non-fat dry milk in PBS for 60 min and incubated with primary antibody mAb110 (1 in 1,000) in PBS containing 0.1% Tween 20 for 60 min. After washing with PBS, the membranes were incubated with horseradish peroxidase-conjugated anti-mouse immunoglobulin (Ig) G antibody (Amersham Biosciences, Buckinghamshire, UK) for 60 min. They were washed again and developed with enhanced chemiluminescence (ECL) western blotting detection reagents (Amersham) and the bands were visualized by exposure to X-ray film (RX-U FUJI Medical, Japan).