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PrP^{Sc} level and incubation time in a transgenic mouse model expressing Borna disease virus phosphoprotein after intracerebral prion infection

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Abstract

Our previous studies have shown that the persistent expression of Borna disease virus phosphoprotein (BDV P) in mice leads to behavioral abnormalities resembling those in BDV-infected animals. In this study, we investigated whether the neurobehavioral abnormalities genetically induced by BDV P influence experimental prion disease. The effect of the phosphoprotein on prion diseases was evaluated based on the incubation time and survival curve, as well as the abnormal isoform of prion protein (PrP^{Sc}) levels in brains of BDV P Tg mice treated with proteinase K (PK) treatment and subjected to western blotting. Increased expression of the BDV P transgene had no effect on the PrP^{Sc} level, incubation time, or survival curve. The abnormalities induced by BDV P are different from those induced by prion diseases, indicating that the signaling cascades induced by the phosphoprotein differ from those induced by prion diseases.

Keywords: Borna disease virus; Prion; Scrapie; PrP; Psychiatric disease

Prion diseases are neurodegenerative disorders characterized by the accumulation of an abnormal isoform of prion protein (PrP^{Sc}) and astrocytosis in the central nervous system (CNS) [1]. The prion diseases include scrapie, bovine spongiform encephalopathy (BSE), Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker disease (GSS), and fatal familial insomnia (FFI). The PrP^{Sc} isoform shows increased β-sheet content and proteinase-K (PK) resistance compared to the cellular isoform of prion protein (PrP^{C}) [20]. The phenomena may be associated with the deposition of amyloid by scrapie-associated fibrils, which is specific to the prion diseases [15]. Therefore, resistance to proteinases such as PK is usually used as an index of prion infections in both clinical diagnosis and laboratory testing [26]. Furthermore, although the protein-only hypothesis has not been finally proved [19], it states that PrP^{Sc} is replicated by autocatalytic conversion of PrP^{C} into PrP^{Sc} [20]. Recently, experiments with protein misfolding cyclic amplification (PMCA) have come very close to proving this hypothesis by producing a prion disease in a natural host using a prion entirely generated *in vitro* [3,35].

Borna disease virus (BDV) is a noncytolytic and neurotrophic virus that belongs to *Mononegavirales* and can infect a broad range of vertebrates, including all warm-blooded animals and possibly also humans [7,21,31]. BDV infection leads to a variety of abnormal behaviors such as anxiety [22], aggression [22], hyperactivity [6,22], abnormal play behavior [18], chronic emotional overactivity [18], inhibition of responses to novel stimuli [6], and abnormal social behavior [18]. Various disorders could also be induced by BDV infection including cognitive deficits [5], reminiscent of autism [11], and schizophrenia [30]. Loss of Purkinje cells [4,6,33] and alterations in cytokine and chemokine gene expression [6,17,28] following neonatal BDV infection have been also demonstrated. Furthermore, epidemiological studies have demonstrated a higher prevalence of BDV infection in psychiatric patients such as schizophrenics than in controls [2,7,14]. Notably, as BDV phosphoprotein (P) is abundant in infected animal brains and interferes with a multifunctional protein, high-mobility group protein box 1 (HMGB1), in neuronal cells [10,16], it is thought to affect neuronal cells in the infected CNS. BDV P transgenic (Tg) mice develop behavioral abnor-

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malities resembling those in BDV-infected animals, and severe neurological disturbances are linked to neurobehavioral disorders. Therefore, the BDV P Tg mouse is thought to be a good experimental psychiatric animal model [29].

Interestingly, a rare mutation in the prion protein (PrP) gene has been discovered in a family with a strong history of psychiatric disease, and was linked to a complex phenotype including typical symptoms of schizophprenia like persecutory delusions and auditory hallucinations [27]. However, other studies found no association of single nucleotide polymorphisms (SNPs) in PrP gene with schizophrenia [23,32]. It is conceivable that psychiatric diseases and prion diseases share several signaling cascades and have some association. However, there was no appropriate experimental system for examining this issue. Here, we studied survival and the accumulation of PrP^{Sc} in brains of scrapie (chandler)-infected BDV P Tg mice.

PrP^{Sc} was intracerebrally (i.c.) inoculated into C57BL6J (wild-type, WT) mice and BDV P Tg mice [9] older than 8 weeks of age. The inoculation (i.c.) was conducted in P3 biohazard facilities as follows. Twenty microliters of inocula including 1% homogenate prepared from the brains of terminally diseased mice with mouse-adapted scrapie (chandler strain) was injected into the cerebral ventricular system of mice using a microsyringe as described previously [8]. The clinical symptoms (tremors and ataxia) were observed for the calculation of incubation time.

Semiquantitative reverse-transcription (RT)-polymerase chain reaction (PCR) and DNA-PCR was performed with the primers P1 (5′-TCA GAC CCA GAC CAG CGA A-3′) and P2 (5′-AGC TGG GGA TAA ATG CGC G-3′) for BDV P (p24), and G1 (5′-ACC ACA GTC CAT GCC ATC AC-3′) and G2 (5′-TCC ACC ACC CTG TTG CTG TA-3′) for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as described previously [24]. The intensity of gel bands was analyzed with Scion Image software (Scion Corp., Frederick, MD).

To discriminate PrP^{Sc} from PrP^{C}, 50 μg of protein per sample was treated in the absence and presence of 20 μg/ml of PK (Merck & Co. Inc., Whitehouse Station, NJ, USA) at 37 °C for 30 min. After termination of the reaction, the product was analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and western blotting.

Brains were removed from dead mice within 24 h and frozen at −80 °C in a refrigerator. The samples were solubilized in radio-immunoprecipitation assay (RIPA) buffer and sonicated at 4 °C. The RIPA buffer was composed of 10 mM Tris–HCl (pH 7.4) containing 1% sodium deoxycholate, 1% Nonidet P-40, 0.1% SDS and 0.15 M sodium chloride. Then, the protein samples {20 μg/lane for PrP [PK(−)]; 50 μg/lane for PrP [PK(+)]} were dissolved in SDS-PAGE sample buffer [70 mM SDS, 10% (w/v) glycerol, 1% 2-mercaptoethanol, 15 mM bromophenol blue, and 62.5 mM Tris–HCl, pH 6.8] and incubated at 100 °C for 10 min before electrophoresis in a 15% polyacrylamide gel containing 0.1% SDS at a constant current of 30 mA for 1 h. After electrophoresis, the proteins in the gel were electrically transferred to a polyvinylidene difluoride (PVDF) membrane (see below).

Proteins separated by SDS-PAGE were electrically transferred onto PVDF membranes (Amersham Biosciences, Piscataway, NJ), which were pretreated with methanol and transfer buffer [48 mM Tris, 39 mM glycine, 20% (v/v) methanol and 1.3 mM SDS] at a constant voltage of 12 V (ca. 100 mA) for 1 h. After the transfer, the membrane was blocked with 5% skim milk (Wako, Osaka, Japan) for 1 h at room temperature with gentle shaking before incubation with a mouse anti-PrP antibody, SAF83 (SPI bio, Montigny le Bretonneux, France) diluted with phosphate-buffered saline (PBS) containing 0.5% (v/v) skim milk and 0.1% (v/v) Tween 20. After 1 h of agitation at room temperature, the membrane was washed three times with PBS containing 1% (v/v) Tween 20 (PBS-T) for 5 min. The membrane was then incubated with horseradish peroxidase (HRP)-conjugated anti-mouse immunoglobulin antibody (Jackson Immunoresearch, West Grove, PA) for 1 h before being thoroughly washed with PBS-T. HRP was detected using an ECL™ kit (Amersham Biosciences) as described in the kit's instructions. The electrophoreogram was recorded on X-ray film (Amersham Biosciences). Each PrP^{Sc} and/or total PrP band intensity was measured using Scion Image for Windows software (Scion Corp., Frederick, MD, USA) in western blotting, and the PrP^{Sc} and/or total PrP level of each brain was estimated as a percentage (compared with the band intensity of cerebral PrP^{C} of the uninfected mouse).

The survival curves were analyzed by the Logrank test using Prism 4 Software (GraphPad Software, San Diego, CA, USA). Data on incubation time was analyzed with Student's *t*-test. The band intensities in the western blotting and RT-PCR analysis were subjected to a non-repeated measure ANOVA followed by the Bonferroni correction test.

In this study, we intracerebrally injected chandler prion into brains of WT mice and BDV P Tg mice and compared the two groups in terms of survival rate, incubation time, and PrP^{Sc} accumulation. First, the survival rate of the groups was compared (Table 1 and Fig. 1). All 11 prion-infected WT mice showed abnormal behavior including tremors and ataxia from 161 days and had died by 219 days. Similarly, all 5 prion-infected BDV P Tg mice showed clinical symptoms of prion disease from 182 days and died by 215 days. The Longman test showed that the survival rate of two groups was not significantly different (Fig. 1). The survival rate reflected a similar tendency in incubation time (Table 1). The incubation times of infected WT mice (197.3 ± 5.6) were not significantly different from those of infected BDV P Tg mice (194.8 ± 5.8) according to Student's *t*-test. As predicted, non-injected WT and BDV P Tg mice sur-

Table 1
Incubation time of wild-type (WT) and BDV P transgenic (Tg) mice infected with scrapie

	Inoculum	Mean incubation time ± S.E.M.[a] (days)	N/N_0[b]
WT	Chandler prion	197.3 ± 5.6	11/11
	Normal brain homogenate	>280	0/10
Tg	Chandler prion	194.8 ± 5.8	5/5
	Normal brain homogenate	>280	0/2

[a] S.E.M., standard error of the mean.
[b] *N*, number of dead animals; N_0, number of inoculated animals.