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Effect of Polylysine on Scrapie Prion Protein Propagation in Spleen during Asymptomatic Stage of Experimental Prion Disease in Mice

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Prion diseases are incurable neurodegenerative disorders. Our previous study demonstrated that polylysine was effective in prolonging the incubation period in a rodent model and in alleviating the scrapie prion protein (PrP^{Sc}) burden in the brain at the terminal stage of the disease. Here, we report that intraperitoneal administration of polylysine suppresses the accumulation of prions in the spleen during the early stages of the disease. This study supports the congruence of PrP^{Sc} inhibition by polylysine in both the spleen and brain.

Keywords: Prion, PrP^{Sc}, animal model, spleen, brain, polylysine

Prion diseases are degenerative disorders of the central nervous system (CNS) [6, 7]. The proteinaceous agents with transmissibility, designated prions, cause a number of neurologic disorders in mammals, such as Creutzfeldt-Jakob disease in humans, bovine spongiform encephalopathy in cattle, and chronic wasting disease in cervids [8, 10]. Although these diseases are regarded rare in humans and animals, the risk of the diseases for public health is considered undeniable [9]. To date, treatment options to cure prion diseases have not been established, although a number of attempts to develop anti-prion therapy have been made [9, 14]. We previously reported the effectiveness of L-lysine polymers as an anti-prion agent [4, 11]. Polylysine was efficacious in delaying the disease onset in mice infected with mouse-adapted scrapie prions. Furthermore, the level of PrP^{Sc}, which comprises the prion pathogen and is generated from the normal cellular prion protein via conformational conversion, was decreased in the brains of mice that received polylysine [11].

Because prion diseases are CNS disorders, anti-prion agents should be active in the brain to suppress prions and their downstream pathophysiological consequences. However, the agents that can inhibit prions in the non-CNS organs, such as spleen, are also valuable because some, if not most, acquired prion diseases occur via prion infection in non-CNS sites and these prions initially propagate in the spleen, lymph nodes, and tonsils at the early stage of the disease progression before they finally reach the brain for

major propagation [1]. Moreover, drugs are commonly delivered through the peripheral system of the body, but not directly to the brain. We hypothesize that the anti-prion agents effective in inhibiting prion propagation in the spleen would be considered beneficial and convenient for the treatment of prion disease at the early stage of the disease progression.

Although our previous studies revealed the potent anti-prion activity of polylysine in brain at the terminal stage of the disease [11], its anti-prion efficacy in the spleen at the early stage has not been investigated. In this study, we tested whether polylysine suppresses prion propagation in the spleen during the asymptomatic stage in a mouse model of prion disease.

Experiments associated with animals were conducted according to the protocols approved by the Institutional Animal Care and Use Committee (2006-0044) and the Biological Safety Office (B06-538M) at the University of Kentucky. Detailed experimental procedures for prion infection, drug administration, and PrP^{Sc} assay by western blotting were described in our previous publications [11]. Five-week-old wild-type CD-1 mice (Harlan, USA) were inoculated via the intraperitoneal route with 30 µl of 1% (w/v) brain homogenate prepared from mice terminally ill

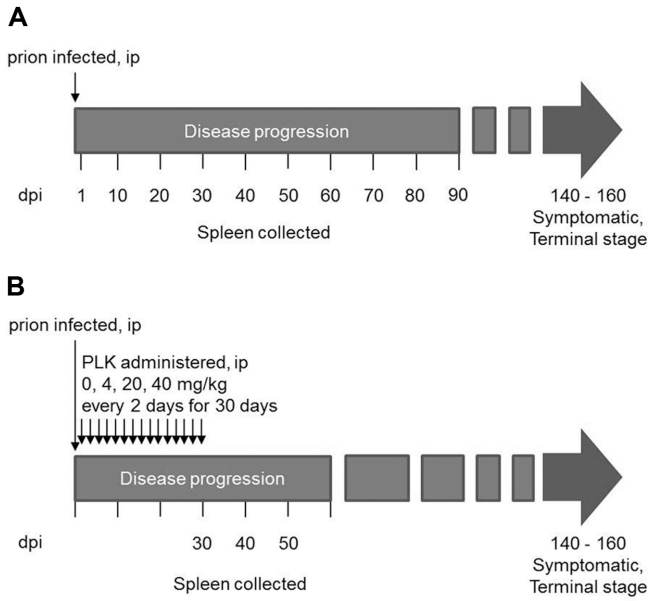

Fig. 1. Scheme of prion inoculation, spleen collection, and polylysine administration in mice.
(**A**) Spleen collection from mice intraperitoneally inoculated with RML prions. (**B**) Spleen collection from mice intraperitoneally inoculated with RML prions and intraperitoneally administered with polylysine. PLK, polylysine; ip, intraperitoneal; dpi, days post-inoculation.

by mouse-adapted scrapie RML prions. For the measurement of PrP^{Sc} level during the time course of disease development, the spleen was collected from infected mice (n = 3/time point) at one and every 10 days until 90 days post-inoculation (dpi) (Fig. 1A). For in vivo efficacy tests, a dose of 8, 20, or 40 mg/kg polylysine was intraperitoneally given to infected mice (n = 3/group) on every other day for 30 days, starting 2 dpi (Fig. 1B). In parallel, another group of infected mice received phosphate-buffered saline (PBS, pH 7.4, Invitrogen, USA) vehicle in the same manner. The polylysine used in this study was poly-L-lysine with the average molecular mass of 30–70 kDa (Sigma-Aldrich, USA). On 30, 40, and 50 dpi, the spleens from the vehicle and polylysine-treated groups were harvested (n = 3/each dose group). Ten percent (w/v) spleen homogenate prepared in 2% Sarkosyl-PBS was used to analyze the proteinase K-resistant PrP^{Sc} level by western blotting according to the procedures described elsewhere [2]. Anti-prion protein monoclonal antibody clone 6D11 (Covance Antibody Products, USA) and anti-β-actin (Ac-15) monoclonal antibody (Santa Cruz Biotechnologies, USA) were used as the primary antibody with 1:30,000 and 1:5,000 dilution, respectively. Peroxidase-conjugated anti-mouse immunoglobulin G antibody (Pierce, USA) was used as the secondary antibody with 1:10,000 dilution. Incubation with the primary and secondary antibodies lasted for 2 and 1 h, respectively. The PrP^{Sc} level was quantified by densitometry using GeneTools software (Syngene, UK). ANOVA was used for statistical analysis. The statistical significance level was the p value less than 0.05.

First, the PrP^{Sc} accumulation in the spleen of prion-infected mice via the intraperitoneal route was investigated during the time course of the disease development. No PrP^{Sc} was detected by western blotting on 1 dpi, suggesting that prions used for inoculation included the PrP^{Sc} level below the detection limit of western blotting (Fig. 2A). A very low level of PrP^{Sc} was accumulated in the spleen as early as 10 dpi and its level gradually, but rapidly, increased thereafter (Figs. 2A and 2B). The level of β-actin was constant among samples. This demonstrates that PrP^{Sc} in spleen can be detected long before prions reach the CNS during the early stage of disease progress, suggesting that such a low level of prions efficiently propagated in the non-CNS organs. This result is comparable to that of previous studies [3, 12], in which accumulation of PrP^{Sc} was detected at the earliest 7–28 dpi in the spleen of mice experimentally inoculated with prions.

Based on this observation, the efficacy of polylysine to inhibit prion propagation in the spleen was investigated.

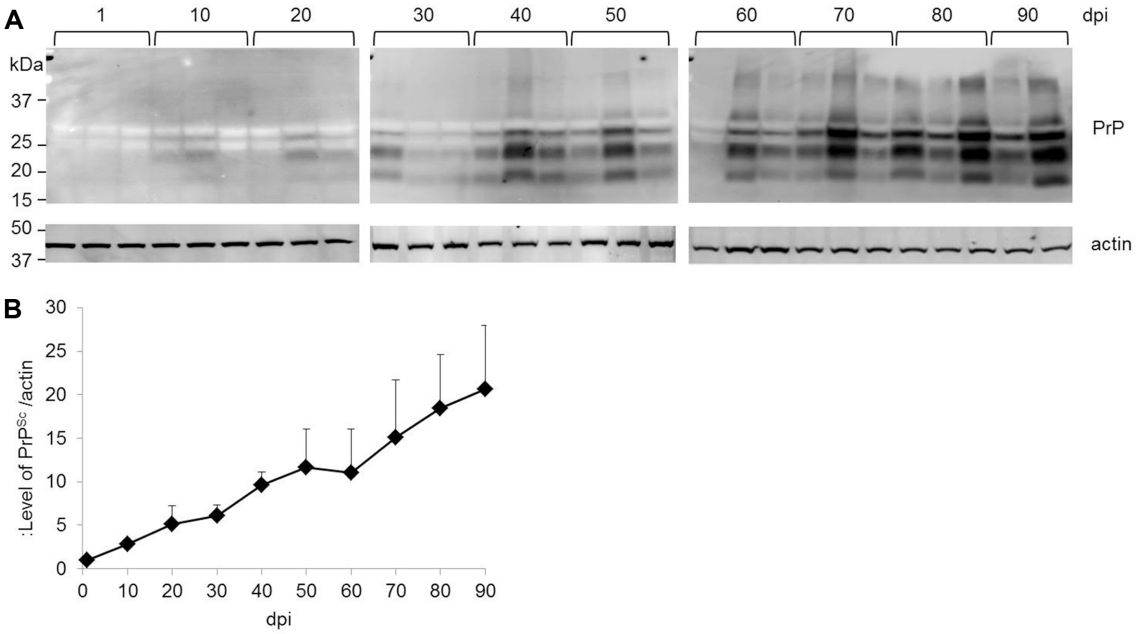

Fig. 2. Kinetics of PrP^{Sc} accumulation in mouse spleen.

(**A**) Western blots of the splenic PrP^{Sc} and β-actin levels in the early time course (1–90 dpi) of the prion disease progression. (**B**) Densitometry of the PrP^{Sc} levels in the spleen. The relative PrP^{Sc} levels were obtained by normalization with the β-actin levels. Multiple bands of PrP^{Sc} representing three different glycosylation states are considered as a whole for PrP^{Sc} in densitometry analysis. The average and standard deviation of relative densitometry values are presented as diamond symbols with error bars.

Western blot analyses of PrP^{Sc} in the spleens collected at 30, 40, and 50 dpi from mice that received 8–40 mg/kg polylysine showed that PrP^{Sc} accumulation was inhibited in some samples with variations compared with that found in the vehicle-treated control group (Figs. 3A–3C). However, densitometry analysis demonstrated that the average PrP^{Sc} levels in the spleens of mice that received no or increasing dosage of polylysine were almost identical at 30 and 40 dpi, whereas the PrP^{Sc} levels in the spleens of polylysine-treated mice were lower than those of vehicle-treated mice at 50 dpi (Fig. 3D). The difference was statistically significant ($p < 0.05$). These results suggest that different intervals after the last polylysine administration affect the effectiveness of polylysine to suppress PrP^{Sc} accumulation in the spleen, because the effectiveness of polylysine was demonstrated at 50 dpi and not until 40 dpi. Investigation of the PrP^{Sc} accumulation rate by plotting the splenic PrP^{Sc}/β-actin ratio relative to control brain PrP^{Sc} during 30–50 dpi revealed that polylysine slowed the kinetics of PrP^{Sc} accumulation. The second-order regression analysis clearly demonstrated the tendency of prion suppression in the spleen by polylysine. This supports that delayed prion accumulation in the spleen by polylysine at the early stage of the disease results in delayed prion accumulation in the brain at the terminal stage [11] presumably via ineffective prion invasion to the CNS.

The regimen for administration of polylysine used in this study was tolerated in mice, without showing aberrant behavioral changes. This corresponds to the previous in vivo toxicity data [13]. Additionally, our earlier in vivo polylysine distribution study reported that polylysine intraperitoneally administered is available in the spleen [5]. Thus, targeting of polylysine in the spleen suffices the observed tendency of prion suppression by polylysine in the spleen.

This study confirms that polylysine is efficacious in inhibiting the accumulation of PrP^{Sc} in the spleen, in which prions multiply prior to neuroinvasion. The beneficial effect of polylysine demonstrated in this study suggests that the inhibition of splenic prion accumulation by polylysine facilitates prion suppression in the brains shown in our previous study [11]. The model used in this study opens up an opportunity to establish a convenient tool to determine the in vivo efficacy of anti-prion therapeutic candidates within a relatively short period of time, because the conventional in vivo model depends on measuring the extension of the incubation period at the terminal stage of the disease.

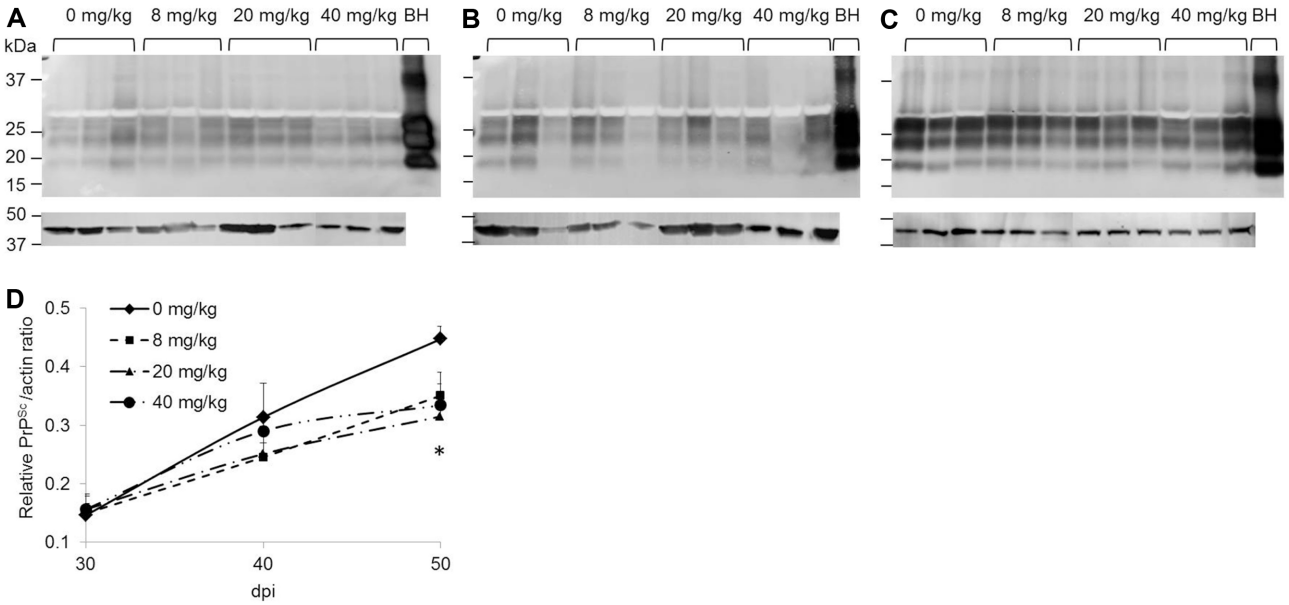

Fig. 3. Comparison of the PrP^{Sc} levels in the spleens of RML prion-infected mice treated with polylysine.
Mice were intraperitoneally inoculated with prions and received 0, 8, 20, or 40 mg/kg polylysine by intraperitoneal injection at every 48 h for 30 days. Western blots of the PrP^{Sc} levels in the spleen collected on 30 (**A**), 40 (**B**), and 50 (**C**) dpi. (**D**) Densitometry of the PrP^{Sc} levels presented in **A**–**C**. Multiple bands of PrP^{Sc} representing three different glycosylation states are considered as a whole for PrP^{Sc} in densitometry analysis. The PrP^{Sc} level was normalized with the β-actin levels. The relative PrP^{Sc} level was presented as a ratio to that of brain obtained at the terminal stage in the mouse model of prion disease. BH, brain homogenate.

Acknowledgments

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