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Commentary & View

Prion interference with multiple prion isolates

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Co-inoculation of prion strains into the same host can result in interference, where replication of one strain hinders the ability of another strain to cause disease. The drowsy (DY) strain of hamster-adapted transmissible mink encephalopathy (TME) extends the incubation period or completely blocks the hyper (HY) strain of TME following intracerebral, intraperitoneal or sciatic nerve routes of inoculation. However, it is not known if the interfering effect of the DY TME agent is exclusive to the HY TME agent by these experimental routes of infection. To address this issue, we show that the DY TME agent can block hamster-adapted chronic wasting disease (HaCWD) and the 263K scrapie agent from causing disease following sciatic nerve inoculation. Additionally, per os inoculation of DY TME agent slightly extends the incubation period of per os superinfected HY TME agent. These studies suggest that prion strain interference can occur by a natural route of infection and may be a more generalized phenomenon of prion strains.

Prion diseases are fatal neurodegenerative diseases that are caused by an abnormal isoform of the prion protein, PrP^{Sc}.[1] Prion strains are hypothesized to be encoded by strain-specific conformations of PrP^{Sc} resulting in strain-specific differences in clinical signs, incubation periods and neuropathology.[2-7] However, a universally agreed upon definition of prion strains does not exist. Interspecies transmission and adaptation of prions to a new host species leads to the emergence of a dominant prion strain, which can be due to selection of strains from a mixture present in the inoculum, or produced upon interspecies transmission.[8,9] Prion strains, when present in the same host, can interfere with each other.

Prion interference was first described in mice where a long incubation period strain 22C extended the incubation period of a short incubation period strain 22A following intracerebral inoculation.[10] Interference between other prion strains has been described in mice and hamsters using rodent-adapted strains of scrapie, TME, Creutzfeldt-Jacob disease and Gerstmannn-Sträussler-Scheinker syndrome following intracerebral, intraperitoneal, intravenous and sciatic nerve routes of inoculation.[10-15] We previously demonstrated the detection of PrP^{Sc} from the long incubation period DY TME agent correlated with its ability to extend the incubation period or completely block the superinfecting short incubation period HY TME agent from causing disease and results in a reduction of HY PrP^{Sc} levels following sciatic nerve inoculation.[12] However, it is not known if a single long incubation period agent (e.g., DY TME) can interfere with more than one short incubation period agent or if interference can occur by a natural route of infection.

To examine the question if one long incubation period agent can extend the incubation period of additional short incubation period agents, hamsters were first inoculated in the sciatic nerve with the DY TME agent 120 days prior to superinfection with the short-incubation period agents HY TME, 263K scrapie and HaCWD.[16-18] The HY TME and 263K scrapie agents have been biologically cloned and have distinct PrP^{Sc} properties.[19,20] The HaCWD agent used in this study is seventh hamster passage that has not been biologically cloned and therefore will be referred to as a prion isolate. Sciatic nerve inoculations were performed as previously described.[11,12] Briefly, hamsters were inoculated with $10^{3.0}$ i.c. LD_{50} of the DY TME agent or equal volume (2 µl of a 1% w/v brain homogenate) of uninfected brain homogenate 120 days prior to superinfection of the same sciatic nerve with either $10^{4.6}$ i.c. LD_{50} of the HY TME agent, $10^{5.2}$ i.c. LD_{50} of the HaCWD agent or $10^{4.6}$ i.c. LD_{50}/g 263K scrapie agent (Bartz J, unpublished data).[16,18,21] Animals were observed three times per week for the onset of clinical signs of HY TME, 263K and HaCWD based on the presence of ataxia and hyperexcitability, while the clinical diagnosis of DY TME was based on the appearance of progressive lethargy.[16-18] The incubation period was calculated as the number of days between the onset of clinical signs of the agent strain that caused disease and the inoculation of that strain. The Student's t-test was used to compare incubation periods.[12] We found that sciatic nerve inoculation of both the HaCWD agent and 263K scrapie agent caused disease with a similar incubation period to animals infected with the HY TME agent (Table 1). In the co-infected hamsters, inoculation of the DY TME agent 120 days prior to superinfection with the HY TME agent resulted in a complete blockage of HY TME agent from causing disease, consistent with previous studies (Table 1).[12] In hamsters inoculated with the DY TME agent 120 days prior to superinfection with the HaCWD or 263K agents, the animals developed clinical signs of DY TME with an incubation period that was not different from the DY TME agent control

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Table 1 **Clinical signs and incubation periods of hamsters inoculated in the sciatic nerve with either the HY TME, HaCWD or 263K scrapie agents, or co-infected with the DY TME agent 120 days prior to superinfection of hamsters with the HY TME, HaCWD or 263K agents**

First inoculation	Interval between inoculations	Second inoculation	Clinical signs	PrP-res migration	A/I[a]	Onset of clinical signs After 1st inoculation	Onset of clinical signs After 2nd inoculation
Mock	120 days	HY TME	HY TME	21 kDa	5/5	n.a.	72 ± 3[b]
Mock	120 days	HaCWD	HaCWD	21 kDa	5/5	n.a.	73 ± 3
Mock	120 days	263K	263K	21 kDa	5/5	n.a.	72 ± 3
DY TME	120 days	Mock	DY TME	19 kDa	4/4	224 ± 2	n.a.
DY TME	120 days	HY TME	DY TME	19 kDa	5/5	222 ± 2[c]	102 ± 2
DY TME	120 days	HaCWD	DY TME	19 kDa	5/5	223 ± 3[c]	103 ± 3
DY TME	120 days	263K	DY TME	19 kDa	5/5	222 ± 2[c]	102 ± 2

[a]Number affected/number inoculated; [b]Average days postinfection ± standard deviation; [c]Incubation period similar compared to control animals inoculated with the DY TME agent alone ($p > 0.05$). n.a., not applicable.

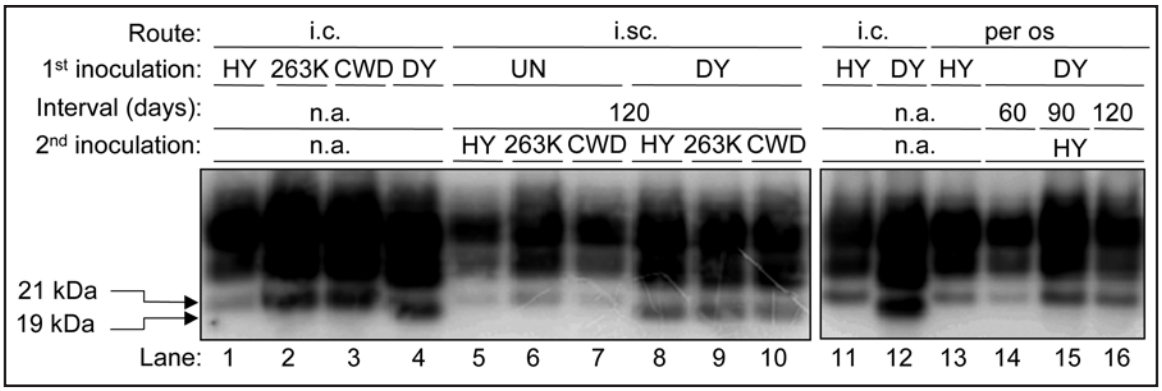

Figure 1. The strain-specific properties of PrP^{Sc} correspond to the clinical diagnosis of disease. Western blot analysis of 250 µg brain equivalents of proteinase K digested brain homogenate from prion-infected hamsters following intracerebral (i.c.), sciatic nerve (i.sc.) or per os inoculation with either the HY TME (HY), DY TME (DY), 263K scrapie (263K), hamster-adapted CWD (CWD) agents or mock-infected (UN). The unglycoyslated PrP^{Sc} glycoform of HY TME, 263K scrapie and hamster-adapted CWD migrates at 21 kDa. The unglycosylated PrP^{Sc} glycoform of DY PrP^{Sc} migrates at 19 kDa. Migration of 19 and 21 kDa PrP^{Sc} are indicated by the arrows on the left of the figure. n.a., not applicable.

group (Table 1). Proteinsase K digestion of brain homogenates and PrP Western blot analysis was performed as previously described.[12] The PrP^{Sc} migration properties were consistent with the clinical diagnosis and all co-infected animals had PrP^{Sc} that migrated similar to PrP^{Sc} from the DY TME agent infected control animal (Fig. 1, lanes 1–10). This data indicates that the DY TME agent can interfere with more than one isolate and that interference in the CNS may be a more generalized phenomenon of prion strains.

To examine the question if prion interference can occur following a natural route of infection, hamsters were first inoculated per os with the DY TME agent and then superinfected per os with the HY TME agent at various time points post DY TME agent infection. Hamsters were per os inoculated by drying the inoculum on a food pellet and feeding this pellet to an individual animal as described previously.[22] For the per os interference experiment, $10^{5.7}$ i.c. LD_{50} of the DY TME agent or an equal volume of uninfected brain homogenate (100 µl of a 10% w/v brain homogenate) was inoculated 60, 90 or 120 days prior to per os superinfection of hamsters with $10^{7.3}$ i.c. LD_{50} of the HY TME agent. A 60 or 90 day interval between DY TME agent infection and HY TME agent superinfection resulted in all of the animals developing clinical signs of HY TME with incubation periods that are similar to control hamsters inoculated with the HY TME agent alone (Table 2; $p > 0.05$). The 120 day interval group, however, developed clinical signs of HY TME with an incubation period that was extended compared to control hamsters inoculated with the HY TME agent alone (Table 2; $p < 0.01$). In all three of the co-infected groups of hamsters, PrP^{Sc} migrated similar to PrP^{Sc} from control hamsters inoculated with the HY TME agent alone (Fig. 1, lanes 11–16). The eight-day extension in the incubation period of HY TME in the 120 day interval co-infected group is consistent with a 1 log reduction in titer.[21] This is the first report of prion interference by the per os route of infection, a likely route of prion infection in natural prion disease and provides further evidence that prion strain interference could occur in natural prion disease.[23-25]

The capacity of the DY TME agent to replicate modulates its ability to interfere with the HY TME agent. TME interference, following sciatic nerve inoculation, occurs in the lumbar spinal cord and DY PrP^{Sc} abundance in this structure correlates with the ability of the DY TME agent to interfere with the HY TME agent.[12] Following extraneural routes of infection, DY TME agent replication and PrP^{Sc} deposition are not detected in spleen or lymph

Table 2 **Clinical signs and incubation periods of hamsters per os inoculated with either the HY TME or DY TME agent, or per os co-infected with the DY TME agent 60, 90 or 120 days prior to superinfection of hamsters with the HY TME agent**

First inoculation	Interval between inoculations	Second inoculation	Clinical signs	PrP-res migration	A/I[a]	Onset of clinical signs After 1st inoculation	Onset of clinical signs After 2nd inoculation
Mock	120 days	HY TME	HY TME	21 kDa	5/5	n.a.	140 ± 5[b]
DY TME	60 days	HY TME	HY TME	21 kDa	5/5	195 ± 6	135 ± 6
DY TME	90 days	HY TME	HY TME	21 kDa	5/5	230 ± 5	140 ± 5
DY TME	120 days	HY TME	HY TME	21 kDa	5/5	269 ± 3	149 ± 3[c]

[a]Number affected/number inoculated; [b]Average days postinfection ± standard deviation; [c]Incubation period extended compared to control animals inoculated with the HY TME agent alone ($p < 0.01$); n.a., not applicable.

nodes, which is the major site of extraneural HY TME agent replication.[11,21,26] The DY TME agent can interfere with the HY TME agent following intraperitoneal and per os infection, suggesting that the DY TME agent is replicating in other locations that are involved in HY TME agent neuroinvasion (Table 2).[11]

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