Prions in Skeletal Muscles of Deer with Chronic Wasting Disease

Rachel C. Angers,[1*] Shawn R. Browning,[1*†] Tanya S. Seward,[2] Christina J. Sigurdson,[4‡] Michael W. Miller,[5] Edward A. Hoover,[4] Glenn C. Telling[1,2,3§]

Prions are transmissible proteinaceous agents of mammals that cause fatal neurodegenerative diseases of the central nervous system (CNS). The presence of infectivity in skeletal muscle of experimentally infected mice raised the possibility that dietary exposure to prions might occur through meat consumption (*1*). Chronic wasting disease (CWD), an enigmatic and contagious prion disease of North American cervids, is of particular concern. The emergence of CWD in an increasingly wide geographic area and the interspecies transmission of bovine spongiform encephalopathy (BSE) to humans as variant Creutzfeldt Jakob disease (vCJD) have raised concerns about zoonotic transmission of CWD.

To test whether skeletal muscle of diseased cervids contained prion infectivity, Tg(CerPrP) mice (*2*) expressing cervid prion protein (CerPrP) were inoculated intracerebrally with extracts prepared from the semitendinosus/semimembranosus muscle group of CWD-affected mule deer or from CWD-negative deer. The availability of CNS materials also allowed for direct comparisons of prion infectivity in skeletal muscle and brain. All skeletal muscle extracts from CWD-affected deer induced progressive neurological dysfunction in Tg(CerPrP) mice, with mean incubation times ranging between 360 and ~490 days, whereas the incubation times of prions from the CNS ranged from ~230 to 280 days (Table 1). For each inoculation group, the diagnosis of prion disease was confirmed by the presence of disease-associated, protease-resistant PrP (PrP^{Sc}) in the brains of multiple infected Tg(CerPrP) mice [see (*3*) for examples]. In contrast, skeletal muscle and brain material from CWD-negative deer failed to induce disease in Tg(CerPrP) mice (Table 1), and PrP^{Sc} was not detected in the brains of asymptomatic mice as late as 523 days after inoculation (*3*).

Our results show that skeletal muscle as well as CNS tissue of deer with CWD contains infectious prions. Similar analyses of skeletal muscle from BSE-affected cattle did not reveal high levels of prion infectivity (*4*). It will be important to assess the cellular location of PrP^{Sc} in muscle. Although PrP^{Sc} has been detected in muscles of scrapie-affected sheep (*5*), previous studies failed to detect PrP^{Sc} by immunohistochemical analysis of skeletal muscle from deer with natural or experimental CWD (*6*, *7*). Because the time of disease onset is inversely proportional to prion dose (*8*), the longer incubation times of prions from skeletal muscle extracts compared with those from matched brain samples indicated that prion titers were lower in muscle than in the CNS, where infectivity titers are known to reach high levels. Although possible effects of CWD strains or strain mixtures on these incubation times cannot be excluded, the variable 360- to ~490-day incubation times suggested a range of prion titers in skeletal muscles of CWD-affected deer. Muscle prion titers at the high end of the range produced the fastest incubation times, which were ~30% longer than the incubation times of prions from the CNS of the same animal. Because all mice in each inoculation group developed disease, prion titers in muscle samples producing the longest incubation times were higher than the end point of the bioassay, defined as the infectious dose at which half the inoculated mice develop disease. Although the risk of exposure to CWD infectivity after consumption of prions in muscle is mitigated by relatively inefficient prion transmission via the oral route (*9*), our results show that semitendinosus/semimembranosus muscle, which is likely to be consumed by humans, is a major source of prion infectivity. Humans consuming or handling meat from CWD-infected deer are therefore at risk to prion exposure.

Table 1. Incubation times after inoculation of Tg(CerPrP) mice with prions from skeletal muscle and brain samples of CWD-affected deer. PBS, phosphate buffered saline.

Inocula	Incubation time, mean days ± SEM (n/n_0)*	
	Skeletal muscle	Brain
	CWD-affected deer	
H92	360 ± 2 (6/6)	283 ± 7 (6/6)
33968	367 ± 9 (8/8)	278 ± 11 (6/6)
5941	427 ± 18 (7/7)	
D10	483 ± 8 (8/8)	231 ± 17 (7/7)
D08	492 ± 4 (7/7)	
Averages	426	264
	Nondiseased deer	
FPS 6.98	>523 (0/6)	
FPS 9.98	>454 (0/7)	>454 (0/6)
None	>490 (0/6)	
PBS	>589 (0/5)	

*The number of mice developing prion disease (n) divided by the original number of inoculated mice (n_0) is shown in parentheses. Mice dying of intercurrent illnesses were excluded.

References and Notes

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Materials and Methods
Fig. S1

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[1]Department of Microbiology, Immunology and Molecular Genetics; [2]Sanders Brown Center on Aging; [3]Department of Neurology, University of Kentucky, Lexington, KY 40536, USA. [4]Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO 80523, USA. [5]Colorado Division of Wildlife, Wildlife Research Center, Fort Collins, CO 80526, USA.

*These authors contributed equally to this work.
†Present address: Department of Infectology, Scripps Research Institute, 5353 Parkside Drive, RF-2, Jupiter, FL 33458, USA.
‡Present address: Institute of Neuropathology, University of Zurich, Schmelzbergstrasse 12, 8091 Zurich, Switzerland.
§To whom correspondence should be addressed. E-mail: gtell2@uky.edu