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Experimental Chronic Wasting Disease (CWD) in the Ferret

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Summary

Chronic wasting disease (CWD), a prion disease of North American deer, elk and moose, affects both free-ranging and captive cervids. The potential host range for CWD remains uncertain. The susceptibility of the ferret to CWD was examined experimentally by administering infectious brain material by the intracerebral (IC) or oral (PO) route. Between 15 and 20 months after IC inoculation, ferrets developed neurological signs consistent with prion disease, including polyphagia, somnolence, piloerection, lordosis and ataxia. Upon first sub-passage of ferret-adapted CWD, the incubation period decreased to 5 months. Spongiform change in the neuropil was most marked in the basal ganglia, thalamus, midbrain and pons. The deposition of PrP^{CWD} was granular and was occasionally closely associated with, or localized within, neurons. There were no plaque-like or perivascular PrP aggregates as seen in CWD-infected cervids. In western blots, the PrP^{CWD} glycoform profile resembled that of CWD in deer, typified by a dominant diglycosylated glycoform. CWD disease in ferrets followed IC but not PO inoculation, even after 31 months of observation. These findings indicate that CWD-infected ferrets share microscopical and biochemical features of CWD in cervids, but appear to be relatively resistant to oral infection by primary CWD inoculum of deer origin.

Keywords: chronic wasting disease; prion; species barrier; transmissible spongiform encephalopathy

Introduction

Chronic wasting disease (CWD) is a prion disease of North American mule deer, white-tailed deer, Rocky Mountain elk and moose (family Cervidae), and is the only prion disease known to naturally affect wildlife. CWD has been recognized for at least three decades in a focal geographical region encompassing north-eastern Colorado and south-eastern Wyoming (Williams and Young, 1980, 1982), wherein its prevalence in free-ranging mule deer has reached up to 30% (Williams, 2005). More recently, this transmissible spongiform encephalopathy (TSE) was discovered in isolated foci over 100 miles beyond this endemic area, most recently as far east as New York (Sigurdson and Miller, 2003). Transmission among cervids is believed to occur horizontally, based on observations with captive deer sharing common pastures and epidemiological models of prevalence and population at risk (Miller *et al.*, 1998, 2000; Miller and Williams, 2003). The mechanism(s) and route(s) of natural

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agent transmission remain unclear, although infectious prions have been demonstrated in saliva and blood of CWD-infected deer (Mathiason *et al.*, 2006). Moreover, whether other species are susceptible to, and/or can act as reservoirs for, CWD has been minimally investigated and thus remains uncertain.

A CWD-susceptible small animal model is crucial in order to study CWD prevention schemes and therapeutics, and to serve as a bioassay for potential transmission vehicles such as saliva, urine or faeces. Animal prion diseases such as scrapie in sheep and goats have been extensively modelled in mice and hamsters, enabling mapping of prion infectivity during the course of disease (Brandner *et al.*, 2000). Wild-type mice have been shown to be resistant to CWD (Williams and Young, 1992; Browning *et al.*, 2004). However, transgenic mice expressing cervid prion protein (PrP) in the central nervous system (CNS) have been shown to be susceptible to CWD after intracerebral inoculation, supporting the importance of the cervid PrP gene in disease susceptibility (Browning *et al.*, 2004; Kong *et al.*, 2005; Tamguney *et al.*, 2006). Bartz *et al.* (1998) showed that ferrets (*Mustela furo*; European polecats) are susceptible to CWD, and that CWD-resistant hamsters became susceptible to ferret-passaged CWD. These results suggest that ferrets may be a surrogate small animal model and may provide insights into the potential for CWD to cross species barriers. The aim of the present study was to determine the susceptibility of ferrets to CWD following exposure by a natural route (orally; PO) and by intracerebral (IC) inoculation.

Materials and Methods

Animals and Experimental Inoculation

Brain homogenate from terminally ill, naturally CWD-infected mule deer was prepared in physiological saline with penicillin-streptomycin (100 U/ml) added.

Nine domestic ferret kits (*Mustela furo*) were administered anaesthesia and intracerebrally-inoculated with 300 µl of a 10% homogenate of CWD-infected mule deer brain given into the left parietal cortex. Two of these ferrets were killed at 3, 6 and 12 months post-inoculation, and the three remaining ferrets were maintained until terminal disease developed. Brain from these terminally diseased ferrets was then pooled and inoculated into three further ferrets.

Nine ferrets were syringe-fed once daily for 4 days with 1.8 ml of a 40% CWD brain homogenate (total dose equivalent to 2.9 g of brain from infected mule deer). Two of these ferrets were killed at 3, 6 and 12 months post-inoculation. Control ferrets ($n = 9$) were inoculated with uninfected mule deer brain by the intracerebral and oral routes described above, and were killed at equivalent time points.

Histology and Immunohistochemistry

Eight sections of brain were examined from each of five intracerebrally-inoculated and four control animals. These sections were taken to include the basal ganglia, cerebral cortex, hippocampus, thalamus, midbrain, pons, cerebellum, and medulla oblongata. Tissue sections were mounted onto positively charged glass slides, dewaxed, and hydrated in preparation for immunohistochemistry (IHC). For exposure of PrP epitopes, sections were pre-treated by hydrated autoclaving for 10 min in a citrate-based antigen retrieval solution, pH 6.0 (Dako, Carpinteria, CA), and cooled for 30 min. The reagents employed sequentially in the immunohistochemical reaction included: monoclonal antibody (Mab) L42, which is specific for the ovine 145–163 PrP epitope (R-Biopharm AG, Darmstadt, Germany) (Vorberg *et al.*, 1999), biotinylated secondary anti-mouse antibody, alkaline phosphatase-streptavidin conjugate, substrate chromogen (Ventana Medical Systems, Tucson, AZ), and haematoxylin counterstain. CWD positive and negative control tissue sections were included in each experiment.

Immunofluorescence Labelling

Tissue sections (6 µm) were mounted onto positively charged glass slides, dewaxed and hydrated. To expose PrP epitopes, sections were heated in a citrate buffer solution (pH 6.0) for 5 min using a pressure cooker, cooled for 5 min, and then treated with 2 µg/ml proteinase K (Roche Applied Science, Indianapolis, Indiana) for 10 min at room temperature (RT) and washed in distilled water. Tissues were next immersed in 3% H_2O_2 in methanol for 15 min to quench endogenous peroxidase, blocked (TSA Kit blocking reagent; PerkinElmer, Boston, MA) and incubated in a mixture of antibody specific for glial fibrillary acidic protein (GFAP; rabbit polyclonal, 14.5 µg/ml; Dako) and anti-PrP monoclonal antibody L42 (0.05 µg/ml) for 30 min. Secondary antibodies were then added sequentially to "visualize" the location of GFAP and PrP within the tissues. For GFAP, the secondary antibody was goat anti-rabbit IgG-Alexa 546 (Invitrogen, Carlsbad, CA) and for PrP the secondary antibody was goat anti-mouse IgG-horseradish peroxidase (HRP) (Zymed, San Francisco, CA) followed by amplification with biotinyl tyramide and detection using