

Assessment of Prospective Preventive Therapies for Chronic Wasting Disease in Mule Deer

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ABSTRACT: We compared prion infection rates among mule deer (*Odocoileus hemionus*) receiving pentosan polysulfate, tannic acid, tetracycline HCl, or no treatment 14 days before to 14 days after (dpi) oral inoculation with tonsil tissue homogenate. All deer were infected, but the rapid disease course (230–603 dpi) suggested our challenge was overwhelming.

Prospects for controlling chronic wasting disease (CWD), a prion disease of North American cervids (Williams, 2005), have dimmed in the absence of management tools other than culling. A vaccine or therapeutic compound preventing cervids from becoming infected with CWD would be a tremendous asset to disease management, especially one that could be easily delivered (e.g., via mineral licks or bait) and that had sustained effects that prevented new infections and reduced shedding in infected individuals. Several compounds showing some efficacy in preventing or retarding the progression of prion infection have been identified (Sim and Caughey, 2010). We tested three compounds that are readily available, have been shown to be safe, and have anti-prion efficacy *in vitro* or *in vivo*: Pentosan polysulfate is a sulfated polysaccharide with anti-inflammatory properties that interferes with peripheral prion propagation; tannic acid is a naturally occurring polyphenol antioxidant; and tetracycline HCl is an antibiotic with prion-decontaminating properties (Ehlers and Diringer, 1984; Caughey and Raymond, 1993; Farquhar et al., 1999; Forloni et al., 2002; Kocisko et al., 2003; Raymond et al., 2006; Sim and Caughey, 2010). We briefly describe outcomes of a small study assessing the effectiveness of these three therapies in preventing prion infection in

experimentally challenged mule deer (*Odocoileus hemionus*).

We used hand-raised mule deer ($n=19$) of both sexes that were about 15 mo old at the beginning of the study (10 September 2003). All deer were homozygous for serine at codon 225 of the *Prnp* gene (225SS), and although they originated from CWD-endemic sources, none had evidence of disease-associated prion protein (PrP^{CWD}) deposits in tonsil biopsies (Wolfe et al., 2002) collected before the study. We randomly assigned deer to one of four treatment groups (Table 1; details below) and confined each group to a separate, outdoor, ~0.04-ha paddock. These paddocks had not housed other deer for >18 yr. All deer received alfalfa hay, pelleted supplement without mammalian protein, mineralized salt blocks, and water *ad libitum* (except as below) following established protocols. Study methods were approved by the Colorado Division of Wildlife Animal Care and Use Committee (file 02-2003).

We applied treatments orally, dosed and delivered as follows: pentosan polysulfate in 0.1-g capsules, hand-fed individually in a dollop of cake icing three times per day; tannic acid dissolved in tap water at a concentration of 20 g/l, provided *ad libitum* in place of drinking water; tetracycline HCl dissolved in tap water at a concentration of 0.5 g/l, provided *ad libitum* in place of drinking water; control (received no treatment). Individual dosing was known for the pentosan polysulfate group but was estimated (=daily group consumption/ n) post hoc for the tannic acid and tetracycline HCl groups based on mean daily water consumption. We began

TABLE 1. Prion infection rate and disease course among groups of mule deer (*Odocoileus hemionus*) receiving pentosan polysulfate, tannic acid, tetracycline HCl, or no treatment before and after oral inoculation with tonsil tissue homogenate.

Treatment	Estimated daily dose/deer (g)	Number infected/total	Disease course (days after inoculation)	
			Mean ^b ±standard error	Range
Pentosan polysulfate	0.3	5 ^a /5	358±32 ^c	294–422 ^c
Tannic acid	96.2	5/5	311±20	252–387
Tetracycline HCl	2.2	5/5	350±55 ^d	280–540 ^d
Untreated control	0	4/4	397±75	230–603

^a Two animals were tonsil biopsy negative at 90 days after inoculation (dpi) but were positive at 270 dpi.

^b Survival did not differ among respective treatment groups and the control group (log rank test, Bonferroni-corrected $P=1$; Yang et al., 2011).

^c One animal in this treatment group that died of unrelated causes 210 days after inoculation was censored.

^d One animal in this treatment group that died of unrelated causes 161 days after inoculation was censored.

daily treatments 14 days before inoculation and continued for 14 days after inoculation (dpi) based on time frames used in similar studies.

For challenge, we inoculated each deer orally with one dose of about 1 ml of a 10% (wt/vol) solution of infectious tonsil tissue (equivalent to about 0.1 g wet weight tonsil tissue/dose). The inoculum was prepared from three mule deer with clinical CWD (1 g tonsil tissue from each deer; 3 g total) by combining and homogenizing the tissue, suspending the macerate in 30 ml of normal saline, decanting, and drawing 1-ml doses of the decanted supernatant.

Deer were observed daily, and those showing behavioral changes, loss of body condition, ataxia, and salivation or polydipsia consistent with signs of clinical CWD (Williams, 2005) were euthanized.

We sampled each deer via tonsil biopsy again at 90 dpi; two deer with negative tonsil biopsies at 90 dpi were resampled at 252 dpi. Deer were removed from the study when found to be biopsy positive, but these individuals were still observed daily to assess health and estimate disease course (time from inoculation to euthanasia or death). We confirmed prion infections postmortem.

None of the three treatments appeared to prevent infection or lengthen disease course (Table 1). Seventeen of the 19 mule deer were biopsy-positive at 90 dpi; the

remaining two (both in the pentosan polysulfate group) were biopsy-positive at 252 dpi. Seventeen of the 19 deer died or were euthanized with evidence of clinical CWD 230–603 dpi (Table 1); two deer died early in the study of causes apparently unrelated to treatment or prion infection.

The rapid disease course in deer after inoculation suggested our challenge may have been overwhelming and thus may have obscured potential treatment effects (e.g., Priola et al., 2003). The overall mean±standard error (SE) disease course (352±26 dpi) was much shorter than the 2–4 yr thought to typify the natural course of CWD (Williams, 2005) or the 673±24-day course produced by orally challenging mule deer with 1 g of undiluted brain homogenate containing about 3 µg of PrP^{CWD}/g homogenate (Miller et al., 2012). We did not measure concentrations of PrP^{CWD} in the tonsil homogenate, but our challenge may have contained 2–74 times more PrP^{CWD} than the aforementioned brain homogenate based on concentrations estimated from other mule deer tonsil tissue (range 68–2,376 µg/g wet weight tissue, mean±SE=672±66 µg/g; O'Rourke et al., 2003). Although a high concentration of infectivity seems to be the most plausible explanation for the rapid disease courses produced by our challenge, it is possible that prions in peripheral tissues have oral infectivity properties different from prions in brain tissue.

Absent any precedent for using tonsil tissue for experimental prion infection in deer, we selected the challenge dose based on a conservative estimate of infectivity in the inoculum to ensure that all individuals became infected. A much lower dose (perhaps 0.001 g) or a more natural exposure source (e.g., contaminated paddocks; Miller et al., 2004) may have provided a more biologically relevant challenge.

Despite the outcome, we believe it worthwhile to continue searching for compounds that could prevent cervids from becoming infected with CWD. From a population management standpoint, compounds that prevent or clear peripheral infection will be more useful than those targeting the central nervous system; from a practical standpoint, such compounds will likely need to be effective when delivered orally. Of the various prospects identified thus far, the large-molecule compounds like sulfated polysaccharides and sulfated fucosylated polysaccharides (Doh-ura et al., 2007), alone or in combination (Sim and Caughey, 2010), seem most promising for application to captive and free-ranging cervids. Seaweed fucoidan may deserve further attention in light of its apparent effects in delaying onset of scrapie in orally inoculated mice (Doh-ura et al., 2007).

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