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Cervical spinal cord and motor unit pathology in a canine model of SOD1-associated amyotrophic lateral sclerosis

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ABSTRACT

Development of effective treatments for amyotrophic lateral sclerosis (ALS) would be facilitated by identification of early events in the pathological cascade of disease progression. Degenerative myelopathy (DM), a naturally occurring disease in dogs, is quite similar to forms of ALS associated with *SOD1* mutations and is likely to be a good model for these forms of the human disease. The sequence of histopathological changes that occur in DM was characterized by analyzing tissue samples obtained from affected dogs euthanized at various stages of disease progression. Cervical spinal cord and the associated spinal nerve roots, ulnar nerve, and the extensor carpi radialis (ECR) muscle were obtained from Pembroke Welsh Corgi dogs (PWCs) with early and late stage DM and from age-matched unaffected PWCs. In early stage disease there was a substantial change in the ratio of the two main muscle fiber types and an increase in mean muscle fiber area in the ECR. DM, even in late stage disease, was not accompanied by changes in the number of motor neuron cell bodies, in the number of axons in the motor or sensory nerve roots, or in the ulnar nerve. In addition, no disease-related denervation of the acetylcholine receptors of the ECR was observed at any stage of the disease. On the other hand, axon densities in both motor and sensory nerve tracts in the cervical cord were reduced in affected dogs. SOD1-immunoreactive aggregates were observed in spinal cord motor neuron cell bodies only in late stage disease. These findings suggest that some of the earliest pathological changes in DM occur in the muscle fibers and upper motor and sensory neuron tracts in the spinal cord. Targeting therapeutic interventions to these early events in the disease are most likely to be effective in slowing disease progression for DM and may translate to therapy of *SOD1*-related forms of ALS.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a group of human neurodegenerative diseases that in most cases are characterized by a late-adult onset and relatively rapid progressive loss of muscle function. Initial muscle weakness progresses to almost total paralysis. At end-stage disease, loss of muscle functions involved with swallowing and respiration ultimately leads to death. Although no underlying genetic or environmental causes have been identified for the majority of ALS patients, mutations in numerous genes have now been associated with some of forms of ALS [1–7]. Among these are mutations in the superoxide dismutase 1 gene (*SOD1*), which encodes the enzyme superoxide dismutase-1 [8,9]. To date, over 150 different *SOD1* mutations have been associated with ALS [10] (http://alsod.iop.kcl.ac.uk/als/). A mutation in only one of the two *SOD1* alleles is sufficient to cause ALS, indicating that the disease results not from a loss of superoxide dismutase function, but rather from toxic effects of the expression of the mutant allele. Among the potential mechanisms proposed to explain this toxicity is that the mutant protein assumes an abnormal conformation leading to its aggregation, particularly in neurons, which in turn causes co-aggregation of other normal proteins. The accumulation of these protein aggregates has been hypothesized to cause dysfunction and death of neurons involved in regulating muscle function and thus resulting in impairment of muscle function.

Degenerative myelopathy (DM) is a disease in dogs that is very similar to the form of ALS caused by *SOD1* mutations [11,12]. DM, which occurs in many dog breeds, has a typical age of onset of 9 years in large breeds [11], and 11 years of age in Pembroke Welsh Corgis (PWCs) [13]. Most DM affected dogs will progress to nonambulatory status within 1 year from onset of signs but can live 3 years before respiratory dysfunction either necessitates euthanasia or results in unassisted death [14]. In the United States, most affected dogs that suffer from DM are euthanized before they reach disease end stage when respiratory distress becomes apparent. Clinical signs and progression are relatively uniform among dogs of the same breed and between breeds, with the primary breed difference being in the average age of onset. This uniformity in

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Table 1
Neurological signs present at time of euthanasia used to grade disease stage among dogs with DM.

Stage[a]	Neurological signs
1	Asymmetric, general proprioceptive ataxia and spastic paresis in pelvic limbs
	Intact spinal reflexes
2	Non-ambulatory paraparesis, paraplegia
	Reduced to absent pelvic limb spinal reflexes Pelvic limb muscle atrophy ±urinary/fecal incontinence
3	Flaccid paraplegia, thoracic limb paresis
	Absent spinal reflexes Severe pelvic limb muscle atrophy Urinary/fecal incontinence
4	Flaccid tetraplegia
	Absent spinal reflexes Severe generalized muscle atrophy Urinary/fecal incontinence Dysphagia, dystonia, respiratory difficulty

[a] For this study dogs with stages 1 and 2 disease were pooled into the "early stage" group and dogs with stages 3 and 4 disease were pooled into the "late stage" group.

disease phenotype may be partly explained by the fact that in all dogs, except some Bernese Mountain Dogs, DM is associated with the same *SOD1* mutation: *SOD1:c.118G* ➔ *A*, which predicts a p.E40K amino acid substitution [15]. Some Bernese Mountain Dogs have an alternate *SOD1*: *c.52A* ➔ *T* mutation associated with DM [16]. The uniformity in DM disease phenotype in dogs contrasts with substantial variability in the phenotypic onset and clinical progression of ALS which in part may be due to variability in the underlying causes, the majority of which have not yet been identified.

Companion dogs that suffer from DM are euthanized at different stages of disease progression at the owners' discretion. The ability to obtain tissues from dogs at all stages of the disease provides an opportunity to study the histopathology of disease progression that is not possible with human autopsy specimens that are for the most part obtained from donors who survived to extreme disease end-stage with the aid of artificial respiratory and nutritional assistance. By studying the central and peripheral nervous system and muscles from dogs at different stages of DM disease progression, it should be possible to gain a better understanding of the natural history of the disease. Such an understanding may allow for the identification of targets for early therapeutic interventions to aid in the treatment of at least some forms of ALS. Therefore, as a follow-up to previous studies [17–20], research was undertaken to characterize potential histopathological DM disease-related changes in the cervical spinal cord and associated nerves and muscle at different stages of disease progression.

2. Materials and methods

2.1. Sample collection and clinical classification

Tissues were dissected and preserved from companion PWCs between 2014 and 2016. Samples were acquired from 29 PWCs. There were 5 sexually intact females, 12 spayed females, 3 sexually intact males, and 9 castrated males. Median age of early disease dogs ($n = 11$) was 13 years (range 10–17 years), late disease dogs ($n = 10$) 13.3 years (range 11.5–13.3 years), and control dogs ($n = 8$) 14.5 years (range 7–16 years).

All samples utilized in the study were collected with the informed consent of the owners at the time the owners made the decision to have their pets euthanized. The studies were approved by the University of Missouri Animal Care and Use Committee. Dogs were tentatively diagnosed with presumptive DM at academic, private specialty or general practices based on clinical presentation and progression of upper and lower motor neuron signs (Table 1) [11]. Further details on sample acquisition and on the diagnoses and DM disease grade determinations are included in the Supplemental Materials section. Each affected dog was assigned to one of the disease stages listed in Table 1 based on the neurological signs the dog was exhibiting at the time of euthanasia. Dogs with stages 1 and 2 disease were classified as early stage and dogs with stages 3 and 4 disease were classified as late stage.

2.2. Motor neuron cell body numbers and SOD1 aggregate content

Portions of the C8 spinal cord segments were embedded in paraffin and 4 μm-thick sections were immunostained for SOD1 and choline acetyl transferase (ChAT, a motor neuron specific marker) and counterstained with hematoxylin as described previously [19]. In most cases, a single section from the sample was subjected to SOD1 immunostaining. To determine motor neuron density, multiple sections of the C8 cord segment were used for quantitative assessment of ChAT-positive neuron cell bodies in the Rexed lamina 9 of the anterior horns as described in detail in the Supplemental materials section.

2.3. Axon numbers in spinal nerve roots and ulnar nerve

Approximately 3 mm long slices of the dorsal (sensory) and ventral (motor) roots were dissected from the spinal nerves of cord segment C7 from each dog in which these nerve roots remained attached to the cord segment during sample collection at necropsy. In addition, a 4 to 5 mm

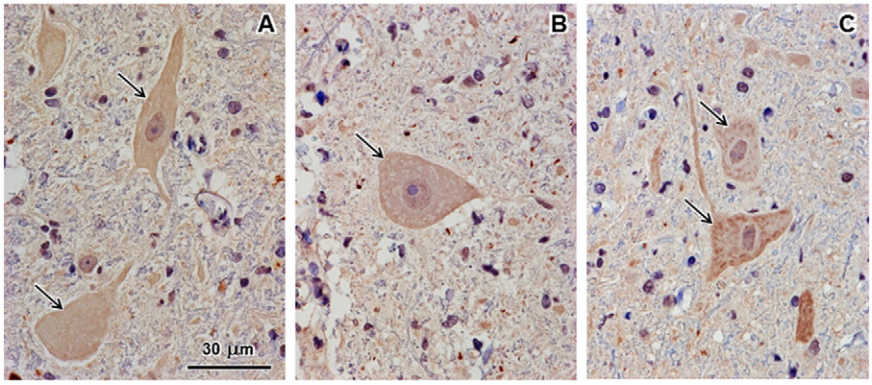

Fig. 1. Sections of the ventral horn of cervical spinal cord segment C8 immunostained to reveal SOD1 from a control PWC (A), a PWC with early stage DM (B), and a PWC with late stage DM (D). Ventral horn neurons indicated with arrows. Dark brown staining inclusions were present in only in motor neurons from PWCs with late stage disease. Bar in (A) indicates magnification of all 3 micrographs.