Effects of degenerative myelopathy on the canine genioglossus muscle

Veterinary Research Scholars Program
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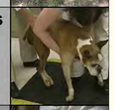
Canine DM

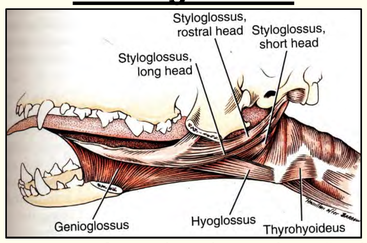
- Canine DM is an adult-onset, progressive neurodegenerative disease.
- There are 4 grades based on severity (see Table 1).

Table 1: Stage of neurological signs in DM dogs

Stage	Neurologic Signs	
1 Early	**UMN Paraparesis** • Progressive general proprioceptive ataxia • Asymmetric spastic paraparesis • Intact spinal reflexes	
2 Early	**Nonambulatory Paraparesis to Paraplegia** • Mild to moderate loss of muscle mass • Reduced to absent spinal reflexes in pelvic limbs • +/- urinary and fecal incontinence	
3 Late	**LMN Paraplegia to Thoracic Limb Paresis** • Signs of thoracic limb paresis • Flaccid paraplegia • Severe loss of muscle mass in pelvic limbs • Urinary and fecal incontinence	
4 Late	**LMN Tetraplegia and Brain Stem Signs** • Flaccid tetraplegia • Difficulty with swallowing and tongue movements • Reduced to absent cutaneous trunci reflex • Generalized and severe loss of muscle mass • Urinary and fecal incontinence	

- Increased variability in muscle fiber size and shape occurs as disease progresses.
- Furthermore, the number of type 2 muscle fibers decrease and type 1 fibers hypertrophy.

Genioglossus



- The genioglossus is necessary for prehension and swallowing food and drink in dogs. It is composed predominantly of type 2 myofibers. In latter stage disease, DM affected dogs develop dysphagia and have difficulty moving the tongue.
- The tongue muscle has not been studied in DM.

Significance

- Swallowing and tongue function are eventually affected, regardless of region of onset, in ALS patients. Respiratory failure in the late stages of the disease is the cause of death for most ALS patients, making it difficult to study tissues during disease progression.
- Canine DM parallels clinical signs of upper motor neuron onset ALS. Furthermore, dogs with DM are euthanized at various stages of disease progression, providing the opportunity to study tissues post-mortem at different disease stages.

Objective and Hypothesis

Our objective is to investigate post-mortem genioglossus samples from age- and breed size-matched controls and DM-affected dogs spanning all disease stages.

We hypothesize that the genioglossus muscle will display pathologic changes characteristic of neuromuscular degeneration with degree of severity correlating to disease stage.

Expectations

Based on findings that intercostal muscles of DM-affected dogs developed a type 1 myofiber predominance corresponding with muscle atrophy, we expect to find a similar pattern in the genioglossus. This would strengthen DM-affected dogs as a model for ALS.

Methods

Genioglossus Samples (Figure A)

- 27 samples from our archived collection of immersion-fixed tongues stored in 10% neutral buffered formalin (NBF).
- Biopsied up to 8 samples per tongue: 4 from horizontal and 4 from oblique compartments.

Tissue Processing (Figure B)

- Biopsies were stored in fresh 10% NBF for 3-5 days before paraffin processing and embedding.
- Each paraffin block contained 4 samples (2 horizontal & 2 oblique) from either the anterior or posterior region of the genioglossus.
- Paraffin blocks and antibodies given to the Veterinary Medical Diagnostic Lab for immunohistochemical staining.

Tissue Examination (Figure C)

- IHC stained slides are currently being examined by light microscopy to identify muscle fiber types.

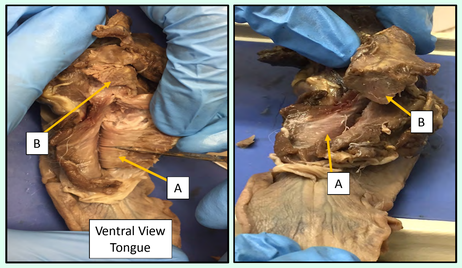


Figure A. Depiction of genioglossus dissection. Images show the orientation of the genioglossus during a dissection. The compartments of the genioglossus are (A) oblique and (B) horizontal.

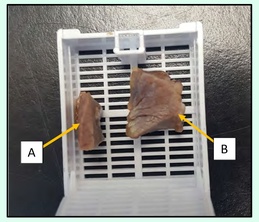


Figure B. Horizontal and oblique genioglossus sections in cassette for tissue processing. Samples collected from archived tongues were placed inside cassettes to be processed and embedded in paraffin. (A) oblique and (B) horizontal.

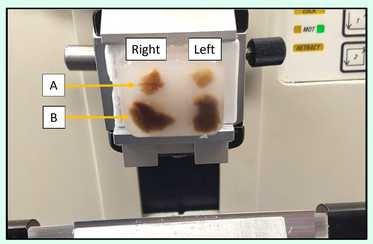


Figure C. Paraffin embedded tissues on microtome. Processed tissue samples were embedded in paraffin then sectioned at 4µm. (A) oblique and (B) horizontal.

Summary and Limitations

- BA-D5 antibody did not stain genioglossus myofibers, even at high concentrations and with various antigen retrieval protocols.
- The ideal dilution for MHC-1 antibody is 1:100 for our formalin-fixed, paraffin embedded genioglossus samples.
- Archived samples had remarkably well preserved morphology post-staining.
- Myofiber changes indicative of DM may not be recognized until terminal disease stage. In other words, tissues even at late stage classification still may not be severe enough to detect neuromuscular degeneration.
- The small sample size, which contains dogs of various breeds, may limit the statistical analysis.

Future Directions

- Quantify markers of muscle degeneration (nuclear internalization, vacuolization, reduced abundance of type 2 fibers, and reduced myofiber diameters) in the genioglossus of dogs with DM and age-matched controls.
- Determine which markers of muscle degeneration in the genioglossus readily distinguish DM from aging versus artifact.
- Determine if breed size correlates with myofiber size, such that larger dogs have larger myofibers than smaller dogs. If a correlation exists, then normalization of myofiber size may be warranted for statistical analysis.

Preliminary Results

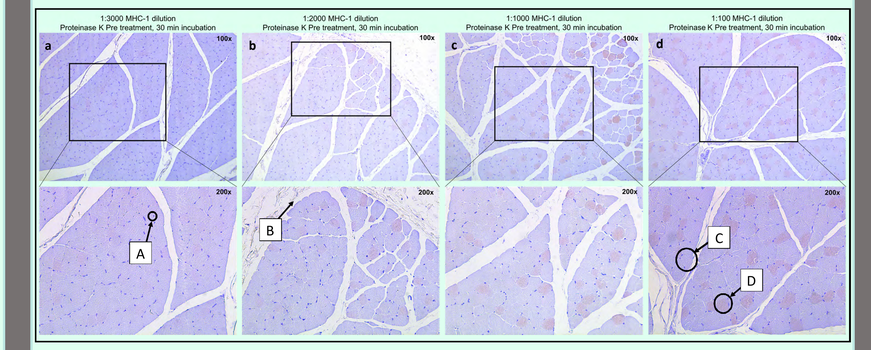


Figure 4. Immunohistochemical staining of control tissue with anti-slow skeletal myosin heavy chain antibody. This figure depicts representative cross sectional images of the horizontal compartment of the genioglossus. We used Myosin Heavy Chain 1 (MHC-1) antibody to target type 1 myofibers. Fibers positive for MHC-1 appear brown. Samples were counterstained with CAT hematoxylin. Notable tissue features include the nuclei of the myofibers (A), connective tissue (B), individual myofibers (C, D). (C) is a type 2 fiber and (D) is a type 1 fiber. All samples shown are control tissues stored in formalin for approximately 2 years.

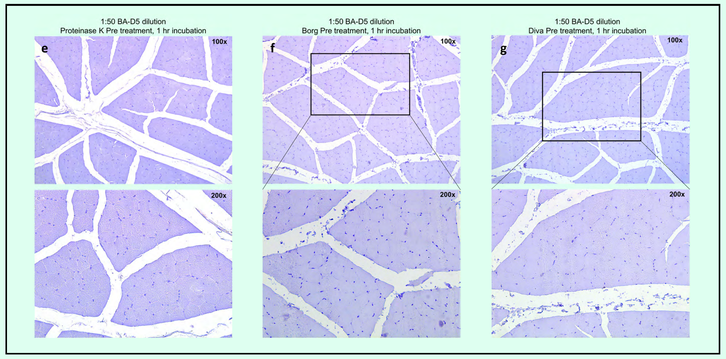


Figure 5. Immunohistochemical staining of control tissues with myosin heavy chain type 1 antibody (BA-D5). This figure depicts representative cross sectional images of the horizontal compartment of the genioglossus. We used Myosin Heavy Chain 1 (MHC-1) antibody to target type 1 myofibers. Fibers positive for MHC-1 appear brown. Samples were counterstained with CAT hematoxylin. We used BA-D5 antibody to target type 1 myofibers. Various pre-treatments were used in an attempt to optimize staining; however, there was no difference in the results. All samples shown are control tissues stored in formalin for approximately 2 years.

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