Familial Motor Neuron Disease in Rottweiler Dogs: Neuropathologic Studies

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Abstract. Two 6-week-old female Rottweiler littermates were evaluated for regurgitation, diminished growth, progressive ataxia, and pelvic limb weakness. Clinical examination indicated a progressive, diffuse, lower motor neuron disorder and megaesophagus. The pups were killed at 6 and 8 weeks of age. Lesions included central chromatolysis and swelling of the perikarya in many large motor neurons in the ventral gray matter of the spinal cord. Some involvement of red, oculomotor, trigeminal motor, and ambiguus nuclei of the brainstem was noted. Ultrastructurally, chromatolytic neurons had excess neurofilaments, and an increase in and enlargement of Golgi complexes. Wallerian-like degeneration was prominent in neuropil of spinal cord and in peripheral nerve. Clinical, histological, and ultrastructural findings were consistent with a progressive motor neuron disease.

Motor neuron diseases are characterized by progressive degeneration of the anterior horn cells of the spinal cord, brainstem, and in some cases the large motor neurons of the cerebral cortex. Skeletal muscle atrophy, decreased segmental spinal reflexes, and motor weakness are results of the diminished motor innervation. Prominent motor neuron diseases in humans are amyotrophic lateral sclerosis (ALS) and progressive muscular atrophy. ALS is a complex of disorders that compromise function of both the lower and upper motor neurons in adults. Despite extensive work to establish ALS as a slow virus or autoimmune disease, the cause remains unknown. Progressive muscular atrophies may be inherited and include the Werdnig-Hoffmann and Wohlfart-Kugelberg-Welander diseases. The infantile form, Werdnig-Hoffmann disease, is inherited as an autosomal recessive trait and is considered to be progressive and usually fatal by age three. The intermediate and juvenile forms, such as Wohlfart-Kugelberg-Welander disease, occur in childhood or adolescence, are more slowly progressive, and are inherited as autosomal recessive, autosomal dominant, or sex-linked recessive traits. Motor neuron diseases are uncommon in dogs. However, inherited neuronopathies have been described in young Brittany spaniels, Swedish Lapland dogs, and pointers. Hereditary canine spinal muscular atrophy in Brittany spaniels has been well-documented as a dominantly inherited motor neuron disease having three phenotypic variants. In the accelerated form, the clinical course resembled that of Werdnig-Hoffmann disease in infants. Pups developed weakness and skeletal muscle atrophy by 6 weeks of age and were quadriparietal by 3 months. In the intermediate form, the pups were weak by 4 to 6 months of age and quadriparietal by 2 to 3 years. In the chronic form, the weakness appeared during the first year of life and was slowly progressive. The intermediate and chronic forms of hereditary canine spinal muscular atrophy were similar to Wohlfart-Kugelberg-Welander disease in juveniles. In this paper we report the histological and ultrastructural findings of a lower motor disease in two Rottweiler littermates having similarities to the accelerated form of hereditary canine spinal muscular atrophy in Brittany spaniels.

Materials and Methods

Pup #2 was euthanized at 6 weeks of age by intravenous administration of T-61 solution (Hoechst, Inc.). Tissues from this dog were immersion fixed in 10% neutral buffered formalin. Pup #1, killed at 8 weeks of age, was anesthetized with intravenous sodium pentothal, given heparin intravenously, and then perfused with a 4% paraformaldehyde washout solution followed by 2.5% glutaraldehyde (both in 0.1 M phosphate buffer). Tissues from both animals were embedded in paraffin, sectioned, and stained with hematoxylin and eosin (HE). Selected sections of the nervous system were also stained with luxol fast blue-periodic acid-schiff, Holmes, Nissl (cresyl violet), and phosphotungstic acid procedures. These sections included several brainstem levels, upper cervical and mid-thoracic spinal cord levels, and the cervical and lumbosacral enlargements of the spinal cord. Tissues from ventral horn of the spinal cord, spinal nerve roots, and sciatic and peroneal nerves from the perfusion-fixed animal (pup #1) were post-fixed in 2% osmium tetroxide, dehydrated, embedded in epon epoxy resin, sectioned at 1 μm and stained with toluidine blue and safranin. Sections selected from these were cut at 80 nm, stained with uranyl acetate and lead citrate and viewed in a JEOL transmission electron microscope at 80 kV. Immersion-fixed spinal cord from a pup without neurological signs served as a control. Fixatives for the latter were 10% neutral buffered formalin.
Fig. 1. Pup #1. Chromatolytic large motor neuron, spinal cord ventral horn. Perikaryal cytoplasm enlarged; nucleus displaced peripherally. Nissl substance diminished in staining intensity, concentration, and number. Epoxy section, toluidine blue-safranin.

Fig. 2. Pup #1. Myelinated nerve fiber in spinal cord ventral horn neuropil, swollen debris-containing axonal segment (arrow). Myelin debris consistent with Wallerian-like degeneration immediately adjacent to fiber (arrowhead), possibly representing focal breakdown of the latter. Epoxy section, toluidine blue-safranin.

Fig. 3. Pup #1. Electron-micrograph of cell body of chromatolytic large ventral horn neuron, spinal cord. Adjacent myelinated nerve fiber undergoing Wallerian-like degeneration (arrow).

Results

Two pups from a litter of seven Rottweiler dogs died soon after birth and were not available for examination. Of the animals included in this study, one female pup (pup #1) regurgitated milk soon after birth. A second female pup (pup #2) began to regurgitate food at 4 weeks of age. The two affected animals were more clumsy and much smaller than their littermates. They were examined at 6 weeks of age because of progressive pelvic limb weakness, decreased growth, and regurgitation of food.

Cranial nerve examinations were normal except for a reduced swallow reflex in pup #1. Both pups demonstrated a slight head tremor as they approached food. Pup #1 was ambulatory but tired easily and had mild pelvic limb weakness while pup #2 was quadriparetic and non-ambulatory. Both animals had mild pelvic limb muscle atrophy, reduced segmental spinal reflexes except for the flexor and perineal reflexes, and cervical muscle weakness as evidenced by the inability to lift the head. Sensation appeared normal.

Esophagrams using liquid barium sulfate revealed a dilated esophagus in both pups. Laboratory analyses on pup #1 were normal except for slight neutrophilia, mild hypoglobulinemia, and increased creatine kinase values. Pup #2 was killed at 6 weeks of age. Pup #1 was observed for 2 weeks and then killed at 8 weeks of age because the pelvic limb weakness progressed rapidly to severe quadriparesis. Clinical findings are reported in detail elsewhere.16

Lesions were consistent in both pups and are described together with the caveat that the study of epoxy embedded sections and electron microscopy were only performed on pup #1. Grossly, muscle mass was reduced and the intrathoracic portion of the esophagus was dilated.

Foci of single or groups of atrophic skeletal muscle fibers were present. In addition there were some hypertrophic and regenerating fibers as well as occasional necrotic ones. Light microscopic examination of the spinal cord revealed lesions at all levels studied. There was central chromatolysis and marked swelling of the perikarya in many (but not all) large (alpha) motor neurons in the ventral horns of the gray matter. These were more prevalent in the two intumescences of the cord supplying motor innervation to the limbs. Detectable Nissl substance frequently had diminished staining intensity and was often present in the periphery of the nerve cell body but was absent from more central regions. In 1 μm thick epoxy sections the chromatolytic neuronal perikaryon was noted to be made up of expansive, pale staining cytoplasm containing...
Fig. 4. Pup #1. Spinal cord ventral horn. Intersecting bundles of excess neurofilaments separate Nissl substance and other organelles in periphery of chromatolytic large motor neuron cell body.

Fig. 5. Pup #1. Prominent Golgi complexes containing numerous vesicles concentrated in perikaryal cytoplasm of chromatolytic neuron from spinal ventral horn. Bundles of neurofilaments separate Golgi complexes, other organelles.

scattered tiny basophilic foci (Fig. 1). The nucleus of involved neurons was displaced, often located beneath the plasma membrane (Fig. 1). Nucleoli were prominent and dark-staining while chromatin appeared dispersed. Occasional markedly distended chromatolytic neuronal cell bodies were vacuolated. Necrosis of motor neurons and some neuronophagia were evident in affected regions.

The neuropil of the ventral horn contained focal axonal swellings. These appeared as elongated, segmental distensions along the course of neurites in the Holmes preparations. No evidence of involvement of proximal portions of axons of chromatolytic neurons was present. One micrometer thick sections of epoxy embedded-material showed swollen axons with thin myelin sheaths and varying quantities of stainable axoplasmic debris (Fig. 2). Occasional myelinated fibers undergoing Wallerian-like degeneration were seen in the neuropil and white matter (Fig. 2).

Cross-sections of the sciatic and peroneal nerves revealed moderate numbers of myelinated fibers undergoing Wallerian-like degeneration. These included occasional swollen fibers with axoplasmic debris. There appeared to be a greater proportion of degenerating fibers in the peroneal (distal) than in the sciatic (proximal) levels of peripheral nerve. Sections of the ventral and dorsal nerve roots also revealed Wallerian-like degeneration.

Swollen, chromatolytic nerve cell bodies were noted in some cranial nerve nuclei of the brainstem. These included the oculomotor, trigeminal motor, and ambiguus nuclei. In addition, occasional involvement of neurons in the red nucleus was also seen. Sections of other regions such as cerebral and cerebellar cortices, thalamus, and hippocampus revealed no such lesions. The intrinsic innervation of the esophagus appeared unaffected.

The chromatolytic neurons noted above had perikaryal pallor and enlargement, and loss of central Nissl bodies when viewed by electron microscopy (Fig. 3). Cytoplasm in these regions was pale and contained increased neurofilaments which sometimes formed intersecting bundles (Fig. 4). Within this meshwork were small residual Nissl bodies, neurotubules, dense bod-
Fig. 6. Pup #1. Cross-section of peroneal nerve. One myelinated fiber (arrow) has intra-axonal aggregation of mitochondria and dense bodies. Other Schwann cells containing debris indicative of advanced Wallerian-like degeneration (arrowheads).

ies, empty or debris-laden membrane bound vesicles, and mitochondria. The latter were prevalent in some cells. Some prominence of Golgi complexes was seen in chromatolytic neurons. These organelles were frequently present in increased numbers, and contained more extensive cisternal and vesicular aggregates than in the control pup (Fig. 5). Both coated and smooth-surfaced vesicles were noted in the enlarged Golgi complexes. The neuronal nucleus was often displaced peripherally in the cell body and had a prominent nucleolus and dispersed chromatin (Fig. 3). Synaptic boutons were on the neuronal surface.

Many swollen axon profiles with attenuated myelin sheaths were seen in the neuropil of the ventral horns of the spinal cord. A small number of these axons were distended with maloriented neurofilaments. The others contained axoplasmic granular material, vesicles, degenerating organelles, membranous bodies, and debris consistent with Wallerian-like degeneration (Fig. 3). Numerous astrocytic filament-filled processes were seen in the ventral horns, associated with the neuronal lesions.

Degenerating myelinated nerve fibers in sciatic and peripheral nerves were confirmed by electron microscopy (Fig. 6). Earlier stages were manifest by intraxonal dense bodies and debris (Fig. 6). Later there was breakdown of axons and their myelin sheaths within phagocytes present inside the Schwann cell basal lamina (Fig. 6). Some of these phagocytes were Schwann cells.

Discussion

We describe a rapidly progressive lower motor neuron disease in two female Rottweiler littermates that demonstrated progressive weakness and skeletal muscle atrophy initially noted at about 4 weeks of age. Lesions were prominent in lower motor neurons and resembled those of similar, genetically determined canine syndromes such as hereditary spinal muscular atrophy in Brittany spaniels and hereditary neuronal abiotrophy in Swedish Lapland dogs, as well as human infantile spinal muscular atrophy (Werdnig-Hoffmann disease).

The most evident lesions in the cell bodies of large motor neurons in our cases were central chromatolysis, excess accumulation of neurofilaments, and hypertrophic and hypertrophic Golgi complexes, with progression to neuronal necrosis. Swollen axons were prominent in regions of affected nerve cell bodies. While small numbers of these were distended with maloriented bundles of neurofilaments, the majority of such swollen axons contained axoplasmic debris and degenerating organelles, and probably represented stages of Wallerian-like degeneration of the fiber. The latter process was better developed in sections of peripheral nerve where degenerating nerve fibers were more prevalent in distal than proximal levels. This suggests a "dying-back" pattern of nerve fiber degeneration in these Rottweilers as in another form of canine motor neuron disease characterized by an inherited defect in lipid metabolism. It may be a reflection of a relatively slowly evolving inability of affected lower motor neurons to maintain their axonal processes.

The pattern of spinal cord ventral horn lesions in the Rottweiler pups differed slightly from those described in hereditary canine spinal muscular atrophy of Brittany spaniels. In the accelerated form of the latter, central chromatolysis of motor neurons and perikaryal accumulation of neurofilaments were seen, as in our dogs. However, we did not see the frequent, massive accumulations of maloriented axonal neurofilaments in proximal portions of axons described in accelerated and intermediate forms of hereditary canine spinal muscular atrophy. A defect in slow axonal transport has been shown to account for these perikaryal and proximal axonal neurofilamentous aggregates in the latter entity.

Central chromatolysis is seen in conditions other than the motor neuron diseases described above. Prominent among these is the axon reaction, a perikaryal response to transection of the axon. The changes in our Rottweiler pups resembled those seen in the axon reaction, except that the separation of axosomatic synapses from affected neurons seen in that condition was not present. In addition, enlargement of Golgi complexes and increased neurofilaments seen in our pups were not typical of the axon reaction. The neuronal chromatolytic change in the pups we report is likely a degradative change in contrast to the anabolic
properties of chromatolytic neurons in the axon reaction.2

The presence of some degenerating myelinated axons in the spinal dorsal roots suggests that populations other than lower motor neurons may be also involved although less extensively. Lesions of sensory neurons have been seen in Werdnig-Hoffmann disease in man,3,17 and in an inherited neuronopathy of Swedish Lapland dogs.15 In the latter condition chromatolysis of neurons was considered secondary to axonal degeneration.

Neurons of the nucleus ambiguus were involved, suggesting a basis for the megaesophagus, particularly in the absence of lesions of the intrinsic innervation of the viscus. Although the hypoglossal and trigeminal motor nuclei were affected, clinical signs relating to these were not evident. Upper motor neurons residing in the red nucleus showed chromatolytic changes in a few cells, but long tract degeneration was not detected, possibly reflecting the small number of affected cells in this nucleus.

The motor neuron disease in these two Rottweiler pups is not the same as the neuroaxonal dystrophy recently described in this breed.7 The latter entity is characterized clinically by slowly progressive cerebellar signs and pathologically by membrane-filled swellings ("spheroids") of distal axonal regions.

References


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