Postnatal cerebellar cortical degeneration in Labrador Retriever puppies

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Postnatal cerebellar cortical degeneration in dogs is believed to be caused by an intrinsic defect in cortical neurons that leads to their premature death (1). The disease is characterized clinically by progressive cerebellar ataxia that usually develops within the first year of life. There is no treatment. Prognosis is generally poor, but lifespan varies according to breed. The pathological findings are dominated by changes in the cerebellar cortex and include various degrees of Purkinje cell loss, granule cell loss, and thinning of the molecular layer (1). Lesions in the brain stem nuclei and spinal cord are also seen in some breeds (2,3). The cause of postnatal cortical degeneration is unknown, although it is suspected to be a biochemical defect in Purkinje and sometimes granule cells that leads to their premature degeneration (1,4). The disease has been observed most commonly in the Kerry Blue Terrier, Gordon Setter, and Rough Coated Collie of Australia, and studies have shown that it is an inherited autosomal recessive disease in these breeds (1-3,5,6). Less commonly affected breeds are the Airedale Terrier, Finnish Harrier, Swedish Lapland Dog, Brittany Spaniel, Bern Running Dog, Jack Russell Terrier, Smooth Haired Fox Terrier, and Beagle (1,7,8). This report is the first clinical and pathological description of cerebellar cortical degeneration in the Labrador Retriever.

The subjects of our study were three Labrador Retriever puppies, two male and one female, from a litter of 12 born January 1, 1988. The puppies were raised by a private breeder in New Jersey and placed in private homes at eight weeks of age. The puppies were 9-17 weeks old when referred to the Animal Medical Center. Histories and pedigrees were provided by the breeder and referring veterinarian. The following diagnostic tests were performed on one or more of the puppies: complete blood count (dogs 1, 2, and 3), serum biochemical analysis (dogs 1, 2, and 3), urine analysis (dogs 1 and 3), fecal flotation (dog 3), cerebrospinal fluid analysis and bacterial culture (dogs 1, 2, and 3), canine distemper titer of serum and cerebrospinal fluid (dogs 2 and 3), serum toxoplasmosis titer (dog 3), and serum *Cryptococcus* capsular antigen titer (dog 3). Abdominal radiography (dog 1), electroencephalography (dog 3), and computed axial tomography (dog 3) were also done.

The dogs were anesthetized with thiopental and euthanized with pentobarbital at one (dog 1), two-and-one-half (dog 2), and four (dog 3) weeks after the onset of signs, and complete necropsies were done. The brain and spinal cord and samples of peripheral nerves and all organs were submitted for histological examination. The tissues were fixed in buffered 10% formalin, processed routinely, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (H&E). Sections of the brain and spinal cord were also stained with luxol fast blue (LFB). Additional sections of the cerebellum were examined immunohistochemically with primary antibodies directed at neurofilament (NF, Biogenex Laboratories, Dublin, California, USA) and glial fibrillary acidic protein (GFAP, Dako Corporation, Santa Barbara, California, USA). Standard avidin-biotin-peroxidase complex (ABC) immunoperoxidase techniques were used (9). Sections of cerebellum from a 12-week-old dog without evidence of neurological disease were prepared similarly to serve as controls.

All three puppies were normal at eight weeks of age when they received their first vaccinations (canine distemper, parvovirus, adenovirus type 2, and parainfluenza). The puppies' parents were clinically normal and were vaccinated before breeding. No common ancestry was revealed by tracing back four generations. This was the bitch's first litter, and her pregnancy and whelping were normal. There was no known neurological disease in the previous offspring of the stud dog. Three out of 12 puppies died within the first two weeks of life from unknown causes. The remaining six dogs were in good health at one-and-one-half years of age according to the owners.

The onset of clinical signs was at nine (puppy 1), 11 (puppy 2), and 17 (puppy 3) weeks of age (mean, 12 weeks). All three puppies had similar clinical signs, which worsened rapidly. In the first two days after onset of signs they exhibited a mildly ataxic gait affecting the pelvic limbs, which worsened on exercise. The gait was characterized by a delayed onset of movement, exaggerated protraction of the pelvic limbs, and a marked abduction of the pelvic limbs while turning. By the fourth day, they had striking truncal ataxia and a base wide stance. By one week after the onset of clinical signs, the thoracic limbs were similarly

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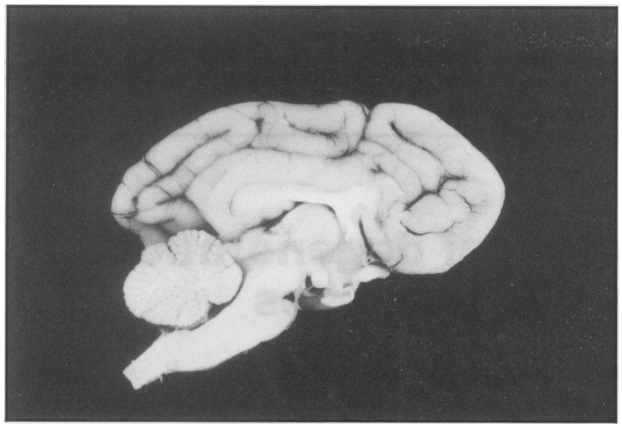

Figure 1. Midsagittal section of the brain, dog 2. Cerebellum is slightly small. Cerebellar folia in the rostral vermis are thinner than normal and sulci are wide.

Figure 2. Cerebellar folia of the rostral vermis, dog 3. Number of Purkinje cells is abnormally low. A solitary Purkinje cell (arrow) can be seen. There is extensive vacuolization in areas of Purkinje cell loss, and granular layer cellularity is reduced (H&E).

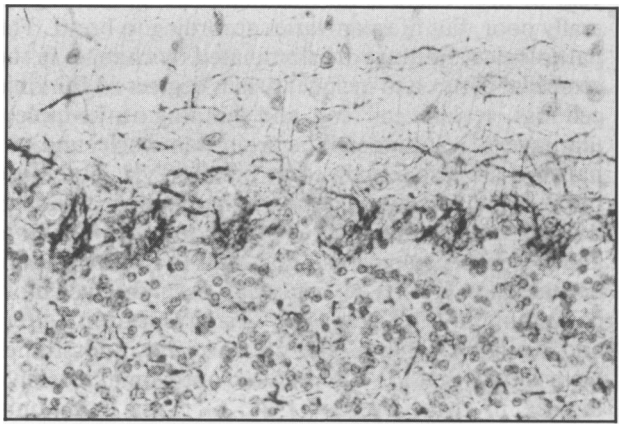

Figure 3. Cerebellar folia of the rostral vermis, dog 3. Notice neurofilament-positive baskets with no Purkinje cells (ABC immunoperoxidase).

affected, and the dysmetria was severe enough to cause the puppies to fall after a couple of meters of ambulation. All limbs were hypermetric when subjected to postural reaction testing. The puppies had intention tremors and wide head excursions. The developed a positional changing nystagmus, characterized predominantly by a downbeat vertical fast phase. An oscillatory component was also observed intermittently. A menace gesture resulted in diminished closure of the eyelids bilaterally. Puppy 1 was euthanized one week after onset of clinical signs. By two-and-one-half weeks, puppies 2 and 3 were unable to walk without assistance. By four weeks, puppy 3 would fall repeatedly, even with assistance.

Results of all diagnostic laboratory tests were within normal limits.

On postmortem examination, gross and histological lesions were limited to the cerebellum in all three dogs. Grossly, the cerebellum was smaller than normal. There was a gradual reduction in size ranging from barely perceptible in dog 1 to slightly smaller than normal in dog 3. In all three, the small size of the cerebellum was most apparent in the rostral portions of the vermis (lingulata cerebelli and lobulis centralis) when the brain was cut midsagittaly (Figure 1). Here, the cerebellar foliar were visibly thinner than normal and the sulci were wide. The remaining portions of the cerebellums were of normal shape and proportion.

Histological lesions were qualitatively similar in the three cerebellums. The lesions differed only in severity, increasing progressively from dog 1 (least severe) to dog 2 (intermediate) to dog 3 (most severe). The cerebellar alterations were diffuse, and essentially all cerebellar folia were affected. However, there was consistent regional variation in severity; folia of the rostral vermis were most severely affected in all three dogs.

The dominant alteration in the three cerebellums was low numbers of Purkinje cells. Cerebellar folia, which had extensive Purkinje cell loss, were thinner than normal and the sulci were wide. Markedly low numbers of Purkinje cells were seen in the rostral vermian folia of dogs 1 and 2, while the rostral vermian folia of dog 3 were nearly devoid of Purkinje cells with only occasional solitary cells remaining (Figure 2). In less severely affected folia, Purkinje cell loss was also extensive, but solitary Purkinje cells and groups of several Purkinje cells separated by zones of Purkinje cell loss were more numerous. Occasional Purkinje cells were degenerate and some were necrotic. Degenerate Purkinje cells were characterized by various degrees of vacuolar change; necrotic Purkinje cells were shrunken and intensely eosinophilic, and their nuclei were pyknotic. The loss of Purkinje cells resulted in small vacuolar foci (empty baskets) and clefts in the Purkinje cell layer. In areas of the most severe Purkinje cell loss, there was moderate to severe reduction in the thickness and cellular density of the granular layer (Figure 2). In addition to loss of Purkinje cells, occasional heterotopic Purkinje cells were seen. Heterotopic Purkinje cells were most often found in the granular layer, and, less frequently, in the subjacent foliar white matter. Swollen eosinophilic axons (presumed to be Purkinje cell axons) were seen multifocally in both the granular layer and foliar white matter. Mild to moderate vacuolar change, gliosis, and loss of myelin staining characterized the foliar white matter. Prominent, swollen dendrites of Purkinje cells were occasionally seen in the molecular layer. Minimal to mild central chromatolysis was observed in neurons of the cerebellar nuclei.

Immunohistochemical staining for neurofilament (NF) revealed marked positive staining of baskets in the Purkinje cell layer (Figure 3). Remaining Purkinje cell bodies were negative for NF. Baskets were evenly distributed in the Purkinje cell layer throughout the cerebellar folia, regardless of whether Purkinje cells were retained within the baskets or lost (empty baskets). Neurofilament staining intensity was greater in empty baskets compared with those in which Purkinje cells were retained. Immunohistochemical staining for NF in the control cerebellum revealed NF-positive baskets containing normal Purkinje cells distributed in the Purkinje cell layer similar to the baskets of the affected cerebellums. The Purkinje cell bodies in the control cerebellum were also negative for NF. Intensity of staining of baskets in the control cerebellum was similar to that of basket cells with retained Purkinje cells in the affected cerebellums. Immunohistochemical staining for glial fibrillary acidic protein (GFAP) revealed the presence of mild to moderately increased numbers of GFAP-positive astrocytes in the foliar white matter compared with the control cerebellum, confirming the presence of gliosis.

Although a genetically inherited disease is strongly suspected in the dogs of this study, we cannot draw this conclusion as yet because of the small number of dogs examined and the lack of selective breeding studies

The clinical manifestations of this disease that developed in our Labrador Retrievers are similar to, but distinct from, those seen in the Kerry Blue Terrier, Australian Rough Coated Collie, and Gordon Setter (1-3,5,6). Our clinical findings differed most markedly with what is seen in the Gordon Setter. In our puppies, the onset of disease was much earlier and the progression was more rapid. Furthermore, unlike the Gordon Setter, the pelvic limbs were affected first with rapid progression to the thoracic limbs. These clinical findings are more consistent with what is seen in the Australian Rough Coated Collie and Kerry Blue Terrier. The nystagmus seen in all three puppies was notable. It has not been reported in the Kerry Blue Terrier or Australian Rough Coated Collie and is an inconsistent finding in the Gordon Setter.

Grossly and histologically, the cerebellar lesions that developed in these Labrador Retrievers increased in severity with increasing age of the dogs and progression of clinical signs. Although all regions of the cerebellar cortices were affected in each dog, the most extensive Purkinje cell loss, granular layer diminution, and foliar white matter gliosis was in the rostral vermis of all three (especially the lingulata cerebelli and lobulus centralis). This area of the cerebellum primarily controls function of the trunk and pelvic limbs (10). Thoracic limb function is partially controlled by the culmen and declive lobules (lobules IV, V, and VI) (15). In Gordon Setters, these lobules are more severely affected. In our dogs, the culmen and declive lobules were affected in the late stages of disease. Retrograde degeneration of brain stem nuclei and spinal cord white matter is seen in Kerry Blue Terriers and Rough Coated Collies. Although this was not observed in our dogs, perhaps similar lesions would have developed if they had lived longer.

Postnatal cerebellar cortical degeneration is an inherited autosomal recessive disease in Kerry Blue Terriers, Rough Coated Collies, and Gordon Setters (1-3,5,6). Although a genetically inherited disease is strongly suspected in the dogs of this study, we cannot draw this conclusion as yet because of the small number of dogs examined and the lack of selective breeding studies. In sporadic cases of cerebellar degeneration, paraneoplastic, toxic, and infectious causes should also be considered. Although neoplasia was not observed in our puppies, human beings with certain malignancies can develop a paraneoplastic syndrome with secondary cerebellar degeneration (4). Also in humans, alcohol, phenytoin, and mercury toxicity can cause selective Purkinje cell degeneration (11). The three puppies in this study had no known toxin exposure, pre- or postnatally. Infection seems unlikely due to lack of inflammatory response as well as negative serological and bacteriological test results. However, certain viruses, such as a murine type C RNA virus, can cause neuronal degeneration without inflammatory changes, so viral causes cannot be excluded (1). By studying more affected litters, we may be able to further characterize this disease process and determine if cerebellar cortical degeneration is a genetic disease in Labrador Retrievers.

Acknowledgments

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