Neuronal Ceroid-Lipofuscinosis in Older Dachshunds

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Abstract. A lysosomal storage disease with accumulation of periodic acid-Schiff- and Sudan black-positive autofluorescent granules in neurons occurred in one 5½- and one 7-year-old dachshund. Ultrastructurally, the storage material consisted of membranous material arranged in stacks and fingerprint patterns. The disease was defined as ceroid-lipofuscinosis, and resembled a previously reported case in an adult dachshund.

Since enzyme defects and resulting metabolic diseases in animals often are identical to those in man, animal models of storage diseases play an important role in biomedical research [6, 7, 12]. Ceroid-lipofuscinosis is a lysosomal storage disease associated with accumulation of lipofuscin and its related pigment ceroid [1, 7]. The molecular pathogenesis of the disease is unknown, but it is thought that the storage product is derived from peroxidation of poly-unsaturated lipids followed by their crosspolymerization [1, 7]. In man, ceroid-lipofuscinosis occurs in young individuals as infantile, late infantile and juvenile subtypes (Batten’s disease), and also in adults (Kuf’s disease) [11]. Animal counterparts of juvenile ceroid-lipofuscinosis have been described in dogs and cats [5, 8–10, 14]. The best-known model is the so-called juvenile amaurotic idiocy in English setters [8–10]. The only animal case of adult ceroid-lipofuscinosis, similar to Kuf’s disease, has been described recently in an adult dachshund [2]. We report a neuronal storage disease in two older dachshunds that differed in some respects from that case. Histological, histochemical and ultrastructural observations, however, proved the storage material to be ceroid-lipofuscin.

Case Histories

Dog 1 was a 7-year-old female longhaired dachshund that had chronic heart failure for several years. For a year before death, the dog had occasional epileptic convulsions that did not respond to anticonvulsive therapy. During the last months of life, personality changes, dullness, polyphagia and polydypsia occurred. The dog was killed. At necropsy, a markedly thickened and practically immobile mitral valve was found. The brain appeared atrophic with marked dilatation of the ventricular system.

Dog 2 was a 5½-year-old male longhaired dachshund that had been treated by the
owner with Mexaform® (CIBA-GEIGY, Basel, Switzerland) (an oxiquinoline compound) for diarrhea. The following day the dog paced compulsively and had several severe generalized convulsions. Its condition deteriorated rapidly into stupor, severe dyspnoea, hematuria and death one day later. Necropsy showed multiple hemorrhages in most organs, pulmonary oedema and a focal area of acute pneumonia. The central nervous system was unaffected.

**Materials and Methods**

The brains, spinal cords, and samples of extraneural tissues from both dogs were fixed in 10% formol saline. Representative blocks were processed for paraffin embedding, cut at 4 μm and stained with hematoxylin and eosin (HE). Special stains on paraffin sections included luxol fast blue-cresyl echt violet, periodic acid–Schiff (PAS), alcian blue, toluidine blue and Sudan black B; on frozen sections, a glycogen stain, PAS, Sudan III, Sudan black B and acid-fast stains. Unstained frozen sections were examined with an ultraviolet microscope. Samples were taken from the formalin-fixed Ammon’s horn of dog 1, washed overnight in buffer, postfixied in 1% osmium tetroxide for 1 hour, dehydrated in graded ethanols and embedded in Spurr low viscosity embedding medium. One-micrometer sections were stained with toluidine blue and examined microscopically. Ultrathin sections were cut from suitable areas, stained with uranyl acetate and lead citrate and examined with the electron microscope.

**Results**

The central nervous systems of both dogs showed marked changes. The lesions were more severe in dog 1 than in dog 2, but the topographical distribution and appearance were identical in both dogs. Many neuronal perikarya were distended with fine granular storage material and had peripherally located nuclei (fig. 1). Between these enlarged neurons were smaller rounded ones with homogeneous cytoplasm and pyknotic nuclei (fig. 1). Neuronal lesions were found in most areas of the central nervous system but were most numerous in the pyriform cortex, Ammon’s horn, amygdaloid nuclei, claustrum, putamen and cerebellar cortex.

In the cerebellum, especially in the lateral parts of the hemispheres, there was severe accumulation of storage material in the granule cells with resulting atrophy of the granule cell layer but relative sparing of the Purkinje cells (fig. 2). Focal areas of neuronal cell loss were apparent in the large pyramidal cell areas in the Ammon’s horn and in the superior olivary nuclei. The storage material was colorless to faintly yellow in unstained sections, stained yellow to bright red with HE, dark blue with luxol fast blue and orange with Sudan III. The material did not stain for glycogen, was alcian blue-negative and not metachromatic with toluidine blue. It was red to magenta with PAS and black with Sudan black B both on frozen and paraffin sections. It stained very faintly acid-fast, and had a brilliant yellow-green autofluorescence.

In dog 2, anoxic neuronal necrosis with microvascular proliferation as described in oxyquinoline intoxication [4, 11] was superimposed on the neuronal storage changes in the Ammon’s horn and lobus pyriformis. Of the extraneural tissue in dog 1, only lung, heart, spleen and liver were available for histologic examination and
were found to be free of storage material. Ceroid-lipofuscin was found in the neurons of the gastrointestinal tract of dog 2. There was no storage material in the lung, liver, pancreas, spleen, lymph nodes, adrenal gland, kidney and urinary bladder.

Ultrastructural examination of the Ammon’s horn of dog 1 showed many osmio-philic membrane-bound bodies in neurons (fig. 3). These cytosomes displaced the other cell organelles and disrupted the normal clumping pattern of the rough endoplasmic reticulum. The cytosomes contained some granular and amorphous, but mostly membranous, material, much of which was ill defined and fuzzy (fig. 4). Many trilaminar and pentalaminar membranes were seen (fig. 4). The membranous material was arranged in small crescent-shaped or parallel stacks (fig. 4, 5) and fingerprint patterns (fig. 5). Cytosomes consisting entirely of parallel stacks of laminated membranes (zebra bodies) were rare. The combination of granular material with a few stacks of membranes within the same cytosome was common. The
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surrounding neuropil was strongly vacuolated and contained many fibrillary astrocytes (fig. 3). There was no storage material in glial cells or in mesenchymal elements.

Discussion

That the lysosomal storage material in our dachshunds was ceroid-lipofuscin was suggested by its light-microscopic appearance in routinely stained sections, where it resembled lipofuscin pigment as described in old age [3], and by its tinctorial properties with special stains [1, 8–10]. The brilliant autofluorescence of the storage material is another characteristic of ceroid-lipofuscin, thought to be due to the presence of a retinoyl complex in the ceroid component [15]. Furthermore, the ultrastructural appearance of some cytosomes containing granular material with stacks of laminated material resembled the fine structure of lipofuscin in normal nerve cells [13]. The other types of cytosomes in our dogs resembled those reported in human and canine ceroid-lipofuscinosis [1, 9, 10]. The general histologic appearance of the storage disease in our dogs was comparable to that in other dogs with
ceroid-lipofuscinosis [2, 5, 9, 10], but there were some differences in distribution of the lesions. Especially striking in our dogs was the marked accumulation of ceroid-lipofuscin and atrophy of the cerebellar granule cell layer with relative sparing of the Purkinje cells. This type of cerebellar atrophy has been seen often, in human ceroid-lipofuscinosis [1] and in English setters with amaurotic idiocy [8]. In two Chihuahua dogs [14] and in one dachshund with ceroid-lipofuscinosis [2], neuronal storage resulted in marked Purkinje cell atrophy with preservation of the granule cells. Such differences in topographical distribution of the lesions may reflect some chemical differences in the storage material. This would be consistent with the opinion that the ceroid-lipofuscinoses in man are neither chemically nor genetically homogeneous [10]. Such heterogeneity also may be reflected by the variation in ultrastructural findings in ceroid-lipofuscinosis in man [2]. In English setters with familial amaurotic idiocy, a reproducible and genetically defined condition, however, ultrastructural differences in the morphology of the storage product between individuals have been noted [10]. Ultrastructural features therefore do not seem to be reliable criteria to establish identity or nonidentity of ceroid-lipofuscin storage diseases. The fine-structural variation in relative quantity of the various more or less typical cytosomes between our cases and a case of adult ceroid-lipofuscinosis previously reported in a dachshund [2] (much larger numbers of zebra bodies in the latter) does not exclude their possible identity.

Clinical features also may help to define subclasses of ceroid-lipofuscinosis [2].
Unfortunately it is impossible to compare closely the clinical aspects in our dogs with those in the previously reported dachshund, since our dogs were not examined neurologically. The disease in our first dog progressed slowly, as did that in the previously reported case [2]. It is remarkable that, although considerable neuronal storage with cerebellar atrophy was found in our second dachshund, no neurologic dysfunction had been apparent to the owner before the dog suffered from acute Meñaform intoxication. The occurrence of severe generalized seizures together with neuronal necrosis of the piriform lobe and Ammon’s horn in dogs after ingestion of oxyquinoline has been well documented [4, 11].

Considering similarities in age and breed of dogs affected, and in many important morphologic features, we believe that the disease here presented is closely related to the lysosomal storage disease previously reported [2] and that it confirms the existence of ceroid-lipofuscinosis in the adult dachshund. Whether this disease has a genetic metabolic basis, or may be acquired, remains an open question. Since neither the selective accumulation of storage material nor resulting tissue destruction, as in the cerebellar cortex, has been known to accompany old-age changes in the canine brain [3], we do not believe that the lesions in these dachshunds can be considered an accelerated senile change. Ceroid-lipofuscinosis in adult dachshunds may be a potential animal model for Kufs’s disease in man.

References


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