Histological Characterization of Joint Lesions in a Feline Model of Sandhoff Disease

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Introduction: Lysosomal storage disorders (LSDs) are characterized by abnormal accumulation of cellular waste due to defects in lysosome function; neurons are most severely affected in many LSDs. GM2 gangliosidoses, a subgroup of the disorders including Sandhoff disease, are caused by a deficiency in the enzyme β-N-acetylhexosaminidase (Hex) and present with severe neurological abnormalities in humans, resulting in death at an early age (1). Advances in treatments to prolong lifespan of affected individuals have allowed time for the development of poorly understood skeletal abnormalities that become apparent later in the course of the disease (2,3). The present study focused on a feline model of infantile Sandhoff disease (4,5) and aimed to characterize the histological changes occurring in the distal femur in these animals.

Methods: Distal femora were collected from eleven cats, six controls (no deficiency in Hex; 4 females, 2 males) and five affected (known deficiency in Hex; 2 males, 3 females). Animal ages ranged from 5-6 months (2 affected, 4 controls) to 21-37 months (3 affected, 2 controls). Three cats from the affected group had been treated with an intracerebral injection of adeno-associated virus vectors expressing the alpha and beta subunits of feline Hex, a treatment that significantly increased the lifespan by reducing the neurological effects of the disease, allowing the development of clinically apparent skeletal abnormalities (dysplasias and luxations). No obvious differences between skeletal tissues from treated vs. untreated affected cats had been observed in a previous microCT study; therefore, tissues from these animals were combined into a single affected group for the present study. All samples were fixed in 10% neutral buffered formalin and decalcified in 10% EDTA; locations for histological evaluation were chosen on previous microCT findings in these animals. Approximately 3-mm thick slabs were cut 1) through the center of the weight bearing area of the femoral condyles, perpendicular to the articular cartilage, in a plane that extended through the proximal limit of the trochlea and 2) in an axial plane (perpendicular to the articular cartilage) through the proximal and distal thirds of the trochlea. Samples were processed routinely, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin.

Results: In sections from the youngest affected cats (5 months of age), all chondrocytes were diffusely vacuolated. The articular-epiphyseal cartilage complex was greatly thickened (~1500 microns in affected vs. ~500 microns in controls), due to marked retention of epiphyseal cartilage. Multiple cartilage canal vessels were present in the retained epiphyseal cartilage of affected cats but were absent in controls. Multiple variably-sized foci of retained cartilage also were present within the trabecular bone throughout the epiphyseal/metaphyseal bone but were absent in controls. The bone marrow exhibited...
decreased cellularity compared with controls. The older affected cats (treated by gene therapy)
exhibited retained growth plate cartilage (absent in controls); the calcified cartilage/deep epiphyseal
cartilage was retained and thickened, sometimes forming large plugs that extended into the
subchondral bone. Retained cartilage also contained multifocal areas of matrix degeneration. Retained
foci of cartilage within trabecular bone were present, but were much reduced in number compared with
sections from the younger animals. Lesions of osteoarthritis, characterized by areas of
fibrillation/degeneration accompanied by chondrocyte clones in articular cartilage and periarticular
osteophytes were present in the femoral condyles of the oldest affected animal (31 months of age).
Cellularity of the bone marrow was moderately to markedly increased compared with controls. The
distal femora of the older control cats exhibited normal histological features. All affected cats exhibited
a decreased amount of trabecular bone, particularly evident in the younger cats, and the shape of the
distal femur was altered compared with controls.

**Discussion:** We conclude that Hex deficiency in cats results in marked chondrocyte vacuolation and,
presumably, reduced/altered function of these cells. As a result, the process of endochondral
ossification is delayed/altered, resulting in changes in bone shape and retention of epiphyseal (young
animals) or epiphyseal/calcified (older animals) cartilage. These changes likely contributed to the
development of osteoarthritis in the oldest affected animal. Prevention of such abnormalities is likely to
require treatment interventions early in life and/or optimized targeting of the epiphyseal cartilage of the
major diarthrodial joints.

**Significance:** The results of this study demonstrate the importance of the feline model of Sandhoff
disease in clarifying the pathogenesis of the skeletal lesions occurring in this disease and in developing
treatments for this disease in humans.
Distal femur from a 5-month-old A) affected and C) control cat; B) vacuolated chondrocytes D) normal chondrocytes