Alterations in neuron morphology in mucopolysaccharidosis type I*  
A Golgi study

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Summary. Morphological changes in neurons with inborn defects of the lysosomal hydrolase, α-L-iduronidase, and with concomitant storage of glycosaminoglycans, were evaluated by Golgi staining in two animal models and compared to a similar study of a child with the same disease. Cortical pyramidal neurons in feline mucopolysaccharidosis type I often displayed axon hillock enlargements (meganeurites) and/or ectopic, secondary neuritic processes sprouting from this same region of the cell. The latter structures were prominent and often appeared longer than similar neurites reported in other neuronal storage diseases. Although most meganeurites were aspiny, a few were observed which possessed spine-like processes or neurites. Other than these morphological changes in cortical pyramidal neurons, few other cell types displayed abnormalities demonstrable by Golgi impregnation. In the canine model of this disorder, abnormal Golgi-impregnated cortical neurons resembled more closely those seen in human mucopolysaccharidosis. That is, they possessed meganeurites which typically were aspiny in appearance. Ectopic neurite growth was not observed on any Golgi-impregnated neurons in the cases of canine or human mucopolysaccharidosis used in this study. The latter finding, given the advanced ages of these cases, is consistent with the view that ectopic neuritogenesis seen in neuronal storage diseases may be subject to a developmental window, albeit one open well beyond the period of early postnatal maturation.

Key words: Neuronal storage disease — Neurite growth — Dendrite — Hurler’s disease — Mucopolysaccharidosis

The mucopolysaccharidoses represent a diverse family of inherited disorders characterized by common defects of lysosomal hydrolases involved in the degradation of dermatan, heparan, chondroitin, and keratan sulfates. As a result of such metabolic errors, incompletely degraded glycosaminoglycans accumulate within cells of various organs, including brain, and are excreted in urine. Clinically these diseases are characterized by widespread multi-system abnormalities, which include skeletal alterations (dysoxosis multiplex) and limited joint mobility, corneal clouding, deafness, atherosclerosis, hepatosplenomegaly, and mental retardation [10]. Seven major forms of mucopolysaccharidosis (MPS) have been described (types I—VII) and subdivided according to differences in enzymatic defects or in specific clinical manifestations. Type I MPS is perhaps the best known form of this disease and three or more distinct clinical subtypes have been recognized (Hurler, Scheie, and Hurler-Scheie intermediate forms). All of these clinical subtypes have been shown to result from a recessively inherited defect in activity of the lysosomal hydrolase, α-L-iduronidase.

Clinical onset in the Hurler subtype occurs after the first year of life and follows an insidiously progressive course leading to death by 10 years of age. Mental retardation is a prominent component of this disease. A leading hypothesis for explaining the onset and progression of neurobehavioral decline in this and other neuronal storage diseases suggests that the development of specific changes in neuronal geometry (meganeurite formation) and in synaptic connectivity underly changes in brain function [13]. Previously reported Golgi studies have indicated that human MPS I is characterized by meganeurite formation but these changes appeared less prominent than in ganglioside storage disease [13]. The present study has used colony-derived feline and canine models of MPS I, in conjunction with a similar case in humans, as a
means to explore in greater detail the morphological changes induced in neurons as a result of α-L-iduronidase deficiency and glycosaminoglycan storage. Results have been compared with Golgi studies of other neuronal storage diseases in humans and animals. A preliminary report of some aspects of this work has been published [22].

Materials and methods

Multiple formalin-fixed slices of brain tissue from two cases each of feline and canine MPS I were utilized in this study. Additionally, Golgi-stained cortical tissues from a 6-year old child with MPS I were available for comparative study. Mutant animals were derived from established research colonies and were confirmed as being deficient in the lysosomal hydrolase, α-L-iduronidase, prior to euthanasia. The feline cases were 2 and 2.5 years of age. Clinically, both displayed abnormal facies, bilateral corneal clouding, and mild hind limb lameness. Normal cranial and spinal nerve reflexes were present and no behavioral abnormalities noted. The lameness was believed due to bilateral hip subluxation. Hepatosplenomegaly was also present. Detailed descriptions of this model are available elsewhere [6, 7]. The canine cases were 1.5 and 4.5 years of age. As in the feline model, these animals also displayed facial dysmorphia, corneal clouding, and skeletal abnormalities. Unlike the cats, however, and more like children with the disease, overall growth was significantly stunted. Again, even though behavioral changes were not readily apparent in these animals, morphological and neurochemical findings suggested analogy with the Hurler subtype of human MPS I. Detailed studies of this canine model of MPS I are also available [4, 16–18]. The older animal used in this study had been part of a bone marrow transplant study but had died 35 days post-treatment. The human case of Hurler’s disease available for this investigation was a child of 6 years of age, who had succumbed to bronchopneumonia following an essentially typical course for this disease. He was normal at birth but by 9 months of age he had demonstrated delayed developmental milestones, skeletal abnormalities, and corneal opacity. At this time a variety of biochemical tests suggested the diagnosis of Hurler’s disease. By 5 years of age he could stand without support, but could not walk, and was otherwise helpless [13]. A brief description of Golgi studies of this case have already been reported [13], and the goal here was to use this material for direct comparative study with the animal models.

Golgi-rapid, Golgi-Kopsch, and Golgi-chloral hydrate procedures were carried out on multiple areas of the CNS from each case described above. Details of these staining methods are given elsewhere [19]. Golgi staining was also performed on normal animals of similar age for comparison. Results were documented by photography and by camera lucida reconstruction.

Results

Human MPS I

Detailed evaluation of Golgi-stained sections of cerebral cortex from human MPS I revealed significant morphological changes in a variety of neuron types (Fig. 1). Meganeurites were commonly encountered on layer III pyramidal cells and although many of these were small and slender, others were prominent and exceeded the volume of adjacent somata. Small spine-like processes were sometimes seen in association with meganeurites and these were most often located distally near the axonal initial segment region. Some neurons without meganeurites also had a few spine-like growths clustered at the axon hillock area. Longer neuritic processes, which are a well-known feature of pyramidal neurons in many storage disorders, were never encountered on cells in this case.

Many Golgi-impregnated pyramidal neurons appeared completely normal and all intrinsic cells encountered, primarily small basket neurons, also appeared essentially unaffected (in terms of Golgi morphology) by the storage disease process. Although dendrites of cortical neurons sometimes appeared shorter than normal, and dendritic spines less dense than expected, establishing whether this was the result of the disease process or due to postmortem delay [30] was not possible in this study.

Feline MPS I

Golgi impregnations of the two cases of feline MPS I were excellent and supplied detailed morphological data on cerebral cortex, including hippocampus, several subcortical regions, thalamus, and cerebellum. Results from the two animals appeared equivalent.

In the cerebral cortex, many pyramidal neurons appeared completely normal and displayed long, radiating apical and basilar dendrites which were richly invested with dendritic spines of normal morphology and distribution. The most common change in pyramidal cell morphology was axon hillock enlargement or meganeurite formation and these were most frequently found on layer II–III pyramidal cells and were spine and neurite free (Fig. 2A, C). Although most meganeurites were small, occasional ones were considerably larger and equalled or exceeded their adjacent soma in volume. Meganeurites bearing spines and/or longer neuritic processes were observed (Fig. 3C, D), but these were much less common than the aspiny variety. In addition to meganeurites, secondary neurites also were seen on axon hillocks (Figs. 2D, 3A, 4A–C) and with two exceptions appeared identical to those described in feline models of gangliosidosis [12, 19]. The exceptions were (i) that the length of the longest neurites observed in these animals exceeded those of previous reports (e.g., see Fig. 4B), and (ii) some pyramidal neurons displayed only a single neurite projecting from the axon hillock (e.g., see Fig. 2D). This single process, however, had all the characteristics of those present in the more complex tangles of neurites of other cells. Non-pyramidal (intrinsic) cells also were impregnated and appeared normal (Fig. 3B).