METACHROMATIC LEUKODYSTROPHY WITHOUT ARYLSULFATASE A DEFICIENCY

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SUMMARY

Two siblings of consanguinous parents were noted to have a neurologic syndrome marked by developmental delay, regression of psychomotor performance, marked spasticity and progressive central nervous system degeneration. Markedly delayed nerve conduction times and a sural nerve biopsy which demonstrated changes typical of metachromatic leukodystrophy (MLD) were evident. Impairment of sulfated glycolipid metabolism was documented by analysis of glycosphingolipid in urinary sediment. In spite of these findings, activities of arylsulfatase A and cerebroside sulfatidase in white blood cells and cultured skin fibroblasts were near normal. However, when intact growing fibroblasts were loaded with $^{35}SO_4$-sulfatide a clear defect in sulfatide cleavage, comparable to that seen in MLD patients, was observed. Thus, these patients represent a new form of sulfatide storage disease - MLD characterized by intact enzyme activity in cell homogenates but defective sulfolipid metabolism in vivo and in intact fibroblasts.

SPECULATION

Since cell homogenates from these patients can cleave sulfatide in the presence of detergents while the patients themselves and their intact cells cannot, some explanation other than decreased activity of the relevant lysosomal enzyme must be invoked to explain this storage disease. The two most plausible hypotheses are that either these patients have a defect which prevents enzyme and substrate interaction in the proper subcellular location, or that these patients are missing the putative glycoprotein "activating factor" necessary for sulfolipid hydrolysis in vivo.

INTRODUCTION

Metachromatic leukodystrophy (MLD) is a well known error of sulfated glycosphingolipid metabolism(8). Deficiency of cerebroside sulfatidase activity in affected patients leads to the intralysosomal accumulation of sulfated glycolipids in a number of tissues. Due to the normally substantial content of such sulfolipids in brain white matter and peripheral nerves, defects in sulfatide degradation lead to tissue storage and consequent neuropathology including hemispheric demyelination as well as changes in the basal ganglia, dentate nucleus, and brainstem. Lesions in the peripheral nervous system are characterized by intralysosomal accumulation of metachromatic material in Schwann cells and machrophages, and by peripheral nerve demyelination.

At least three distinct clinical syndromes of MLD have been delineated. These are often classified by their age of onset as the late infantile form (onset 1-4 years), the juvenile form (onset 4-12 years), and the adult form (onset in the second decade or later). The first two clinical types feature early normal development followed by gait disturbance, long tract signs, incoordination, ataxia, peripheral neuropathy, and progressive dementia. In the adult form of MLD, psychosis and dementia may be first noted and motor signs may not appear until much later in the course of the disease.

Cerebroside sulfatidase activity in tissues and body fluids has been equated with arylsulfatase A activity as assayed with artificial substrates(16). In all well documented cases of metachromatic leukodystrophy studied to date, tissue accumulation of sulfatides and deficiency of cerebroside sulfatidase and of arylsulfatase A activities have been observed (1,17). As with many other inborn errors of metabolism, however, biochemical and genetic heterogeneity is anticipated. We report here, the occurrence of metachromatic leukodystrophy in two siblings in the face of near normal levels of cerebroside sulfatidase and arylsulfatase A activities.

CASE REPORTS

Case #1:

Y.F., the propositus in the family, first presented to the Harbor-UCLA Medical Center at five years of age for evaluation of progressive neurologic deterioration. The patient was the product of a nine month pregnancy to a Gravida 6, Para 5, AB 1, Mexican-American female. The patient's parents were first cousins. Labor and delivery, and the neonatal period were unremarkable. The patient had an entirely benign early childhood with the exception of a questionable febrile convulsion at three months of age. At approximately four and one-half years of age, the patient was first noted to exhibit several behavioral abnormalities, such as gnashing of her teeth and wringing of her hands. Following this, mental regression proceeded fairly rapidly. She could not feed herself as she had done before, she had difficulty dressing herself, and she became very withdrawn and hostile to other children. She was no longer able to ride her bicycle and she began to wander off by herself. Changes in motor function were also noted, in that her gait became abnormal and a decrease in fine motor ability was appreciated. Floppiness of the right arm was also described. Approximately three weeks prior to initial evaluation, a right sided focal motor seizure was noted. Initial physical examination demonstrated a well developed, normal appearing female child. She was in the 45th percentile for weight and 10th percentile for height. The general physical examination was unremarkable. The pupils reacted slowly but equally and consensually to light and no funduscopic abnormalities were appreciated. She walked with a slight shuffle and tended to ambulate on her toes. Cranial nerves and motor function were intact and cerebellar testing was felt to be normal. Reflexes were decreased throughout. Initial laboratory examination, including hemogram, urinalysis and blood chemistries were unremarkable. A lumbar puncture was performed which revealed a CSF protein of 44 mg%, 33% of which was IgG globulin. A brain scan was normal and an electroencephalogram showed only generalized slowing. A computerized tomographic brain scan was normal. During the ensuing year the patient deteriorated dramatically. The child stopped walking and talking and began having intermittent generalized motor seizures which were relatively resistant to anticonvulsive therapy. By six years of age, complete regression of neurologic cortical function to less than a six month old level was observed. Cranial nerves were grossly intact. Motor examination demonstrated a marked increase in tone, especially in the upper extremities with flexion contractures being noted. At this time, hyperreflexia was observed but with flexor-plantar responses. A number of primitive cortical reflexes were present. Slowed nerve conduction was evident with conduction velocities of 28.5 cm per second in the median nerve, 30 cm per second in the ulnar nerve, and 28 cm per second in the peroneal nerve. A sural nerve biopsy was performed under local anesthesia.

Case #2:

E.F. is the nineteen year old brother of Y.F. He was the product of an entirely normal pregnancy and delivery and his early development was normal. He was said to have been in good health and achieved his early developmental milestones at appropriate ages. At six years of age he had a generalized seizure and began to forget things rather easily. His deterioration was comparable to that described for his sister, except that it proceeded somewhat more slowly. By age eleven he had lost all apparent intellectual function and was institutionalized. At nineteen years of age the patient had been uncommunicative for more than ten years, was incontinent, non-ambulatory and totally unable to respond to commands. The physical examination demonstrated a markedly spastic, uncommunicative male who appeared considerably younger than his stated age. Intact cranial nerves, but extreme spasticity with hyperreflexia were evident upon examination. Due to family considerations this patient was not studied in as much detail as his younger sibling.

MATERIALS AND METHODS

A segment of sural nerve was divided into two portions: a) a portion fixed in buffered formalin for preparation for light microscopic examination, and b) a portion fixed in cold 2.5% glutaraldehyde in cacodylate buffer for electron microscopy. The formalin-fixed portion was divided into two parts: one for dehydration and embedding in paraffin and the other for frozen section. Paraffin sections were stained by hematoxylin and eosin, phosphotungstic acid hematoxylin, Masson's trichrome, Kluver-Barrera's luxol fast blue, and Bodian's methods. Frozen sections were stained with toludine blue and acidified cresyl violet methods for metachromasia and photographed immediately. The specimen for electron microscopy was dehydrated in graded alcohol and propylene oxide, embedded in Epon 812, and one micron sections were stained with toluidine blue. Thin sections were examined with a Hitachi HU 11C electron microscope.

Twenty-four hour excretion of glycosphingolipids in urinary sediment was determined by the methods of Desnick, et al (5,4). This technique involves extraction of the sediment with chloroform:methanol (2:1), separation of the glycosphingolipid fraction by silicic acid chromatography, mild alkaline methanolysis, separation of individual glycosphingolipids by thin-layer chromatography, and quantitiation by gas-liquid chromatography of the trimethylsilyl methyl glycosides.

Skin fibroblasts were obtained from 3-4 mm punch biopsies by conventional methods. Cells were grown either in Eagle's Minimal Essential Medium (MEM) with 10% fetal calf serum as previously described, or in HEPES buffered MEM. The HEPES buffered (pH 7.4) cultures were equilibrated with air and incubated at 37° C. The other cultures were maintained in 5% CO_2 in air (11).

Arylsulfatase A activities were determined in white blood cells and fibroblasts by minor modifications of the methods of Baum, et al (2). Kinetic studies, pH-activity profiles and heat inactivation kinetics were performed by standard techniques. Polyacrylamide gel electrophoresis, isoelectric focusing and DEAE cellulose column chromatography were done as previously described(22). Enzyme activity with $^{35}SO_4$- cerebroside sulfatide as substrate were assayed in fibroblast homogenates in the presence of taurodeoxycholate by published procedures (20) using $^{35}SO_4$- sulfatide prepared from rat brain (10). Studies of $^{35}SO_4$-sulfatide loading of intact fibroblasts was done by the method of Porter, et al (21) with appropriate controls run concurrently in each experiment.

RESULTS

Histopathologic examination of the proband's (Y.F.) sural nerve biopsy demonstrated features of metachromatic leukodystrophy. Frozen sections stained with toluidine blue showed the nerve to contain brownish metachromatic granules (Figure 1). Electron microscopic examination of the same tissue revealed myeloid bodies, granular inclusions of stored material in Schwann cell cytoplasm, and concentric granular degeneration of myelin lamellae along with preserved axons, consistent with a segmental demyelination process. In table 1, the results of urine sediment glycolipid analysis (Y.F.) is given along with the values for two classical MLD patients, a concurrently run control, and the normal range. Monohexosyl and lactosl sulfatide are markedly elevated in Y.F.'s sediment to levels comparable to those in known MLD patients. The level of most other lipids are near or within the normal range. The presence of leukocytes in the specimen obtained from Y.F. by catheterization may account for the minimal elevation of lactosyl ceramide. (R. Desnick, unpublished observation).

Nitrocatechol sulfatase A activity of leukocytes and fibroblast homogenates in both patients was consistently below the normal range and generally corresponded to the intermediate values seen in MLD heterozygotes without clinical symptomatology (table 2). Similar results were observed with the natural substrate, cerebroside sulfate, in the presence of taurodeoxycholate (table 3). Enzyme activity in the parents was somewhat reduced as well. All of these studies were repeated on several occasions.

Since there was an apparent discrepancy between the clinical features of these patients and their arylsulfatase A activities, further studies were undertaken to characterize their sulfatases in an effort to demonstrate some structural abnormality of the enzyme molecules. As seen in figure 2 and table 4, the affinity for the artificial substrate nitrocatechol sulfate (apparent Km), the pH-activity profile, and the pH and heat stabilities of the patient's arylsulfatase A were all comparable to controls. Furthermore, polyacrylamide gel electrophoresis at two different pH's, isoelectrical focussing, and DEAE cellulose chromatography failed to provide any evidence of a structural mutation in the arylsulfatase A gene. Experiments in which the amount of taurodeoxycholate in the cerebroside sulfatidase assay were varied, likewise did not discriminate between normal and patient enzyme, with maximum activity being reached at 50-70 µg of taurodeoxycholate per assay tube in each.

Previous studies have provided evidence that there is a reduced rate of sulfatide hydrolysis by intact cultured fibroblasts derived from individuals with genetically determined impairments of sulfatide metabolism (21). Such kinetic studies can clearly differentiate patients with metachromatic leukodystrophy from normals or heterozygotes. Furthermore, the degree of in vitro abnormality can be correlated with the age of onset of symptomatology (13). The rate of sulfatide hydrolysis was much reduced in the proband when studied

on several occasions and gave data comparable to adult MLD patients (figure 3). Similar deficits in sulfatide hydrolysis were observed in E.F.'s (case #2) cells. In contrast, the mother of the propositi had a normal rate of sulfatide degradation.

DISCUSSION

In recent years, substantial progress has been made in the elucidation of the pathogenesis of the lysosomal storage diseases. In most instances, a genetically determined deficiency of a specific hydrolase has been identified as being etiologically related to the accumulation of a given macromolecule. A few perplexing diseases such as mucolipidosis II (I-cell disease), mucolipidosis III, and multiple sulfatase deficiency in which more than one enzyme activity is reduced, remain to be fully explained.

It generally has been presumed that when a mutation acts to lower the effective activity of a given lysosomal enzyme below some critical point, such that the step it catalyzes becomes rate-limiting in the pathway of degradation of a macromolecule, then intralysosomal accumulation of that macromolecule may ensue. Several recent observations, however, suggest that difficulties may be encountered in applying this explanation to all observed clinical situations. Several clinically normal heterozygotes for lysosomal storage disease genes have been recognized who have very low levels of in vitro enzyme activity, similar to those seen in affected homozygotes (15,7,25). Furthermore, numerous discrepancies in enzyme activity when assays employing artificial substrates are compared to those utilizing natural substrates, have been encountered (19,24,6,23). The present cases call attention to the fact that a lysosomal storage disease may occur in the presence of apparently functional enzymes.

Homozygosity of a mutant recessive gene seems likely as the cause of the clinical abnormality in these two siblings whose parents are first cousins. The neurologic abnormalities and their progression in these children differed from other MLD patients in that dementia and seizures were more prominent than motor disturbances and that cerebrospinal fluid protein levels were not markedly elevated. Intralysosomal storage of sulfatide is apparent by histochemical and electronmicroscopic examination of sural nerve biopsy material and is corroborated by the massive content of sulfatides in the urinary sediment. In spite of this, arylsulfatase A and cerebroside sulfatidase assays in cell homogenates were observed in a range which should not produce impairment of sulfolipid metabolism. Although an abnormally increased rate of sulfatide synthesis, or the production of a structurally abnormal sulfatide resistant to enzymatic hydrolysis are possible explanations for these observations, a clear defect in the degradation of normal exogeneous sulfatide by the patient's cells makes these considerations unlikely. An intrinsically abnormal enzyme which fails to act at in vivo substrate concentrations but is active at the saturating levels of substrate used in the assay systems has also been ruled out by appropriate studies.

The principal laboratory abnormality in these patients is impaired sulfatide hydrolysis in intact cells with relatively normal enzymatic activities in homogenates in the presence of detergent. At least two explanations may be invoked to explain these findings. The first is that problem of intracellular enzyme localization or transport of sulfatide may be operative, such that enzyme and substrate do not interact. A second hypothesis is that the defect in these patients is the result of abnormal "activator-factor" action. A number of intermediate molecular weight glycoproteins have been described which appear to function as detergents or substrate solubilyzers of glycosphingolipid substrates and presumably are required for natural substrate hydrolysis in vivo (17,9,12,14,18). Several such factors have been reported which appear to have some specificity for particular enzymes and/or their substrates. A specific activator factor for cerebroside sulfatidase has been known for some time. Recently, Conzelmann and Sandhoff (3) have provided evidence that patients with the AB variant of GM_2 gangliosidosis lack such a factor activity needed for in vivo. The absence of this factor with preservation of enzyme activity would account for the observed ability to hydrolyze substrate with cell homogenates in the presence of bile salts which supplant the function of the factor. This theory would also explain why the intact fibroblasts or the patients cannot effect this reaction and consequently store sulfatide.

The proposed cerebroside sulfatidase activator factor defect may be demonstratable in liver or kidney tissue if these become available for study. Alternatively, experiments designed to correct abnormal sulfatide metabolism of cultured cells by treatment with exogenous purified factor, may provide evidence in support of this hypothesis. Elucidation of this defect should add to our understanding of lysosomal physiology. Furthermore, these cases suggest that in specific instances the evaluation of patients with potential storage diseases, histopathology or analysis of stored material may be productive even if enzymatic studies are normal.

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TABLE 1

Concentrations of Glycosphingolipids in Urinary Sediment

Glycosphingolipid	Case # 1 Y.F.	Classic MLD D.P.	K.M.	Normal Range (n=20)
Glucosyl Ceramide	30	38	29	11-87
Galactosyl Ceramide	n.d.	n.d.	n.d.	<10
Lactosyl Ceramide	193	76	59	8-54
Trihexosyl Ceramide	57	24	20	7-38
Tetrahexosyl Ceramide	27	20	17	5-30
G_{M3} Ganglioside	35	36	20	5-30
Monohexosyl Sulfatide	595	863	842	<10
Lactosyl Sulfatide	35	84	146	n.d.

TABLE 2

Nitrocatechol Sulfatase A Activity*
in Leukocytes and Fibroblasts

Subject	Leukocytes	Fibroblasts
Y.F.	24	550
E.F.	21	454
Mother	36	1300
Father	66	450
Typical MLD	<10	<50
Normal Range	50-100	1000-2000

* Activity in nmoles hydrolyzed per mg protein per hour.

TABLE 3

Cerebroside Sulfatidase Activity
in Fibroblast Homogenates

Subject	Activity (nmoles/mg protein/hr.)
E.F.	87
Y.F.	68
Mother	124
Typical MLD	<5
Control 1	230
Control 2	195

TABLE 4

Properties of Proband's
Arylsulfatase A

Parameter	Y.F.	Control
pH activity optimum	5.5	5.5
Heat stability ($T_{\frac{1}{2}}$ @ 62°C)	60 min.	60 min.
$T_{\frac{1}{2}}$ @ 37° pH 3.0	20 min.	20 min.
K_m	4.5×10^{-3}M	5.7×10^{-3}M
Isoelectric focussing	equivalent	
DEAE chromatography	equivalent	
Polyacrylamide gel electrophoresis	equivalent	

Figure 1:

Transverse section of a fascicle of sural nerve reproduced in black and white print from 35 mm Kodachrome. The dark granules scattered through the nerve represent material showing metachromasia. Frozen section, toluidine blue stain, x40 (original magnification).

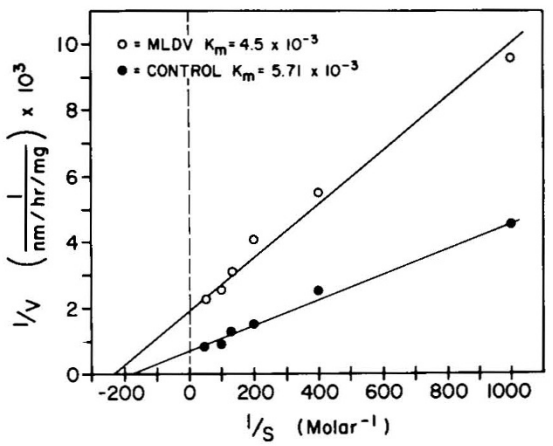

Figure 2:

A comparison of the relative affinity of the patient's fibroblast extract (○ - ○) and control fibroblast extract (● - ●) for the artificial substrate nitrocatechol sulfate was made. Assays were performed at varying substrate concentrations for one hour as described in the text. In spite of the lower activity of the patient extract, apparent Km's for this substrate are comparable.

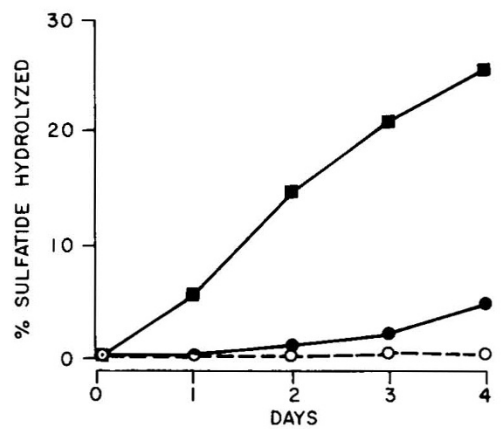

Figure 3:

The ability of intact, growing fibroblasts from the patient (● - ●) to hydrolyze exogenous sulfatide was studied and compared to control cells (■ - ■) and cells from a patient with classical late infantile MLD (○ - ○). MEM-HEPES media containing (^{35}S) sulfatide was added to near-confluent cultures on day 0 as described in the text. The media was analyzed for free (^{35}S) sulfate for 4 consecutive days and the results are expressed as % sulfatide hydrolyzed.

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