A CORRELATION OF INTRACELLULAR CEREBROSIDE SULFATASE ACTIVITY IN FIBROBLASTS WITH LATENCY IN METACHROMATIC LEUKODYSTROPHY

Myna T. Porter, Arvan L. Fluharty, Joe Trammell, and Hayato Kihara

Research Department, Pacific State Hospital, Pomona, California 91768

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SUMMARY

Despite the absence of cerebroside sulfatase activity in cell-free preparations, fibroblasts in culture derived from patients with metachromatic leukodystrophy were capable of hydrolyzing exogenous cerebroside sulfate. Moreover, the degree of whole-cell sulfatase activity was directly correlated to the age of onset of clinical symptoms in the patients from whom the fibroblasts were derived. Intact fibroblasts from patients with the earliest manifesting form, late infantile metachromatic leukodystrophy, did not hydrolyze any cerebroside sulfate, while fibroblasts from patients with later manifesting forms hydrolyzed appreciable amounts of the sulfolipid.

INTRODUCTION

The metachromatic leukodystrophies (MLD) are a group of human genetic disorders which are characterized by the deposition of cerebroside sulfate (sulfatide) rich granules in the central and peripheral nervous systems with resultant progressive neurological degeneration. The disease has been grouped into three sub-types, late infantile, juvenile, and adult, based on differing ages of onset of clinical manifestations and rates of progress. Each of these forms appears to be an independent autosomal recessive disorder with only one form appearing in any kinship. A generalized deficiency of arylsulfatase A is associated with all forms of MLD, but biochemical differentiation among the forms has been difficult to demonstrate. Analogy with other enzyme deficiency disorders indicates that age of onset should be dependent on the degree of enzyme deficiency (1-4), however, attempts to establish this correlation in MLD have been largely unsuccessful. Based on 4-nitrocatechol sulfatase activity, Stumpf and Austin reported that arylsulfatase A in the

urine from juvenile MLD patients, while markedly reduced below control levels, was present in significantly greater quantities than in urine from late infantile patients and that the kinetic properties of the enzyme from patients showed subtle differences from that of control subjects (5). The present communication reports the demonstration of a positive correlation between latency of clinical symptoms in patients with MLD and the cerebroside sulfatase activity of intact fibroblasts derived from these patients.

MATERIALS AND METHODS

Fibroblast cultures were initiated from skin biopsies and maintained as described previously (6). Cell-free preparations were obtained from harvested cells by freezing and thawing 6 times and centrifuging for 1 minute in a Spinco Microfuge. Protein concentration (7) and enzyme activity were determined on the supernatant fluid. Arylsulfatase A activity was measured using either 4-nitrocatechol sulfate (8,9) or ^{35}S-sulfatide as substrate. The reaction mixture for the latter contained 70 nmoles ^{35}S-sulfatide (1.3 x 10^3 cpm/nmole); 20 μmoles sodium acetate buffer, pH 4.5; 8 μmoles sodium chloride; 250 μg sodium taurodeoxycholate; and dialyzed cell extract in a total volume of 0.2 ml. After incubation at 37° for 2 hours, the mixture was partitioned in chloroform-methanol-aqueous sodium chloride (10) with carrier sulfate and the radioactivity of inorganic ^{35}S-sulfate in the aqueous phase determined (a manuscript describing the development of this procedure is in preparation). Methods for the preparation of ^{35}S-sulfatide and estimation of intracellular accumulation and hydrolysis of sulfatide have been described (6).

Control fibroblasts were derived from normal adults and from children afflicted with disorders other than MLD. Late infantile MLD cultures were obtained from children who began to exhibit clinical symptoms at age 2½ years or earlier. It is of interest that patient LI-4 was biopsied at 4 months of age because of a family history of MLD. At that time metachromatic granules were present in peripheral nerves but no overt clinical signs of

the disease were apparent. This child subsequently became symptomatic before 12 months of age and died before his second birthday (11). To emphasize the pertinent findings in this report, we have arbitrarily designated cultures from patients with other forms of MLD as variants rather than as juvenile or adult forms. The sources of the variant cultures and references to their clinical description are indicated in Table I. All skin biopsies were obtained after onset of clinical symptoms with the exception of LI-4.

RESULTS

Fibroblast arylsulfatase A activity toward both 4-nitrocatechol sulfate and cerebroside sulfate (Table I) confirms and extends earlier observations that MLD cells have reduced activity toward both substrates (9, 13). Extracts of MLD cells had uniformly low nitrocatechol sulfatase activity while cerebroside sulfatase activity was below the limit of sensitivity. No differen-

TABLE I

ARYLSULFATASE A ACTIVITY IN CELL-FREE EXTRACTS OF FIBROBLASTS

Cell Strain		Age of onset years	Specific Activity (nmoles/hr/mg protein) 4-Nitrocatechol sulfate	Cerebroside sulfate
Control (13 strains)			1,400 ± 500 (800-2700)[a]	50 ± 20 (10-100)[a]
Late Infantile MLD	LI-1	1.5	30,50,100	<0.05[b]
	LI-2	2	50,90,70	<0.05
	LI-3	2.5	30,80	<0.05
	LI-4	1	20	--
	LI-5	1.5	100	<0.05
Variant MLD	J-1[c]	6.5	40,90,80	<0.05
	J-2[d]	12	3	<0.05
	A-1[e]	16.5	30,80	<0.05

[a]Mean ± one standard deviation (range in parenthesis).
[b]Limit of sensitivity of procedure.
[c]Received from Dr. James Austin, patient 5 in ref. 5.
[d]Received from Dr. Jules G. Leroy, ref. 12.
[e]Received from Dr. James Austin, patient 6 in ref. 5.

tiation into MLD types could be made on this basis. Activities of acid glycosidases and arylsulfatase B in MLD cells fell within the range obtained in the control cells.

In contrast, culturing intact cells in the presence of ^{35}S-sulfatide provided evidence that some MLD fibroblasts do in fact have the ability to metabolize sulfatide (Fig. 1). The control culture accumulated very little sulfatide but excreted significant amounts of inorganic ^{35}S-sulfate into the medium. Hydrolysis of the sulfatide proceeded linearly before leveling off by day 6. Late infantile MLD cells accumulated sulfatide in amounts exceeding normal by approximately 40-fold while production of inorganic sulfate was

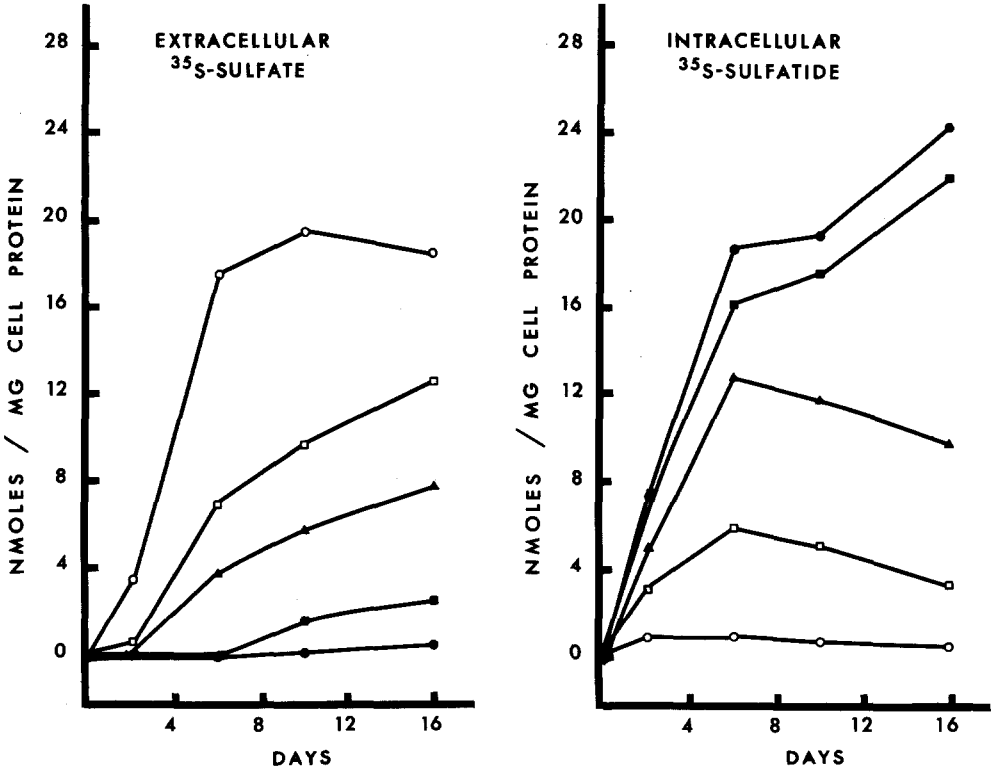

Figure 1. Uptake and hydrolysis of ^{35}S-cerebroside sulfate by cultured fibroblasts. Petri dishes (60 mm) were plated with fibroblasts (300,000/dish) in 3 ml growth medium containing ^{35}S-cerebroside sulfate (23 nmoles/ml; 3.6 x 10^3 cpm/nmole). On the days indicated one dish of each cell strain was analyzed for intracellular sulfatide and ^{35}S-sulfate in the medium. Cell strains: LI-1, ●; J-1, ■; J-2, ▲; A-1, □; control, ○.

negligible as described previously (6). Variant MLD cells, however, in addition to accumulating sulfatide intracellularly, all produced significant amounts of inorganic sulfate, the identity of which was confirmed by chromatography and radioautography.

DISCUSSION

Despite the apparent absence of cerebroside sulfatase activity in cell-free preparations, the present data clearly indicate that intact MLD variant fibroblasts can indeed hydrolyze sulfatide. Moreover, the degree of intracellular accumulation of sulfatide and elaboration of inorganic sulfate into the medium correlated with the latency of appearance of the disease symptoms in the patients from whom the cultures were derived.

A wide range of nitrocatechol sulfatase and sulfatidase activities was observed in extracts of control fibroblasts, but the ratio of the two activities was relatively constant from strain to strain. All MLD cultures showed a small amount of nitrocatechol sulfatase activity, but no hydrolysis of the natural substrate, sulfatide, could be detected. These data suggest that limited hydrolysis of the synthetic substrate by MLD cells is not due to arylsulfatase A, but is an artifact resulting from the limited selectivity of the assay. Conditions of the assay are optimized to favor arylsulfatase A (8) and salts are included to inhibit B activity, but it is quite probable that complete selectivity is not achieved. Thus the use of 4-nitrocatechol sulfate for the detection and characterization of residual arylsulfatase A in crude preparations of MLD tissue is of questionable value. Use of natural substrate, sulfatide, eliminates the problem of selectivity as it is hydrolyzed exclusively by arylsulfatase A.

The apparent discrepancies in sulfatide hydrolyzing capacity of whole cells and cell-free extracts of MLD variants have not as yet been resolved. The data in Figure 1 suggest that the differences among the various cell strains may be due to affinity of the enzyme for the substrate. Normal cells produced inorganic sulfate after accumulating small amounts of intracellular

sulfatide. MLD cells exhibited lag periods during which greater intracellular concentrations of sulfatide were reached before hydrolysis commenced. The concentration attained and the duration of the lag period were inversely related to the age of onset of clinical symptoms. In the late infantile cells, despite the high intracellular sulfatide concentrations achieved on extended incubations, virtually no hydrolysis was observed with several different cultures.

Attempts to demonstrate a K_m effect in cell-free extracts, however, have been unsuccessful. No sulfatidase activity in extracts of any MLD cells could be demonstrated even when the substrate concentration was increased up to 100-fold and incubation extended from 2 to 5 hours. In comparison with the data of the 6th day (Fig. 1), cell-free preparations of the control strain hydrolyzed sulfatide at a rate approximately 400 times that of intact cells. By extension, cell-free preparations of A-1 and J-2 should then have specific activities of 17 and 11 nmoles per hour per mg protein, respectively. Although these levels of activity would be easily detected in the cerebroside sulfate assay system, no hydrolysis of sulfatide could be demonstrated in either the supernatant fluids or pellets of the MLD lysates. It must then be concluded that enzyme activity was lost during extraction.

Neuwelt, et al., reported that MLD tissue extracts contain immunoreactive proteins which precipitate anti-arylsulfatase A serum (14). Their findings and the present data with fibroblasts provide evidence that arylsulfatase A proteins are produced in some types of MLD. These mutant proteins appear to be similar in that they all exhibit extreme lability under normal extraction procedures but they may differ among themselves in affinity for cerebroside sulfate or in some other parameter which determines latency of clinical onset. The data in Figure 1 and the wide range of reported latencies in clinical manifestations suggest that the metachromatic leukodystrophies result from a broad spectrum of

cerebroside sulfatase deficiency ranging from virtual absence to an appreciable percentage of normal levels in those cases of late onset of symptoms. Investigations into the extraction of active cerebroside sulfatase from MLD fibroblasts are in progress. Stabilization of these enzymes should facilitate the elucidation of biochemical differentiation of the metachromatic leukodystrophies.

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