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Sanfilippo Syndrome: Profound Deficiency of Alpha-Acetylglucosaminidase Activity in Organs and Skin Fibroblasts from Type-B Patients

(genetic disease/mucopolysaccharidosis/lysosomes/enzyme)

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ABSTRACT Cultured skin fibroblasts from two patients with Sanfilippo syndrome, Type B were strikingly deficient in α-acetylglucosaminidase activity (α-2-acetamido-2-deoxy-D-glucoside acetamidodeoxyglucohydrolase, EC 3.2.1.X). A similar deficiency was found in frozen organs from two other patients. A partial deficiency of α-acetylglucosaminidase was found in cultured skin fibroblasts from both parents of one patient. Soluble endogenous inhibitors did not account for the enzyme deficiency. Other lysosomal hydrolases were normal or increased in cultured fibroblasts from patients with this disease. No deficiency of α-acetylglucosaminidase is present in other genetic mucopolysaccharidoses, including Sanfilippo Type A.

The Sanfilippo syndrome is an inborn error of mucopolysaccharide metabolism characterized by severe progressive mental retardation, bony deformities, and excessive urinary excretion of heparan sulphate. The disorder is clinically distinct from related hereditary mucopolysaccharide storage diseases, such as Hurler's and Hunter's syndromes, and has been categorized as mucopolysaccharidosis Type 3 by McKusick (1). Analyses of pedigrees indicate that the disorder is transmitted as an autosomal recessive trait.

Kresse *et al.* (2) have demonstrated an impaired rate of degradation of acid mucopolysaccharides in cultured skin fibroblasts from patients with Sanfilippo syndrome. The defect in degradation can be corrected by addition of a macromolecular factor (corrective factor) to cultures of cells from patients with this disease. Corrective factor occurs in medium in which normal skin fibroblasts have been grown, and also in human urine.

Sanfilippo fibroblasts can be subclassified into two types, based on deficiencies of two different corrective factors (2). Fibroblasts from Sanfilippo Type A secrete a factor into the medium that corrects the abnormality of mucopolysaccharide degradation in fibroblasts from Sanfilippo Type-B patients and vice versa. The two types of factor have been partially characterized; they are probably proteins with molecular weights of about 200,000, possessing different thermostability characteristics and different ionic charges at pH 8.5 (2).

I report here that activity of the lysosomal glycohydrolase α-acetylglucosaminidase (α-2-acetamido-2-deoxy-D-glucoside acetamidodeoxyglucohydrolase, EC 3.2.1.X, α-acetylglucosaminidase) is nearly absent in frozen organs and in cultured skin fibroblasts from patients with Type-B Sanfilippo syndrome.

MATERIALS AND METHODS

Kidney tissue was available from one patient with Sanfilippo syndrome; liver tissue was available from this patient and two others. Unfortunately, fibroblasts were not available from these three patients, and they were not classified as Sanfilippo Type A or B. Thus far, there are no evident clinical distinguishing features between Type-A and Type-B syndromes. One of these patients has been presented in detail as the prototype of Sanfilippo syndrome by McKusick (1). Frozen organs from patients with various other mucopolysaccharide storage diseases were also available. Tissues from controls and patients were stored frozen for periods of 2 months to 5 years before assay.

Skin fibroblast cultures were obtained from eight Sanfilippo patients, three with Type A, two with Type B, and three not subclassified. The subclassifications were made in the laboratory of Dr. Elizabeth Neufeld, National Institute of Health, Bethesda, Md. Fibroblast cultures from patients with other mucopolysaccharidoses were also available, as well as from parents of one of the patients.

All fibroblast cultures were grown as described (3–5), and were subcultured at least once before enzyme assay. Control fibroblast cultures were obtained from clinically normal children and adults of both sexes. Amniotic fluid cell cultures were taken from women undergoing amniocentesis during midpregnancy; they were selected from subjects in which a phenotypically normal baby was delivered. Lysosomal hydrolase enzyme activities increase progressively with time after subculture (4). Unless otherwise stated, enzyme assays were made 14 days after subculture; subcultures were begun at similar cell densities.

Fibroblast pellets were obtained by scraping cells from culture flasks, centrifuging at 3000 × *g* for 10 min, aspirating the supernatant medium, suspending the pellet in 0.95 M NaCl, centrifuging, aspirating the supernatant saline solution, and freezing the cell pellet at −20° for 1 day to 3 weeks before enzyme assay.

β-Galactosidase, β-acetylglucosaminidase, β-glucuronidase, β-glucosidase, α-galactosidase, and α-mannosidase were assayed in liver and fibroblast cultures with 4-methylumbelliferyl substrates (3–5).

Assay of *p*-nitrophenyl-α-L-fucosidase activity in fibroblast cultures was as follows. The cell pellet was weighed and homogenized in 5 volumes of distilled water in a ground-glass homogenizer. To 20 μl of homogenate was added 50 μl of *p*-nitrophenyl-α-L-fucopyranoside (1.5 mM in citrate–phosphate buffer, 0.1 M, pH 5.8), and the sample was incubated at 37° for 2 and 4 hr. The released *p*-nitrophenol was estimated as described below for assay of α-acetylglucosaminidase.

Assay of α-acetylglucosaminidase in liver, kidney, and cultured fibroblast pellets was as follows. The tissue or pellet was weighed and homogenized in 5 volumes of distilled water

in a ground-glass homogenizer. To 20 μl of homogenate was added 50 μl of *p*-nitrophenyl-α-D-*N*-acetylglucosaminide (1.0 mM in citrate–phosphate buffer, pH 4.5, 0.1 M with respect to phosphate), and the sample was incubated at 37° for 2 and 4 hr. The reaction was stopped by addition of 100 μl of 3% trichloroacetic acid, after which it was centrifuged at 3000 × *g* for 10 min. The supernatant was then aspirated, taking care not to disturb the sediment; 350 μl of glycine–carbonate buffer (0.25 M, pH 10) was added to the supernatant, and absorbance was read at 420 nm on a spectrophotometer. Tissue blanks for each sample were prepared in the same manner, with distilled water substituted for the substrate. Final absorbances were obtained by subtraction of tissue-blank readings from sample readings.

A pH–activity curve was determined for α-acetylglucosaminidase in human liver tissue by the use of homogenates prepared as above; however, the final pH of the homogenate was varied between 3.5 and 7.0 with citrate–phosphate buffers (0.1–0.2 M). The pH optimum of the enzyme in liver and cultured fibroblasts was 4.5. Under the conditions described, activity of α-acetylglucosaminidase was linear over 4 hr of incubation. When the homogenate dilution was varied, the activity was not linear. Weissmann *et al.* (6) have described similar results in studies of α-acetylglucosaminidase from pig liver; they attribute the nonlinearity to endogenous inhibitors, which they could precipitate with ammonium sulfate.

When homogenate protein concentrations were kept constant from one sample to another by maintaining the same ratio of tissue wet weight to homogenate diluent (1:5), assays of duplicates agreed within ±10%. I did not attempt to optimize the concentration of *p*-nitrophenyl-α-D-*N*-acetylglucosaminide, due to the limited quantities of substrate available.

RESULTS

In frozen liver tissue from two patients with Sanfilippo syndrome, activities of α-acetylglucosaminidase were less than 10% of normal. A similar diminution was found in the kidney of one of these patients. Elevated activity of α-acetylglucosaminidase was present in the liver tissue from one Sanfilippo patient (Table 1).

TABLE 1. *Alpha-acetylglucosaminidase in frozen organs*

Subjects	α-Acetylglucosaminidase
Liver	
Controls (13)	3.4 (1.8–6.3)
Mucopolysaccharidoses	
Hurler's	37.0
Hurler's	27.0
Hunter's	3.7
Sanfilippo (D.F.)*	0.4
Sanfilippo(W.S.)*	0.3
Sanfilippo(C.L.)*	13.0
Kidney	
Controls (2)	3.9, 9.6
Sanfilippo (D.F.)	0.2

Enzyme activities are expressed as nmol of *p*-nitrophenol cleaved per mg of protein per hr at 37°. Average and range of control values are given: numbers of controls studied are in parentheses.

* Patients were not classified as to Type A or Type B. For description of D. F. see (1).

TABLE 2. *Enzymatic activities in cultured skin fibroblasts*

Subjects	α-Acetylglucosaminidase (*A*)	α-Fucosidase (*B*)	Ratio *B*/*A*
Controls (17)	5.4 (2.1–12.6)	18.4 (2.3–41.9)	3.4 (2.0–6.3)
Mother of P.L.	2.4	26	10.6
Father of P.L.	2.8	34	12.1
Mucopolysaccharidoses			
Sanfilippo			
Type B (P.L.)	0.07	21	300
Type B (S.)	0.07	9	130
Type A (S.P.)	6.6	47	
Type A (M.P.)	7.2	52	
Type A (R.N.)	3.9	28	
Average and (range)	5.9(3.9–7.2)	42(28–52)	7
Unclassified (M-1)	3.4	11	
Unclassified (M-2)	2.7	9	
Unclassified (A.J.)	4.5	32	
Average and (range)	3.5(2.7–4.5)	17(9–32)	5
Hurler's	4.1	19	
Hunter's	4.5	16	
Hunter's	2.7	9	
Hunter's	5.3	22	
Average and (range)	4.2(2.7–4.5)	16(9–22)	4
Amniotic cultures (from normal pregnancies) (6)	4.6 (1.7–9.4)	55 (35–74, 3 cases)	12

Enzyme activities are expressed as nmol of *p*-nitrophenol cleaved per mg of protein per hr at 37°. Average and range of control values are given; numbers of cases studied are in parentheses.

When liver homogenates from two patients were mixed in equal proportions with those from controls or with those from patients with other mucopolysaccharidoses, the activities of α-acetylglucosaminidase were the average of the two starting homogenates. These results indicated that soluble endogenous inhibitors did not account for the profound reduction of α-acetylglucosaminidase in the two patients tested. The activity of α-acetylglucosaminidase was not diminished in liver tissue from patients with Hurler's and Hunter's diseases (nor from one Sanfilippo patient), indicating that mucopolysaccharide storage *per se* was not responsible for the deficiency of α-acetylglucosaminidase activity.

Activities of β-acetylglucosaminidase, β-glucuronidase, β-glucosidase, α-galactosidase, and α-mannosidase were either normal or elevated in liver tissue from the Sanfilippo patients. Activities of β-galactosidase were reduced to 30–50% of control values in all patients with mucopolysaccharide storage diseases.

α-Acetylglucosaminidase activity in fibroblast cultures from two Type-B Sanfilippo patients was barely detectable; values were less than 0.07 nmol cleaved per mg of protein per hr. A more sensitive assay technique will be necessary to find whether any α-acetylglucosaminidase activity is present in fibroblasts from such patients.

In one experiment, α-acetylglucosaminidase was assayed in fibroblasts from a single patient at 10, 14, and 21 days after subculture; the same degree of deficiency was found at each time. The deficiency of α-acetylglucosaminidase was present in cultures from the same patient, grown over a 3-month period (more than 10 cell generations). Mixture of fibroblast homogenates from controls (including those with Type-A Sanfilippo syndrome with those from Type-B Sanfilippo patients demonstrated that soluble endogenous inhibitors did not account for the striking deficiency of α-acetylglucosaminidase activity.

Activities of β-galactosidase, β-acetylglucosaminidase, β-glucuronidase, β-glucosidase, α-galactosidase, α-mannosidase, and α-fucosidase were within the normal range in fibroblasts from Type-B patients. No deficiency of α-acetylglucosaminidase activity was present in fibroblasts from patients with other mucopolysaccharidoses, including those with Type-A Sanfilippo (Table 2).

The specific activities of α-acetylglucosaminidase in cultured fibroblasts from both parents of one patient were in the low-normal range (Table 2). When ratios of α-acetylglucosaminidase to α-fucosidase activities were calculated, they were abnormally high (Table 2), indicating a deficiency of α-acetylglucosaminidase relative to α-fucosidase in cells from the heterozygous parents.

α-Acetylglucosaminidase activity was readily demonstrated in amniotic fluid cells, at levels similar to those found in cultured skin fibroblasts (Table 2). α-Fucosidase activity was higher in amniotic cells than in skin fibroblasts.

DISCUSSION

The data suggest that a deficiency of α-acetylglucosaminidase* is the primary enzyme defect in Type-B Sanfilippo syndrome. The defect is specific for α-acetylglucosaminidase. The enzyme is a lysosomal glycohydrolase, distinctly different from β-acetylglucosaminidase, α- and β-glucosidase, α-mannosidase, β-glucuronidase, α-L-fucosidase, β-D-xylosidase, α-acetylgalactosaminidase (6), and hyaluronidase (7). The defect persists over many cell generations. The enzyme is partially deficient in cells from obligate heterozygotes. The mucopolysaccharide that accumulates in Sanfilippo syndrome possesses α-linked *N*-acetylglucosamine residues (8), and appears to be stored in lysosomes (9). The deficiency of a lysosomal degradative enzyme, probably involved in heparan sulfate degradation, fits well with the impairment of mucopolysaccharide degradation (2) in cells from patients with Type-B Sanfilippo syndrome.

The Type-B correction factor is probably identical to α-acetylglucosaminidase. This factor, isolated from human urine, is destroyed by heating to 70° in 0.95 N NaCl for 10 min (2). In homogenates of cultured skin fibroblasts, prepared as described for assay above, I have found that the activity of α-acetylglucosaminidase is destroyed by heating at 70° for 10 min. If α-acetylglucosaminidase and Type-B correction factor are identical, the purified enzyme may prove to have clinical utility.

Recently, Kresse and Neufeld (10) have purified the Type-A corrective factor 850-fold from normal human urine, and have shown it to possess no α-acetylglucosaminidase activity, emphasizing again the genotype specificity of the two types of Sanfilippo syndrome. The Type-A corrective factor appears to be a heparan sulfate sulfatase (10).

The fact that activity for α-acetylglucosaminidase is present in normal amniotic fluid cells taken in midpregnancy indicates that Type-B Sanfilippo syndrome, a fatal hereditary disorder with a 25% recurrence risk, may be diagnosed prenatally by amniocentesis and enzyme assay, in the same manner as is now used for Tay-Sachs disease (11).

* Recently, I have found that the activity of α-acetylglucosaminidase with phenyl-α-D-*N*-acetylglucosaminide as substrate is also markedly diminished in tissues from patients with Type-B Sanfilippo syndrome.

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