SIALIDASE DEFICIENCY IN ADULT-TYPE NEURONAL STORAGE DISEASE

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1. Introduction

Recently, an adult-type neuronal storage disease, characterized by myoclonus, cerebellar ataxia, convulsive seizure, cherry red spots, skeletal dysplasia, mild gargoyle face, angiokeratoma and inguinal hernia was reported as 'A late form of familial amaurotic idiocy' [1] or 'Mucolipidosis' [2] and this disease is transmitted as autosomal recessive trait. Enzymatic study of this disease revealed decreased activity of β-galactosidase in leukocytes and cultured skin fibroblasts and suggested this disease was GM_1 gangliosidosis [3]. However, this possibility was ruled out by lipid analysis of affected sympathetic ganglia which showed marked accumulation of GM_3 and GM_2 ganglioside and not GM_1 ganglioside [3]. Together with accumulation of ganglioside in the nervous system, an increase of sialyl oligosaccharides in the urine of the patient [3] led us to study the sialyl compound metabolism in cultured fibroblasts and leukocytes. We report here a defect of α-*N*-acetylneuraminidase with increase of sialyl compound in fibroblasts of a patient with this disorder.

2. Materials and methods

Reagents: *N*-acetylneuraminic acid (NANA), fetuin (type III) and sialyllactose (type II) were obtained from Sigma Chemical Co., St. Louis. Sialyllactose was separated into two structural isomers, α-*N*-acetylneuraminosyl-(2→3)-lactose and α-*N*-acetylneuraminosyl-(2→6)-lactose by the method in [4]. [^{3}H]-sialyllactitol was obtained from New England Nuclear, Dreieich. (Content of radiochemical isomers: α-*N*-acetylneuraminosyl-(2→3)-lactitol, 90%; α-*N*-acetylneuraminosyl-(2→6)-lactitol, 10%; Spec act. 17.5 μCi/μmol.)

Fibroblasts were cultured from a skin biopsy of a patient with adult-type neuronal storage disease and normal controls. Clinical details of the patient were reported separately [3]. Sialic acid content of the tissue was determined by the method in [5], and protein was determined by the method in [6].

GM_3 ganglioside sialidase was assayed by the method in [7] with slight modification. We used GM_3 ganglioside, tritiated at the sphingosine base, as a substrate. After incubation, liberated lactosylceramide was separated from GM_3 ganglioside by TLC and the radioactivity of lactosylceramide was determined by a liquid scintillation counter. GD_{1a} ganglioside sialidase was assayed as follows: The 100 μl incubation volume contained 10 nmol [^{3}H]GD_{1a} ganglioside (spec. act. 61 μCi/μmol), cell homogenate containing 100 μg protein and 15 μmol acetate buffer (pH 4.2). The mixture was incubated for 1 h at 37°C and the [^{3}H]-GM_1 ganglioside formed, was determined by the method in [8]. Fetuin sialidase activity was assayed by the method in [9]. The sialic acid, liberated from fetuin was measured by GLC method [10] or mass fragmentography (M.S. et al., in preparation). Sialyllactose sialidase activity was assayed by the method in [11] for lysosomal sialidase, using α-*N*-acetylneuraminosyl-(2→3)-lactose. Liberated sialic acid was measured as above. Sialyllactitol sialidase was assayed

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography

Table 1
Sialidase activity with GM_3 and GD_{1a} ganglioside

	Leukocytes		Fibroblasts	
	GM_3	GD_{1a}	GM_3	GD_{1a}
Patient	0.64	1.48 ± 0.35[a]	0.12	0.58 ± 0.16[a]
Control (n=4)	0.56 ± 0.09	1.69 ± 0.28	0.14 ± 0.01	0.49 ± 0.26

[a] Mean value of 4 determinations

Enzyme activity: nmol/mg protein/h

as follows: The 100 μl incubation volume contained 100 nmol [^{3}H]sialyllactitol, cell homogenate containing 100–390 μg protein and 15 μmol acetate buffer (pH 4.2). The mixture was incubated for 1 h at 37°C and the [^{3}H]lactitol formed, was determined by the method in [12].

3. Results

GM_3 ganglioside and GD_{1a} ganglioside sialidase activity were normal in both fibroblasts and leukocytes of the patient (table 1). Total sialic acid content of cultured fibroblasts from the patient was increased to 2.4-fold of normal control (table 2). Activity of fetuin sialidase was assayed by incubation for 24 h at 37°C. Profound deficiency of this enzyme was found in the fibroblasts of the patient (table 3). Activity of the sialyllactose sialidase was assayed by incubation for 12 h at 37°C, using α-*N*-acetylneuraminosyl-(2→3)-lactose as a substrate. This enzyme was also deficient in the fibroblasts of the patient (table 3). In addition, sialidase deficiency was confirmed with [^{3}H]sialyllactitol as a substrate in the fibroblasts of the patient (table 3).

Table 2
Sialic acid content of fibroblasts

	nmol NANA/mg protein
Patient	17.1 ± 3.9[a]
Control (n=8)	7.1 ± 1.8

[a] Mean value of duplicate determinations of 4 different subcultures

Table 3
Sialidase activity of fibroblasts with sialyllactose, [^{3}H]sialyllactitol and fetuin

	Sialyllactose	Sialyllactitol	Fetuin
Patient	0.1	0.32 ± 0.04[a]	0.04
Control (n=4)	4.1 ± 0.9	6.8 ± 0.7	3.7 ± 1.0

[a] Mean value of 4 determinations

Enzyme activity: nmol/mg protein/h

4. Discussion

We examined one patient with an adult-type neuronal storage disease whose clinical and pathological findings were reported in [3].

Analytical data suggested that GM_3 ganglioside was accumulated in the sympathetic ganglia [3], and sialic acid content in skin cultured fibroblasts and sialyl oligosaccharides in urine were increased in the patient [3]. From these findings, the disturbances of sialyl compounds (ganglioside, sialyl glycoprotein or sialyl oligosaccharide) were supposed to exist in the patient.

The activities of the GM_3 ganglioside and GD_{1a} ganglioside sialidases were not decreased in this disease and the accumulation of GM_3 ganglioside was found not attributable to the primary defect of this sialidase. Similar ganglioside accumulation in neurons was reported in Hurler's syndrome [13–16] and mucopolysaccharidosis III [17], but the relationship of the lipid disturbance to the primary defect of mucopolysaccharide metabolism remained unclear.

The sialidase activities using fetuin, α-*N*-acetylneuraminosyl-(2→3)-lactose and α-*N*-acetylneur-

aminosyl-(2→3)-lactitol as substrates were severely decreased in the cultured fibroblasts of the patient. The terminal oligosaccharide structure of fetuin was reported to be α-*N*-acetylneuraminosyl-(2→3)-β-galactosyl-(1→4)-*N*-acetylglucosamine [18]. This data suggests an α-neuraminidase, which hydrolyses the α-*N*-acetylneuraminosyl-(2→3)-galactoside moiety of sialyl oligosaccharides and glycoproteins, is deficient in this disease.

Recently, patients with neuraminidase deficiency have been reported [19,20] who had the same neurological findings as our case, but lacked bony deformity and β-galactosidase deficiency, which were characteristic of our case. In contrast to our case, fetuin sialidase activity remained about 1/4th of normal control in their case. This discrepancy in this enzyme activity may explain the clinical differences in these two disorders.

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