Acidic Glycosaminoglycans in Liver from Five Patients with Mucopolysaccharidosis and Mucopolipidosis

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MINAMI, R., ABO K., TSUGAWA, S., OYANAGI, K. and NAKAO T. *Acidic Glycosaminoglycans in Liver from Five Patients with Mucopolysaccharidosis and Mucopolipidosis*. Tohoku J. exp. Med., 1981, 134 (2), 215-220 — Content, composition and molecular weight distribution of acidic glycosaminoglycans (GAGs) were determined in liver from five patients with genetic lysosomal storage diseases (Hurler syndrome, Hunter syndrome of severe type, Morquio syndrome, GM₁-gangliosidosis type II and I-cell disease). There was a 30- to 40-fold increase in GAGs content of liver from patients with Hurler and Hunter syndromes. The GAGs accumulated in the livers consisted mainly of heparan sulfate and dermatan sulfate, and had a much lower molecular weight than those from control liver. The major GAG accumulated in liver from Morquio syndrome was keratan sulfate, which was not found in the livers from control and other patients, and chondroitin-6-sulfate was also increased. The content and the composition of liver GAGs from GM₁-gangliosidosis and I-cell disease were similar to those of control liver. However, there was about a 33-fold increase in the amount of hexose on the liver GAGs from GM₁-gangliosidosis. A molar ratio of sialic acid to hexosamine was 1.35 for the liver GAGs from I-cell disease and that of hexose to hexosamine was 8.47, while they were 0.46 and 2.32, respectively, for the control liver. ——— acidic glycosaminoglycans; mucopolysaccharidosis; mucopolipidosis

Mucopolysaccharidosis (MPS) and mucopolipidosis (ML) are genetic lysosomal storage diseases. The respective specific enzyme deficiencies have recently been discovered except for mucopolipidosis II (I-cell disease) and III. Systematic investigations of acidic glycosaminoglycans (GAGs) accumulated in liver from patients with MPS are scanty and almost restricted to Hurler, Hunter and Sanfilippo syndromes. We have previously reported the accumulation of keratan sulfate (KS), which was not found in the control liver, in the liver affected by Morquio syndrome (Minami et al. 1979). On the other hand, it has been shown in the literature (Callahan and Wolfe 1970; Tsay and Dawson 1973) that KS-like substance was accumulated in the liver from GM₁-gangliosidosis.

In the present report, we have extended our study on liver GAGs from three

patients with MPS (Hurler, Hunter and Morquio syndromes) and two patients with ML (GM₁-gangliosidosis and I-cell disease).

MATERIALS AND METHODS

The liver tissues were obtained from six control individuals between 1 month and 13 years of age without evidence of liver disease and five patients with genetic lysosomal storage disease (Hurler syndrome, Hunter syndrome of severe type, Morquio syndrome, GM₁-gangliosidosis type II and I-cell disease) at autopsies. The diagnosis in all patients had been determined by typical clinical pictures and enzymatic findings. The livers were stored at -70°C until analysis.

The liver tissues were homogenized with 19 volumes of chloroform/methanol (2:1, v/v). After filtration, the residue was again treated with a reversed ratio of chloroform/methanol (1:2, v/v). The delipidized residue was incubated at 37°C in order to remove the solvent. The dry residue was digested with papain at 60°C for 24 hr. For each 1 g of dried tissue, 1–2 mg of the enzyme (20–40 units) in 4.5 ml of 0.1 M sodium acetate buffer, pH 5.5, containing 5 mM EDTA and 5 mM cysteine was used. The digested suspension was centrifuged at 3,000 rpm for 30 min. The precipitate was resuspended in the same buffer and the digestion was repeated. The volume of the combined supernatant was concentrated to a small volume. The concentrated supernatant was chromatographed on a 3×60 cm of Dowex 1×2 (200 to 400 mesh, Cl⁻ form) and eluted with water, 0.25 M and 4.0 M NaCl. 0.25 M and 4.0 M NaCl fractions were desalted on a Sephadex G-25 column, and further each fraction was concentrated (Brunngraber et al. 1971; Minami et al. 1979). The absence of GAGs in water and 0.25 M NaCl fractions was confirmed by electrophoresis. Finally, 4.0 M NaCl fraction was analyzed for liver GAGs.

Electrophoretic separation of GAGs was performed by the method of Seno et al. (1970).

In order to determine relative proportions of chondroitin-4-sulfate (C4S), chondroitin-6-sulfate (C6S), dermatan sulfate (DS) and hyaluronic acid (HA), the method I of Saito et al. (1968) using chondroitinase ABC and AC, and the method of Ohya and Kaneko (1970) using streptomyces hyaluronidase were employed. The following procedure was developed for measurement of heparan sulfate (HS) and KS. Electrophoretic separation of the chondroitinase ABC-undigested GAGs was performed, and HS and KS were estimated by a densitometer.

The molecular weight distribution of GAGs was determined by the method of Constantopoulos et al. (1969). 600 µg of GAGs as uronic acid were applied to a 0.8×80 cm column of Sephadex G-200, and eluted with 0.25 M NaCl. 2.0 ml of fractions were collected, and analyzed for uronic acid and hexose.

Uronic acid was determined by the Carbazole reaction (Dische 1947), hexose by the method of Smith and Montgomery (1956), total sulfate by the method of Dodgson and Price (1962), N-sulfate by the method of Lagunoff and Warren (1962), hexosamine by the method of Svennerholm (1956) and sialic acid by the Warren assay (1959).

RESULTS

The results of analyses of GAGs content are summarized in Table 1. There was at least a 40-fold increase in the GAGs content (as uronic acid) of liver from Hurler syndrome and a 30-fold increase in Hunter syndrome, compared with the GAGs content of control liver. The amount of GAGs as uronic acid in the liver from Morquio syndrome was 2 times greater than that of control liver, and the amount as hexosamine was 4 times greater. The amount of GAGs in the liver from GM₁-gangliosidosis was at a normal level, but there was about a 33-fold increase

TABLE 1. *Analytical data on liver acidic glycosaminoglycans in control and genetic lysosomal storage diseases*

	mg/g wet weight					
	Hexosamine	Uronic acid	Hexose	Total sulfate	N-sulfate	Sialic acid
Hurler syndrome	4.22 (1.00)	4.15 (0.90)	1.65 (0.39)	1.22 (0.54)	0.95 (0.42)	0.09 (0.01)
Hunter syndrome (severe type)	3.14 (1.00)	3.00 (0.88)	1.82 (0.58)	1.07 (0.63)	0.72 (0.43)	0.08 (0.02)
Morquio syndrome	0.44 (1.00)	0.24 (0.50)	0.53 (1.20)	0.33 (1.41)	0.16 (0.68)	0.05 (0.07)
GM ₁ -gangliosidosis (type II)	0.14 (1.00)	0.20 (1.32)	6.99 (7.05)	0.06 (0.83)	0.04 (0.51)	0.03 (0.17)
I-cell disease	0.03 (1.00)	0.06 (1.82)	0.26 (8.47)	0.03 (1.88)	0.02 (1.35)	0.07 (1.35)
Control (<i>n</i> =6)	0.09 (1.00)	0.08 (0.82)	0.21 (2.32)	0.03 (0.70)	0.02 (0.38)	0.06 (0.46)
Range	0.06-0.14	0.06-0.11	0.13-0.29	0.02-0.05	0.01-0.04	0.03-0.14

Molar ratios based on hexosamine=1.00 are given in the parentheses.

in the amount of hexose in GAGs. The GAGs content in the liver from I-cell disease was also at a normal level, but a molar ratio of hexose to hexosamine was 8.47 and that of sialic acid to hexosamine was 1.35, as compared with 2.32 and 0.46 for the control liver, respectively.

The composition of liver GAGs of control and the patients is shown in Table 2. The liver GAGs in Hurler and Hunter syndromes consisted mainly of HS and DS. In the liver affected by Morquio syndrome, KS comprised 33.9% of the total GAGs, while KS was not detected in the livers from control individuals and other patients. C6S was also markedly increased in the liver from Morquio syndrome. On the other hand, the composition of the liver GAGs from GM₁-gangliosidosis and I-cell disease was similar to that of control liver.

TABLE 2. *Composition of liver glycosaminoglycans in control and genetic lysosomal storage diseases*

	Hyaluronic acid (%)	Chondroitin 4-sulfate(%)	Chondroitin 6-sulfate(%)	Dermatan sulfate (%)	Heparan sulfate (%)	Keratan sulfate (%)
Hurler syndrome	trace	2.2	2.3	31.4	64.1	n.d.
Hunter syndrome (severe type)	1.1	1.4	2.1	30.8	64.6	n.d.
Morquio syndrome	trace	6.4	15.5	1.6	33.9	39.9
GM ₁ -gangliosidosis (type II)	0.3	1.4	0.3	1.3	96.7	n.d.
I-cell disease	2.0	0.7	0.8	3.1	93.4	n.d.
Control (<i>n</i> =6)	7.0	2.7	3.8	2.4	84.1	n.d.
Range	1.3-13.0	0.9-4.7	trace-7.8	0.8-7.3	74.8-93.2	

n.d., not detected.

Fig. 1 shows the molecular weight distribution of liver GAGs in the control and the patients. The elution curve of control liver consisted of two peaks. In the livers of Hurler and Hunter syndromes, the elution patterns of GAGs showed a considerable shift towards the small molecular weight. The other three patients exhibited the elution patterns similar to that of control concerning uronic acid on GAGs. The elution patterns, however, concerning hexose on GAGs from Morquio syndrome and I-cell disease displayed to be shifted towards the small molecular weight, while the elution curve from GM₁-gangliosidosis showed the equal distribution between the small molecular weight and the large molecular weight.

Fig. 1. Sephadex G-200 gel filtration elution patterns for acidic GAGs from liver of control individual and patients with genetic lysosomal storage diseases. ●, uronic acid; ○, hexose; ↑, blue dextran. A: Control. B: Hurler syndrome. C: Hunter syndrome (severe type). D: Morquio syndrome. E: GM₁-gangliosidosis (type II). F: I-cell disease.

DISCUSSION

In accordance with the literature (Gordon and Haust 1971; Constantopoulos et al. 1976), large amounts of HS and DS were accumulated in the livers affected by Hurler and Hunter syndromes. The content and the composition of the GAGs accumulated in the liver from Hurler syndrome were similar to those of Hunter syndrome. Those results might not be explained by a deficiency of the respective specific enzymes required for the liberation of idurosulfate from GAGs, because the unequal distribution of HS and DS in various tissues and in urine from

Hurler and Hunter syndromes was reported (Constantopoulos and Dekaban 1975; Constantopoulos et al. 1976; Kofoed et al. 1978), and because the content and the composition of GAGs in tissues from Hurler and Scheie syndromes which are an α -L-iduronidase deficiency were quite different (Constantopoulos et al. 1976).

We have previously reported the accumulation of KS in the liver affected by Morquio syndrome (Minami et al. 1979), while KS was not detected in the liver from GM₁-gangliosidosis by this procedure on isolation of liver GAGs. However, the GAGs fraction in GM₁-gangliosidosis contained markedly hexose. The increased hexose content might result from galactose in the linkage region between GAGs and peptides chain, because galactose cannot be liberated from GAGs in GM₁-gangliosidosis owing to a deficiency of β -galactosidase.

Although the accumulation of GAGs in fibroblasts from patients with I-cell disease was reported (Schmickel et al. 1975), the liver from I-cell disease showed normal content and composition of GAGs except for a molar ratio of hexose to hexosamine and that of sialic acid to hexosamine. The increased hexose and sialic acid contents might result from the secondarily reduced β -galactosidase (Leroy et al. 1972) and sialidase (Thomas et al. 1976) activities, though the results remain still unresolved.

From the results of our investigations, we think that GAGs are not accumulated in the livers from GM₁-gangliosidosis or I-cell disease, unlike the livers from MPS, though patients with GM₁-gangliosidosis and I-cell disease have severe bony deformities similar to those of patients with MPS.

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