Molecular Genetics and Metabolism Reports 7 (2016) 87–91

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: www.elsevier.com/locate/ymgmr

Short Communication

The levels of urinary glycosaminoglycans of patients with attenuated and severe type of mucopolysaccharidosis II determined by liquid chromatography-tandem mass spectrometry

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ARTICLE INFO

Article history:
Received 27 March 2016
Accepted 27 March 2016
Available online 22 April 2016

Keywords:
Mucopolysaccharidosis
Glycosaminoglycans
LC-MS/MS
Hunter syndrome

ABSTRACT

Glycosaminoglycans (GAGs) play important roles on the regulation of extracellular signaling, neuronal development, and cartilage maintenance. The extracellular concentration of total GAGs has been used as an established measure for the diagnosis of mucopolysaccharidoses (MPSs). Heparan sulfate (HS), Dermatan sulfate (DS) and chondroitin sulfate are known to be elevated in the GAGs under pathological conditions associated with MPS. Furthermore, the selective accumulation of disease-specific one of, or a combination of, them has also been used for the estimation of subtypes of MPS. A previously developed method [Auray-Blais C et al. Molecular Genetics and Metabolism 102 (2011) 49–56.] measures the concentration of GAGs using liquid chromatography with tandem mass spectrometry (LC-MS/MS) with higher precision. To ask whether the selective accumulation of HS and DS in the urine of MPS II patients discriminate the attenuated and severe type of MPS II, we examined the concentrations of HS and DS by this methodology. Compared to the healthy controls, we found a marked elevation of HS and DS in all of the MPS II-affected patients. Among patients who received ERT with confirmed elevation of antibody titer, the concentrations of HS in the urine of patients with attenuated type were lower than those with severe type of MPS II. In these patients, the concentrations of DS by LC-MS/MS and of total GAG by DMB failed to depend on the accumulation of antibody. These results suggest that the LC-MS/MS method employed in this study might discriminate the subtypes of MPS II in different clinical background.

1. Introduction

Mucopolysaccharidoses (MPSs) are lysosomal storage disorders (LSDs) characterized by inherited deficiencies of lysosomal enzymes essential for the degradation of mucopolysaccharides [1]. A failure of this metabolism induces selective accumulation of glycosaminoglycans (GAGs) in the blood, urine, and body tissues. Multiple enzymes are involved in clinical presentations of MPS subtypes, thus a defect or significant impairment of the responsible enzyme activity leads to the accumulation of selective GAGs including heparan sulfate (HS), dermatan sulfate (DS) and chondroitin sulfate (CS). Among all MPS subtypes, only MPS II is an X-linked disorder, while the others are autosomal recessive. The clinical manifestation of MPSs involves skeletal deformities, progressive coarse facial features, and organomegaly with mental retardation in some MPS subtypes. The diagnosis based on clinical presentation is normally inconclusive for all MPS subtypes; thus, biochemical and/or genetic confirmatory examination are always required. Among various assays, biochemical measurement of enzyme activity has been accepted as the most promising measure, because some MPS subtypes such as MPS III and IV are caused by four and two distinct enzymes, respectively.

Several therapeutic strategies for MPSs are currently available. The most widely used treatment is enzyme replacement therapy (ERT), which supplies the deficient enzyme intravenously [2–3]. This therapy improves most systemic manifestation, whereas this is known to be ineffective for intellectual decline and bone destruction under current formulation. To overcome this, a recent study indicated the efficacy of intrathecal administration of iduronate-2-sulfatase (IDS) enzyme due to the significant attenuation of accumulating GAGs in the cerebrospinal fluids (CSFs) [4]. Additionally, a recombinant IDS enzyme that is fused to the fragment of antibody against human insulin receptor has been developed [5]. This strategy aims to facilitate the penetration of IDS enzyme through blood brain barrier via the receptor. In addition to these

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http://dx.doi.org/10.1016/j.ymgmr.2016.03.009

Table 1
Recovery of the spiked GAGs from the urine of a healthy subject.

Additive	Added concentration (μg/mL)	Recovered concentration (μg/mL)	Recovery[a] (%)	Replicate *n*
HS	10	8.2 ± 0.7	82 ± 7	5
DS	10	8.9 ± 0.5	89 ± 5	5
CS[b]	10	16.6 ± 4.7	166 ± 47	3

Data were expressed as mean ± SD in at least triplicate determination.
[a] Recovery is defined as the concentration of recovered GAGs/the concentration of added GAGs.
[b] Endogenous concentration of CS was 60.0 μg/mL.

renovated enzyme agents, hematopoietic stem cell transplantation has also been performed [6–7]. A recent study demonstrated that the levels of total GAGs in the CSFs discriminates the patients of MPS II with and without cognitive impairment [8].

For diagnostic purposes, the levels of GAGs in clinical specimens have been determined by several methods. One procedure involves alkolytic pretreatment of GAGs followed by the measurement of concentrations of GAG-derived disaccharides using LC-MS/MS [9–14]. Based on this technique, the effectiveness of ERT for MPS II has been clearly shown in the several studies [10–11,14]. Similarly, the detection of a trace amount of HS in the CSF of MPS I-affected individuals has also been reported [9]. Thus, we hypothesized whether the disease subtypes of MPS, such as the attenuated and severe type of MPS II, could be identified using this LC-MS/MS assay.

2. Experimental procedure

2.1. Reagents

HS, DS, CS and methanolic 3N-HCl were obtained from Sigma Aldrich (St Louis, MO). Acetonitrile and methanol were purchased from Fischer Scientific (Tokyo, Japan). Isopropanol was purchased from Wako Pure Chemicals (Tokyo, Japan). Deionized water was obtained from a Milli-Q water system (Millipore, Milford, MA). Ammonium acetate and formic acid were purchased from Kanto Chemical (Tokyo, Japan). The other reagents used in this study were of the highest grade commercially available.

2.2. Patients

After informed consent was obtained, urine samples were collected from MPS patients whose diagnosis was confirmed by the detection of either defective or significantly impaired enzyme activity and/or by mutation analysis. We examined the urinary specimens collected from 8 MPS II patients (4 patients of attenuated type and 4 patients of severe type, respectively) and 4 healthy controls. All of the patients with MPS II have been received ERT. Ages of the patients of attenuated and severe type of MPS II as well as those of the healthy controls at sample collection were 10–40, 10–17, and 8–43, respectively. The mean ages of ERT initiation for the patients with attenuated and severe type of MPS II were 15.0 and 6.5 years old, respectively. The mean duration of ERT for the patients with attenuated and severe type of MPS II were 5.5 and 6.5 years, respectively. The mean ages of sample collection of the patients with attenuated and severe type of MPS II were 21.5 and 13.0 years old, respectively. Among 4 and 4 patients of the attenuated and severe type of MPS II, 2 and 2 patients were positive for antibody against IDS. Two patients of the attenuated type of MPS II had not been determined the antibody titer and two patients of severe type of MPS II were reported negative for antibody production.

2.3. Approval by institutional Research Ethics Board

This study was approved by the Research Ethics Board of the National Center for Child Health and Development.

2.4. Sample preparation and analysis by LC-MS/MS

The preparation and analysis of urine samples by LC-MS/MS assay has been previously reported [11–12]. In brief, an aliquot of urine samples (50 μL) was evaporated to dryness using a vacuum centrifuge (model CVE3100, EYELA, Tokyo, Japan). The residue was incubated with methanolic 3N-HCl (100 μL) at 65 °C for 75 min, followed by dryness with nitrogen. The residue was then dissolved in the mobile phase of UPLC (100 μL). An aliquot (0.5 μL) was injected into a Quattro Premier mass spectrometer equipped with an Acquity UPLC system (Waters Corporation, Milford, MA). Disaccharides were separated on an Atlantis T3 C18 column (1.7 μm, 2.1 × 50 mm, Waters Corporation) over 10 min at 0.1 mL/min as described in Supplementary Tables 1–3. The data were acquired by multiple reaction monitoring (MRM) using the specific transitions corresponding to HS, DS and CS, respectively. Before injection, all of the clinical samples were passed through a disposable filter (Fast Remover for Protein, 0.45 μm, GL Sciences, Tokyo, Japan) to minimize the ion suppression for measurement by endogenous contaminants.

2.5. Total GAG assay by 1,9-dimethylmethylene blue (DMB)-based colorimetric method

Total GAG was determined using the Blyscan kit (Blyscan Sulfated Glycosaminoglycan Assay), a colorimetric procedure using 1,9-dimethylmethylene blue (DMB) (Biocolor Ltd., Northern Ireland, UK) [12].

2.6. Creatinine assay

Urinary creatinine concentrations were determined spectrophotometrically using the Creatinine Colorimetric Assay kit (Cayman Chemicals, Ann Arbor, MI) [15].

2.7. Statistical analysis

Data were expressed as either mean ± SD or mean ± SEM, where indicated. Mean values of the two groups were compared using a Student's *t*-test and the difference was considered statistically significant when $P < 0.05$.

3. Results and discussion

3.1. Validation of methodology

To exclude the possibility that the co-migration of endogenous materials with HS might take place, we first examined whether the spiked HS could be quantitatively recovered in our experimental procedure. As shown in Table 1, the known amount of HS (i.e. 10 μg/mL) spiked into

Table 2
The values of intra- and interday CV (%) for the concentrations of HS, DS and CS of the MPS II-affected individuals using LC-MS/MS.

MPS II	Sample collection (y)	Intraday CV (%)			Interday CV (%)
		HS ($n = 5$)	DS ($n = 5$)	CS ($n = 5$)	HS ($n = 3$)
Attenuated type	23	4.8	1.0	3.9	12.9
	10	12.7	1.5	2.2	28.1
	13	5.0	2.3	3.3	14.3
	40	7.1	2.3	0.3	9.7
Severe type	17	7.6	1.5	3.0	8.0
	10	9.9	2.4	3.1	15.5
	14	9.9	3.4	5.2	13.4
	11	5.7	1.3	3.8	9.8

CV, coefficient of variance.