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Evaluation of cerebrospinal fluid heparan sulfate as a biomarker of neuropathology in a murine model of mucopolysaccharidosis type II using high-sensitivity LC/MS/MS

Noboru Tanaka*, Sachiho Kida, Masafumi Kinoshita, Hideto Morimoto, Tadao Shibasaki, Katsuhiko Tachibana, Ryuji Yamamoto

Research Division, JCR Pharmaceuticals, 2-2-9 Murotani, Nishi-ku, Kobe 651-2241, Japan

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ABSTRACT

Mucopolysaccharidosis type II (MPS II or Hunter syndrome) is a lysosomal storage disorder caused by a deficiency of iduronate-2-sulfatase (IDS), an enzyme that catabolizes glycosaminoglycans (GAGs) including heparan sulfate (HS) and dermatan sulfate (DS). GAG accumulation leads to severe neurological and somatic impairments. At present, the most common treatment for MPS II is intravenous enzyme replacement therapy; however, the inability of recombinant IDS to cross the blood-brain barrier (BBB) restricts therapeutic efficacy for neurological manifestations. We recently developed a BBB-penetrating IDS fusion protein, JR-141, and demonstrated its ability to reduce GAG accumulation in the brain of human transferrin receptor knock-in and *Ids* knock-out mice (*TFRC*-KI/*Ids*-KO), an animal model of MPS II, following intravenous administration. Given the impossibility of measuring GAG accumulation in the brains of human patients with MPS II, we hypothesized that GAG content in the cerebrospinal fluid (CSF) might serve as an indicator of brain GAG burden. To test this hypothesis, we optimized a high-sensitivity method for quantifying HS and DS in low-volume samples by combining acidic methanolysis and liquid chromatography-tandem mass spectrometry (LC/MS/MS). We employed this method to quantify HS and DS in samples from *TFRC*-KI/*Ids*-KO mice and revealed that HS but not DS accumulated in the central nerve system (CNS). Moreover, concentrations of HS in CSF correlated with those in brain. Finally, intravenous treatment with JR-141 reduced levels of HS in the CSF and brain in *TFRC*-KI/*Ids*-KO mice. These results suggest that CSF HS content may be a useful biomarker for evaluating the brain GAG accumulation and the therapeutic efficacy of drugs in patients with MPS II.

1. Introduction

Mucopolysaccharidosis type II (MPS II; also known as Hunter syndrome) is caused by deficiency of the lysosomal enzyme iduronate-2-sulfatase (IDS) [1, 2]. MPS II is an X-linked autosomal recessive disorder that is pathologically characterized by the body-wide accumulation of glycosaminoglycans (GAGs), mostly heparan sulfate (HS) and dermatan sulfate (DS). Defects in GAG lysosomal storage principally damage the heart, lung, bone, muscle, gut, skin, and brain [3]. However, patients with MPS II exhibit significant heterogeneity in clinical manifestations. Patients with severe MPS II exhibit progressive neurological impairments mainly characterized by cognitive dysfunction [4]. At present, the available treatment options for MPS II include allogeneic hematopoietic stem cell transplantation and enzyme replacement therapy (ERT) [5, 6]. ERT is the most widely used approach; it facilitates GAG degradation and stabilizes visceral symptoms of MPS II [7–10], but is ineffective for the management of neurological complications due to the inability of recombinant human IDS (hIDS) to cross the blood-brain barrier (BBB) [8, 11].

Recently, we developed a BBB-penetrating technology "J-Brain Cargo®" and structured a BBB-penetrating fusion protein composed of an anti-human transferrin receptor (hTfR) antibody and intact hIDS [12]. After intravenous administration to hTfR knock-in (*TFRC*-KI) mice, this fusion protein, JR-141, crosses the BBB via receptor-mediated transcytosis of transferrin and distributes in the brain. In a previous study, we found that JR-141 significantly decreased brain and peripheral GAG accumulation in the *TFRC*-KI/*Ids*-KO murine model of MPS II [12]. These results suggest that JR-141 may exert therapeutic effects on GAG accumulation in the brain of MPS II patients.

Evaluating the efficacy of JR-141 requires the direct measurement

* Corresponding author.
E-mail address: tanaka-nobor@jcrpharm.co.jp (N. Tanaka).

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of GAG concentrations in the brain; however, this is not a practical approach in humans. A limited number of reports have quantified GAGs in the cerebrospinal fluid (CSF) of patients with lysosomal storage diseases [13–16], but the exact relationship between concentrations in the CSF and brain are unclear. Here, we examined the usefulness of CSF GAG concentrations as a biomarker for brain GAG accumulation in *TFRC*-KI/*Ids*-KO mice. For this purpose, we optimized a previously reported highly sensitive method for quantifying HS and DS in low-volume samples using liquid chromatography-tandem mass spectrometry (LC/MS/MS) [16–18].

2. Experimental procedures

2.1. Reagents

Heparan sulfate was purchased from Iduron Ltd. (Manchester, UK). Methanolic 3 M HCl, methanol-d_4, ammonium hydroxide, acetyl chloride, and dermatan sulfate were obtained from Sigma Aldrich Co. (St Louis, MO). 2,2-Dimethoxypropane was obtained from Tokyo Kasei Company Ltd. (Tokyo, Japan). Acetonitrile and ammonium acetate were obtained from Wako Pure Chemical Industries (Osaka, Japan). Recombinant human iduronate-2-sulfatase (idursulfase, Elaprase®) was obtained from Shire Human Genetic Therapies Inc. (Cambridge, MA).

2.2. Animals

TFRC-KI/*Ids*-KO mice were generated as described previously [12]. Briefly, *Ids*-KO mice were interbred with *TFRC*-KI mice. *TFRC*-KI mice were also used as wild-type controls. The analyses used 9–12-week-old male mice. All animal experiments were conducted in accordance with the Guidelines for Animal Experiments at JCR Pharmaceuticals Co., Ltd.

2.3. JR-141 administration

JR-141 was produced as described previously [12]. Briefly, the JR-141 fusion protein was designed as hIDS fused to a humanized anti-hTfR antibody at the carboxyl terminus of the heavy chain. JR-141 was stably expressed in Chinese hamster ovary (CHO) cells and the released fusion protein was purified from conditioned medium using conventional procedures. JR-141 was administered intravenously to *TFRC*-KI/*Ids*-KO mice via the tail vein once per week for 8 weeks (n = 4–5 per group). Idursulfase, which is currently licensed as an intravenous treatment for MPS II, was also used as a control treatment. One week after the last administration, animals were sacrificed for the collection of CSF, serum, brain, and heart tissue. Urine collection was performed for 3 consecutive days after the last administration and the samples were subjected to HS and DS determination.

2.4. Internal standard preparations

Internal standards were derived in-house from deuteriomethanolysis of GAG standards comprising HS and DS, essentially as described previously [16, 17]. ^{2}HCl was formed in $C^2H_3O^2H$ by adding 240 µL acetyl chloride to 1.5 mL methanol-d_4. For the preparation of HS and DS internal standards, 40 µL of HS and DS solutions (5.0 mg/mL) were dried under nitrogen and the residues were reconstituted with 400 µL of deuterated solvent and incubated at 65 °C for 75 min. The solution was again dried under nitrogen, the residues were reconstituted with 500 µL of MeCN/water (9/1, *v*/v), and the solution was sonicated for 30 min. HS- or DS-internal standards were stored at less than −15 °C.

2.5. Experimental LC/MS/MS parameters

LC/MS/MS was performed on a QTRAP5500 mass spectrometer (AB Sciex, Foster City, CA) equipped with a Nexera system with

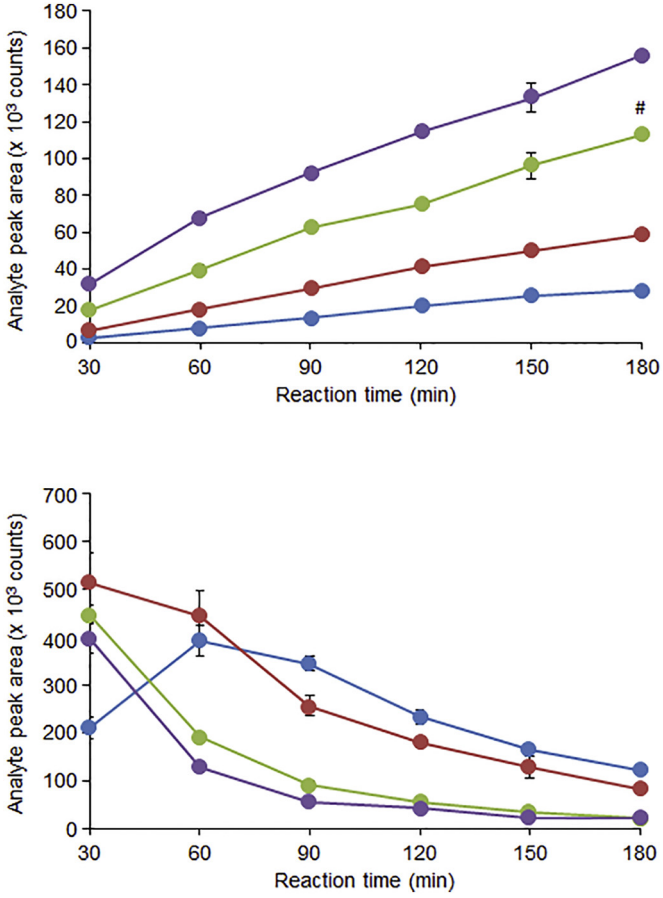

Fig. 1. Optimization of acidic methanolysis of heparan sulfate (HS) and dermatan sulfate (DS).
HS and DS were hydrolyzed by acidic methanolysis at 65–80 °C for 30–180 min and the products were reconstituted with HS and DS internal standards and subjected to LC/MS/MS. Analyte peak areas are shown for HS (upper panel) and DS (lower panel). Values are expressed as the mean ± SD of 3 measurements. #, mean of 2 measurements. Blue, red, green, and purple circles indicate 65, 70, 75, and 80 °C, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

autosampler (Shimadzu, Kyoto, Japan). The LC/MS/MS parameters were determined as described previously [16], with several modifications to improve the sensitivity. In detail, an Acquity UPLC® BEH Amide column (1.7 µm, 2.1 mm × 150 mm; Waters Corp.) was heated to 60 °C under a flow rate of 0.4 mL/min with a stepwise elution from mobile phase A (50 mmol/L ammonium formate in water) to mobile phase B (MeCN/50 mmol/L ammonium formate in water [97/3, v/v]) as follows: (1) elution for 0–5 min with 100% of B; (2) 7.0 min of a linear gradient of 100–80% of B; (3) elution for 7.0–12.5 min with 80% of B; (4) wash for 2 min with 30% of B; and finally, (5) column equilibration for 5 min with 100% of B. The eluate was directly infused into the mass spectrometer and the MS/MS analysis was performed in positive ion mode using electrospray ionization. The parameters of the mass spectrometer were set as follows: curtain gas, 30.0 psi; collision gas, 8.0 psi; ion source gas 1, 70.0 psi; ion source gas 2, 70.0 psi; ion spray voltage, 5500 V; declustering potential, 45 V; entrance potential, 10 V; and heater gas temperature, 425 °C. The collision energies were set at 21 V for HS and 12 V for DS. Data acquisition was performed by selected reaction monitoring using the protonated molecular ion transition mass-to-charge ratio (m/z) 384.1 > 162.1 for HS-derived disaccharides and m/z 426.1 > 236.1 for DS-derived disaccharides. The transitions m/z 390.2 > 162.1 corresponding to [$^2H_6$]HS-derived disaccharides (HS-internal standard) and m/z 432.2 > 239.2 for [$^2H_6$]DS