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A Cerebrospinal Fluid Collection Study in Pediatric and Adult Patients With Hunter Syndrome

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Abstract

Hunter syndrome (mucopolysaccharidosis II [MPS II]) is characterized by lysosomal glycosaminoglycan (GAG) accumulation. Although a majority of patients with MPS II experience neurocognitive involvement, few data are available on cerebrospinal fluid (CSF) GAG levels in these patients. This study measured GAG levels in CSF collected from 9 patients with MPS II, including 4 adults (aged ≥ 18 years) with normal cognition, and 5 children, 3 of them with cognitive impairment. The CSF total GAG levels were generally higher in the 3 patients with cognitive impairment (range 842.9-2360.9 ng/mL) versus those with normal cognitive status (range 356.8-1181.1 ng/mL). Heparan sulfate levels, as measured by mass spectrometry, generally followed a similar pattern, with patients with the severe phenotype having the highest values. These data, limited by small sample size, suggest CSF GAG levels and heparan sulfate levels may be higher in patients with cognitive impairment versus patients with cognitively intact MPS II.

Keywords

cerebrospinal fluid, glycosaminoglycan levels, lumbar puncture, mucopolysaccharidosis II, cognitive impairment

Introduction

Hunter syndrome, also known as mucopolysaccharidosis II (MPS II), is a rare lysosomal storage disorder caused by deficiency of iduronate-2-sulfatase, which leads to progressive accumulation of glycosaminoglycans (GAGs), mostly heparan sulfate (HS) and dermatan sulfate (DS), in nearly all organs and body tissues.[1] Accumulation of HS and DS affects multiple organ systems and manifests clinically as an array of signs and symptoms, including facial dysmorphism, organomegaly, joint stiffness and contractures, pulmonary dysfunction, myocardial enlargement, and valvular dysfunction, with decreased life expectancy.[2,3] An estimated two-thirds of patients have the severe phenotype characterized by cognitive impairment and death typically in the second decade of life. The remaining third of patients, with an attenuated phenotype, maintain intact mental function, are usually diagnosed later and experience slower disease progression with life expectancy of 20 to 30 years or longer.[2,4]

Limited data are available on cerebrospinal fluid (CSF) GAG levels in patients with MPS II, including whether these levels differ between patients with the severe and attenuated phenotypes.[5,6] Clarification of these factors could facilitate diagnosis and treatment of neurocognitive impairment in MPS II and contribute to understanding its pathophysiology.

The HGT-HIT-072 study (NCT01449240) was conducted to determine the levels of CSF GAG, including DS and HS, in

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adult and pediatric patients with MPS II, and the correlation of these levels with the patients' demographic and clinical characteristics, and cognitive status.

Methods

Patients

Adult (aged ≥18 years) and pediatric (aged <18 years) patients with documented MPS II were enrolled. All patients were being treated with intravenous idursulfase. Patients receiving investigational enzyme replacement therapy with intrathecal idursulfase were excluded. Pediatric patients were eligible to participate only if they had been scheduled before screening to undergo a nonstudy-related lumbar puncture (LP) or other medical or diagnostic procedure requiring general anesthesia. Adult patients voluntarily consented to have an elective LP. Adult patients were required to have an intelligence quotient of ≥78 determined through cognitive assessment at or within 3 months before screening/baseline. No formal cognitive testing was required for pediatric patients. All patients or their legally authorized representatives were required to voluntarily sign an institutional review board/independent ethics committee–approved informed consent form before CSF sample collection.

Patients excluded at screening had a history of complications from previous LPs or posed technical challenges in performing one; had received a hematopoietic stem cell transplant; had taken aspirin, nonsteroidal anti-inflammatory drugs, or other medications that could affect blood clot formation within 7 days before LP; or had ingested such medications within 7 days before any study-related procedure in which a change in potential blood clot formation would be deleterious.

Study Design

Study procedures complied with Good Clinical Practice as described in the 21 Code of Federal Regulations (CFR) Parts 50, 56, and 312 and the International Conference on Harmonisation Guidelines as well as with the ethical principles described in the Declaration of Helsinki. Informed consent from all patients and institutional review board/independent ethics board approval for the study were obtained. The study had a 2-week screening/baseline period for laboratory and cognitive assessments followed by CSF collection (via LP or other previously scheduled procedure, allowing access to CSF such as intracranial pressure monitoring device insertion [pressure bolt procedure] or cervical spinal cord decompression). A telephone follow-up for safety evaluation was conducted approximately 1 week after CSF collection (day 8).

Screening/baseline procedures included physical examination, medical history, and vital signs for all patients. Baseline serum chemistry, hematology, and urinalysis were performed only for adult patients undergoing elective LP. Adult patients also underwent cognitive evaluation by a certified psychologist or by qualified staff under the supervision of a licensed psychologist, using the Wechsler Adult Intelligence Scale–Fourth Edition (WAIS-IV).[7] Although cognitive evaluation of pediatric patients was not required, investigators were asked to record their opinion about the patient's cognitive status. For pediatric patients unable to complete screening/baseline procedures because of their previously scheduled, nonstudy-related procedure, medical history and safety data were collected by review of medical charts. Patients undergoing LP were monitored for at least 2 hours after the procedure before discharge.

Outcome and Safety Measures

The primary outcome measure was total GAG concentration in CSF. Safety measures consisted of documentation of adverse events (AEs) and evaluation of each AE by investigators for severity, seriousness, and relatedness to study procedures.

Pharmacodynamic/Biomarker Analysis

Total CSF GAG concentrations were determined using a thrombin activity assay. The CSF samples were preincubated with human heparin cofactor II (HC II) and then incubated with a fixed amount of thrombin and 0.5 mmol/L chromogenic substrate S-2238 in assay buffer. Glycosaminoglycan in CSF samples binds to HC II, which in turn accelerates thrombin inactivation. The GAG concentrations were calculated from a DS calibration curve. Non-DS GAG concentrations were determined by the same method after treatment of the samples with chondroitinase B, which specifically cleaves DS. The level of total HS in CSF was determined by liquid chromatography–tandem mass spectrometry (LC-MS/MS). Briefly, HS in the CSF was first extracted using an anion-exchange resin and then digested by a combination of enzymes including heparinases I, II, and III. The resultant HS disaccharides were labeled with ^{12}C-4-*N*-butylaniline by reductive amination and then analyzed by LC-MS/MS. The disaccharides were quantified based on a calibration curve generated using 6 commercially available disaccharide standards that are the most abundant in human CSF HS. The recovery of the analyte was assessed using bovine kidney HS. Briefly, HS was spiked in CSF before and after the extraction step and the abundance of the digestion-generated disaccharides compared in order to calculate a recovery factor. This recovery factor was used to correct for the loss incurred during the analysis of patients' samples.

Results

Patient Disposition

Ten patients met the study inclusion criteria and were enrolled in the study at 7 centers, 5 in the United States and 2 in the United Kingdom (Figure 1). All 10 enrolled patients completed the study. Of these patients, 8 had evaluable CSF samples and were included in both the safety and pharmacodynamic populations. Of the remaining 2 patients, 1 patient had an unsuccessful LP and the other consented to provide a retrospective CSF

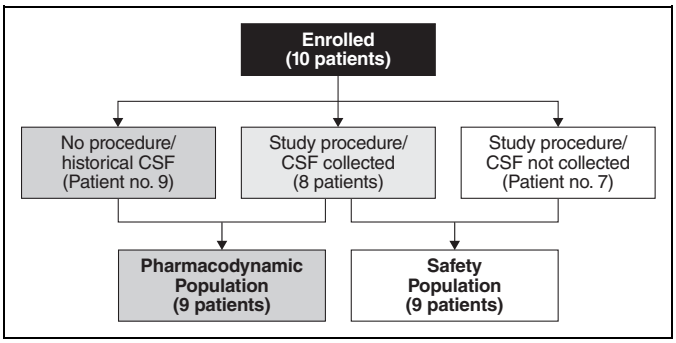

Figure 1. Patient disposition. CSF, cerebrospinal fluid.

sample for GAG analysis. The first patient with the unsuccessful LP was evaluated as part of the safety population but not of the pharmacodynamic population, and the second was part of the pharmacodynamic population but not of the safety evaluation. Thus, both the safety and pharmacodynamic populations had 9 patients, 8 of them overlapping.

Patient Characteristics

Patient baseline demographic and clinical characteristics are shown in Table 1. The study population included 5 adult (aged $\geq$18 years) and 5 pediatric (aged <18 years) patients. The mean ages for the adult and pediatric groups were 27.9 and 7.8 years, respectively. Scores of the WAIS-IV, administered to the adult patients only, ranged from 88 to 111 with a mean score of 99.6. Cognitive status among the pediatric patients, recorded as either normal or abnormal based on the investigator's judgment, was abnormal in 3 patients and normal in 2 patients.

All 9 patients in the safety population were receiving intravenous idursulfase. Other commonly used medications by therapeutic class ($\geq$3 [33%] patients) included anilides and natural opium alkaloids, serotonin antagonists, amides, benzodiazepine derivatives, melatonin receptor agonists, and other general anesthetics. The commonly used medications reflected the surgical/medical treatment profile of the population.

Analysis of GAG

All 5 adult patients underwent LP, although the CSF collection was unsuccessful in 1 who was included in the safety population only, and another patient required 2 LPs, the second of which was successful (Table 1). Among the 9 patients, including all 5 children with CSF samples (pharmacodynamic population), the volume of CSF collected per patient was 1.5 to 16.0 mL.

The primary outcome measure (mean total GAG concentration in the CSF) was 816.8 ng/mL (95% confidence interval 316.5-1317.1 ng/mL). Total GAG concentration range was 356.8 to 2360.9 ng/mL. Among the children only, the CSF total GAG concentration range was 356.8 to 2360.9 ng/mL. For the adults only, GAG concentration range was 381.5 to 1181.1 ng/mL.

The 3 children with cognitive impairment had GAG levels of 842.9, 939.7, and 2360.9 ng/mL in the CSF compared with 356.8 to 1181.1 ng/mL in the 6 patients with normal cognition (4 adults and 2 children). Figure 2 provides a scatterplot of these CSF GAG levels by age at CSF collection and cognitive status. Some correlation between cognitive assessment score and total CSF GAG level can be observed. However, the limited sample size did not allow for general conclusions about the relationships between cognitive status and total GAG concentration.

Non-DS GAG levels in CSF were below the lower limit of quantification (36.7 ng/mL) in 7 of 9 patients. In the 2 other patients, the non-DS GAG levels in CSF were 61.2 ng/mL in an adult and 63.1 ng/mL in a child. Activation of HC II by non-DS GAG has been shown to be less potent than activation by DS. The thrombin activity assay is more specific for DS measurement but less sensitive for non-DS GAG quantification. Therefore, the relative abundance of DS versus non-DS GAG in patients with MPS II remains to be determined.

Levels of CSF HS were determined by mass spectrometry. The levels of HS were quantifiable in all analyzed samples. Our preliminary data suggests for those aged 2 to 18 years, the normal CSF HS levels range from <0.25 to 0.44 μmol/L (Shire data on file). All patients in this study had levels above this range. The cognitively intact patients had, in general, lower HS values (5 of 6 patients had levels 0.8-1.7 μmol/L) than the patients with cognitive impairment (2.3-4.3 μmol/L), however, the highest recorded value (9.57 μmol/L) was in a cognitively intact adult patient.

Safety

Four (44.4%) of 9 patients experienced at least 1 AE, and at least 1 AE was judged related to the procedure. No severe or life-threatening AEs, and no deaths or discontinuations due to AEs, occurred during the study. Most common AEs reported overall were headache and procedural pain (n = 2, with 2 events each). The 1 serious AE reported was a procedure-related moderate headache in an adult patient, which resolved without sequelae.

Discussion

The results of this study contribute much-needed data regarding the levels of CSF GAG and HS in pediatric and adult patients with MPS II and their association with cognitive status.

The data, although limited because of the small sample size, suggest that patients with normal cognitive development/attenuated MPS II generally have lower CSF GAG levels than those with the severe phenotype, as indicated by investigator-determined abnormal cognition. Of the 6 patients, adult and pediatric, whose cognitive status was rated as normal, 5 had CSF GAG values from 360 to 460 ng/mL, while 1 patient had a value of 1181.1 ng/mL. In contrast, all 3 pediatric patients with abnormal cognition had values >840 ng/mL. The levels of HS, as measured by the

Table 1. Patient Baseline Characteristics and CSF GAG Results.

Patient	Age (y, y) at Onset MPS II Symptoms	Age (y, y) at MPS II Diagnosis	Age (y, y) at CSF Sample Collection	Height, cm	Weight, kg	Cognitive Assessment (Method)	CSF GAG, ng/mL	CSF Non-DS, ng/mL	CSF HS, μmol/L
1	3.0	4.2	8.0	127.6	23.9	Normal[a]	373.4	<36.7[b]	1.74
2	4.0	5.8	16.2	148.4	67.2	Normal[a]	356.8	<36.7[b]	0.80
3	n/k	4.8	21.4	160.4	61.6	88 (WAIS-IV)[c]	459.3	<36.7[b]	1.42
4	0.8	1.4	25.4	137.0	56.8	93 (WAIS-IV)[c]	455.2	61.2	9.57
5	3.0	3.9	26.5	152.5	63.2	105 (WAIS-IV)[c]	1181.1	<36.7[b]	1.48
6[d]	6.9	6.9	29.5	131.2	57.2	101 (WAIS-IV)[c]	381.5	<36.7[b]	1.38
7[e]	n/k	3.5	36.8	166.9	87.4	111 (WAIS-IV)[c]	–	–	–
8	1.0	1.3	4.1	114.0	23.0	Abnormal[a]	842.9	<36.7[b]	2.94
9[f]	n/k	3.3	4.6	110.0	23.8	Abnormal[a]	2360.9	63.1	4.26
10	1.0	3.5	6.3	120.0	30.8	Abnormal[a]	939.7	<36.7[b]	2.32

Abbreviations: CSF, cerebrospinal fluid; DS, dermatan sulfate; GAG, glycosaminoglycan; HS, heparan sulfate; MPS, mucopolysaccharidosis II; n/k, not known; WAIS-IV, Wechsler Adult Intelligence Scale–Fourth Edition.
[a]Cognitive status of all pediatric patients (aged <18 years) was determined by investigator opinion.
[b]Lower limit of quantification.
[c]Per study eligibility criteria, adult patients (aged ≥18 years) were required to have an IQ ≥78.
[d]Patient underwent 2 lumbar puncture procedures, the second of which was successful.
[e]Patient evaluable for safety assessment only.
[f]Patient evaluable for pharmacodynamic assessment only.

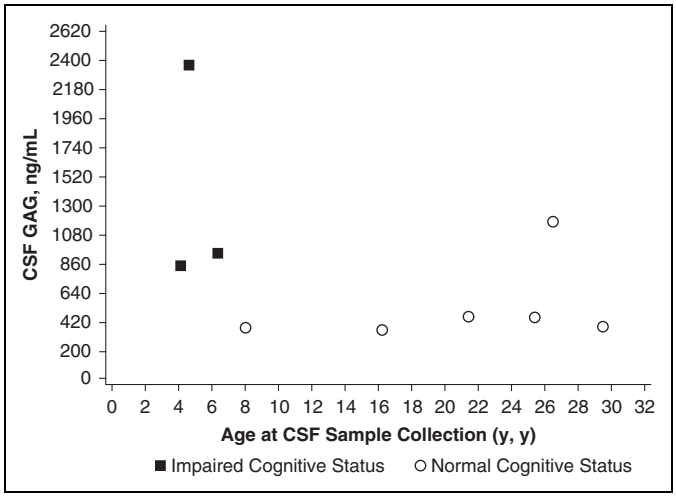

Figure 2. The CSF GAG concentrations by age and cognitive status (pharmacodynamic population). CSF, cerebrospinal fluid; GAG, glycosaminoglycan.

LC-MS/MS method, generally followed a similar pattern; all patients with MPS II had abnormally high values compared with healthy individuals, and the patients with the severe phenotype had the higher values. However, the highest HS value was observed in a cognitively intact adult (but who was not the same subject as the cognitively intact adult with the highest total CSF GAG value). These findings are consistent with the observations by Dekaban et al[6] who found that the levels of CSF GAG were higher in patients with cognitive impairment having MPS than in cognitively intact patients.

Any biochemical means of predicting MPS II phenotype as early as possible would be a valuable asset for optimal patient management. Predicting whether a child with MPS II will develop cognitive impairment is presently a difficult task.[2,8,9] Genetic mutational analysis is often of limited value,[2] and although from the clinical perspective some early signs predictive of neurological involvement have been identified, including sleep disturbance, increased activity, behavior problems, seizure-like behavior, perseverative chewing, and unsuccessful bowel and bladder training,[10] their usefulness is limited by their lack of specificity. Similarly, although brain imaging has identified higher rates of brain atrophy, enlarged ventricles, and severe white matter lesions in patients with cognitive impairment versus cognitively intact patients, these indicators did not predict the severe phenotype because they also occurred in some patients without cognitive impairment.[11,12] Thus, when a patient has a novel or very rare mutation and no family history of MPS II, the clinician often has no choice but to employ a wait-and-see approach with close monitoring of cognitive development.

The present data suggest that clinicians may be able to diagnose the severest form of MPS II biochemically, by measuring CSF GAG levels. Larger studies are needed to confirm the validity of these findings in the population with MPS II and to further explore the clinical significance of CSF GAG accumulation in MPS II.

In summary, analysis of GAG levels in CSF in patients with MPS II indicated that the levels were generally higher in pediatric patients with abnormal cognition than in cognitively intact adult and pediatric patients.

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