Mucopolysaccharidosis type I (MPS I): Assessment of disease severity, therapeutic options and early diagnosis

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Heparan sulfate and dermatan sulfate derived disaccharides and non-reducing end disaccharides in MPS I patients prior to initiation of therapy

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Chapter 7

ABSTRACT

Background Mucopolysaccharidosis type I (MPS I) is a lysosomal storage disorder characterized by progressive multi-organ dysfunction caused by continued storage of the glycosaminoglycans (GAGs) heparan sulfate and dermatan sulfate. Its three phenotypic subgroups range from severe (the Hurler phenotype, MPS I-H) to attenuated (the Hurler-Scheie and Scheie phenotypes, MPS I-H/S and MPS I-S). Early assessment of disease severity is important for optimal treatment decision making. However, the predictive value of clinical signs and symptoms and genotype is often poor. The availability of a reliable biomarker would provide a tool for disease prognostication after diagnosis. Here, we study whether the concentrations of heparan sulfate and dermatan sulfate derived disaccharides (HS and DS, respectively) and non-reducing end (NRE) disaccharides in plasma and urine samples of MPS I patients collected prior to the initiation of treatment correlate with clinical disease severity. We compare these results with levels of total urinary GAGs (uGAGs).

Methods Heparan and dermatan sulfate in plasma and urine were enzymatically digested into disaccharides. Concentrations of HS, DS and NRE disaccharides were determined by HPLC-MS/MS analysis in plasma of 22 MPS I patients and urine of 15 MPS I patients. Results of total uGAG levels, as measured by the dimethylene blue (DMB) test, were available from 23 patients. All samples were obtained prior to initiation of therapy. Levels were corrected for the normal age-related decline.

Results The age-corrected levels of HS and DS and total uGAGs were clearly elevated in MPS I patients prior to initiation of treatment compared to control subjects. Plasma HS and DS levels were significantly elevated in MPS I-H patients compared to the other subgroups, however, separation was not complete. Urinary HS and DS levels showed an inferior correlation with the phenotype. Significant differences between the total uGAG levels of the three phenotypic subgroups were shown, and distinction between different phenotypes was better than for plasma HS and DS. Heparan sulfate derived NRE disaccharides in plasma and urine correlated well with HS levels. No significant differences were shown between the different phenotypic subgroups.

Conclusions Plasma HS and DS levels and total uGAGs correlate with MPS I disease severity. Total uGAGs showed the best distinction between different phenotypes in this studied group of patients.
INTRODUCTION

Mucopolysaccharidosis type I (MPS I; OMIM 252800) is a rare autosomal recessive lysosomal storage disorder (LSD). The progressive signs and symptoms in MPS I result from continued accumulation of the glycosaminoglycans (GAGs) heparan sulfate and dermatan sulfate, which is caused by a deficiency of the lysosomal hydrolase α-L-iduronidase (IDUA; EC 3.2.1.76) [1]. The MPS I phenotype is represented by a broad spectrum, ranging from severe (the Hurler phenotype, MPS I-H) to attenuated (the Hurler-Scheie and Scheie phenotypes, MPS I-H/S and MPS I-S). MPS I-H patients have an early disease onset, usually in infancy, and develop severe symptoms, including progressive pulmonary, cardiac and central nervous system (CNS) disease, if left untreated [1,2,3].

Early diagnosis and timely treatment (hematopoietic stem cell transplantation, HSCT), preferably before the onset of irreversible CNS-disease, is essential to protect these patients from a relentless progressive disease course with likely mortality during the first two decades of life [4]. In patients with attenuated disease phenotypes, the diagnosis is generally suspected because of a combination of -often aspecific- signs and symptoms, such as skeletal and joint disease, upper airway disease, hepatosplenomegaly, hearing deficits, and both umbilical and inguinal hernias. In addition, more severely affected MPS I-H/S patients can have (mild to moderate) intellectual disability. Disease onset and progression in patients with attenuated disease is slower than that observed among severely affected patients, and in MPS I-S, life expectancy can be normal, even in the absence of disease modifying treatment. However, attenuated MPS I patients can become significantly debilitated over the course of their disease, mainly because of progressive musculoskeletal symptoms [1,2,3]. Early start of the treatment of choice in this group of patients, which is intravenous enzyme replacement therapy (ERT) with recombinant enzyme (laronidase, Aldurazyme®), is likely to prevent or at least minimize irreversible damage [5].

The clinical heterogeneity of MPS I symptoms complicates prediction of the phenotype and subsequent decision making on the optimal treatment strategy. This difficulty is illustrated by the remarkable variability in assessments of disease severity, even among experts [6]. While a reliable clinical severity scale is lacking, tools to predict the MPS I phenotype are warranted. It is essential to try to distinguish patients with potential CNS involvement from patients with more attenuated phenotypes, because HSCT should be performed in the former group without delay [4]. Early prediction of the phenotype is even more pressing, considering the advent of newborn screening (NBS) for MPS I in the near future [7].

Prediction of the phenotype based on the genotype is, unfortunately, often difficult in MPS I. Although several (mainly nonsense) mutations in the IDUA gene strongly correlate with a severe phenotype if present in homozygous or compound heterozygous combination, many mutations are ‘private’ and are therefore not informative for predicting clinical severity [8,9]. In addition, relating residual IDUA-activity to the severity of an MPS I
patient’s phenotype is difficult. One study showed no or almost no residual enzyme activity in fibroblasts from both severely affected and attenuated patients [10]. In contrast, another study showed that a ratio of IDUA-activity to trisaccharides derived from heparan sulfate and dermatan sulfate, both measured in cultured fibroblasts, may discriminate between MPS I patients with and without CNS involvement [11]. However, this latter methodology is complex and has not been demonstrated in unselected and larger groups of patients. An alternative approach is the use of biochemical markers in body fluids, to study correlation between marker concentration(s) and disease severity. No clear association between the total excretion of urinary GAGs (uGAGs), currently the biomarker most frequently used for diagnostic testing of MPS I, and the total body disease burden has been reported to date, and there is controversy about whether total uGAGs may primarily reflect GAG storage in kidneys, rather than their storage in other, clinically affected, organs [12,13]. Over recent years, several new and promising biomarkers for MPS I have been studied, including the heparin cofactor II-thrombin (HCII-T) complex in serum [14,15]. In addition, the urinary dermatan sulfate:chondroitin sulfate ratio has shown promise for monitoring efficacy in treating MPS I [16,17], and this ratio may be also be used for disease prognostication.

We recently described a method for reliably assessing disaccharides derived from the primary storage substrates heparan sulfate and dermatan sulfate, in plasma and urine, based on the method originally described by Oguma and co-workers [18], and we showed that these disaccharides might be used for monitoring treatment efficacy in MPS I [19]. Here, we report on the concentrations of these heparan sulfate and dermatan sulfate derived disaccharides (HS and DS, respectively) in the plasma and urine of MPS I patients collected prior to initiation of treatment, and we evaluate their discriminative value for differentiating between the different phenotypic subgroups. We hypothesized that blood might be a more appropriate compartment to investigate for potential biomarkers, which led us to study the correlation between plasma HS and DS levels and the phenotypic severity. In addition, we have compared these results with total uGAGs. Finally, we have measured levels of the non-reducing end (NRE) disaccharides of the accumulating GAG chains. It was previously shown that levels of these NRE disaccharides are present in abundance in MPS I patients compared to control subjects and can be used to differentiate between different types of MPS [20]. We have now investigated the potential role of disaccharides in the evaluation of disease severity.

MATERIALS AND METHODS

Patients

All patients had a confirmed diagnosis of MPS I as documented by enzymatic and genetic analyses. Plasma samples, which were used for measurement of HS and DS and NRE
disaccharides were collected prior to the initiation of treatment in 22 patients, who were classified at diagnosis or later in the course of their disease on the basis of predictive genotypes and/or clinical signs, symptoms and disease course as MPS I-H (n=12), MPS I-H/S (n=4), or MPS I-S (n=6). The median ages of patients at the time of blood sampling were 1.21 years for MPS I-H (range 0.75-7.08 years), 3.96 years for MPS I-H/S (range 3.92-22.42 years) and 20.9 years for MPS I-S (range 9.25-35.33 years). Urine samples for measurement of HS and DS and NRE disaccharides had been collected from 15 of the 22 patients (5 MPS I-H, 4 MPS I-H/S, 6 MPS I-S). The median ages of patients at the time of urine collection were 1.17 years for MPS I-H (range 0.92-7.08 years), 3.96 years for MPS I-H/S (range 3.92-22.42 years) and 13.62 years for MPS I-S (range 2.25-30.75 years). The results of total uGAG levels, as measured by the dimethylene blue (DMB) test, were available from 23 patients (12 MPS I-H, 4 MPS I-H/S, 7 MPS I-S). The median ages of patients at the time of urine collection were 1.21 years for MPS I-H (range 0.75-7.08 years), 3.96 years for MPS I-H/S (range 3.92-22.42 years) and 14.5 years for MPS I-S (range 2.25-35.33 years). Samples had been stored at -80 °C until analysis.

Control plasma and urine samples
Plasma samples of 35 controls (range 0.08-52.6 years) and urine samples of 63 controls (range 0.01-54.7 years) were collected, which were used for measurement of HS and DS. Urine samples of 97 controls were obtained for measurement of total uGAGs (range 0.01-77.9 years).

HS and DS assays
Levels of HS in plasma and urine were represented by the sum of the heparan sulfate derived disaccharides obtained after enzymatic digestion of heparan sulfate by heparinase I, II and III, followed by quantitation by HPLC-MS/MS analysis as described previously [18,19]. Levels of DS in plasma and urine samples were represented by the sum of the dermatan sulfate derived disaccharides obtained after enzymatic digestion of dermatan sulfate by chondroitinase B, followed by quantitation by HPLC-MS/MS analysis as described previously [18,19]. The enzymatic digestion of heparan sulfate and dermatan sulfate also results in the liberation of NRE disaccharides. The detection of NRE disaccharides generated in this manner was carried out during the same run as the heparan and dermatan sulfate derived disaccharides, and was quantified in the MRM acquisition mode using the transitions m/z 396.1>193.1 for I0A0, and 514.0>434.0 for I0S6. Values for the NRE disaccharides are given as the ratio of the NRE disaccharide peak area to the internal standard peak area of each sample. The identity of these disaccharides was confirmed by adding recombinant IDUA to the samples, which leads to the loss of generated NRE disaccharides.
Measurement of total urinary GAGs

Total uGAGs were measured by the dimethylene blue (DMB) test, which involves binding of GAGs to the dye DMB followed by a spectrophotometric analysis of the GAG-DMB complex [21].

Data analysis

The data obtained were analyzed with the Kruskal-Wallis test. When there was a statistically significant difference, the Mann-Whitney U-test was used as a post-hoc test on each pair of groups. The criterion for statistical significance was $p<0.05$ (two-sided). All data analyses were performed using SPSS (version 19.0, SPSS Inc., Chicago, IL).

RESULTS

Plasma HS and DS levels at baseline and in controls

The concentrations of HS and DS in plasma in control subjects decrease significantly during the first years of life and stabilize from an age of approximately 15 and 5 years, respectively (Fig. 1 and 2). All MPS I patients had significantly elevated HS and DS plasma levels at baseline compared with the controls (Table 1, Fig. 1 and 2). All HS and DS levels in plasma of the patients were subsequently corrected for age by subtracting the upper value of the 95% prediction interval for age of the reference values, which was considered to be the upper limit of normal (Fig. 3 and 4). Kruskal-Wallis analysis showed there was a significant difference between the age-corrected levels of plasma HS and DS for the three groups ($p=0.001$). Mann-Whitney tests were used to follow-up on these findings. No significant differences between the age-corrected HS and DS levels of the MPS I-H/S patients and the MPS I-S patients were detected ($p=0.055$ and 0.136, respectively). However, age-corrected HS and DS levels of the MPS I-H patients were significantly elevated compared to the MPS I-H/S patients ($p=0.008$ and 0.011, respectively) and the MPS I-S patients (both $p=0.001$). Age-corrected plasma HS levels were 1.3 to 7.8-fold higher in all but one of the MPS I-H patients compared to the other patient groups (Fig. 3). HS levels did not distinguish this

Table 1. Median plasma and urinary HS and DS levels and total uGAG levels

<table>
<thead>
<tr>
<th></th>
<th>Median (range) plasma HS levels (ng/ml)</th>
<th>Median (range) plasma DS levels (ng/ml)</th>
<th>Median (range) urinary HS levels (µg/mmolcreat)</th>
<th>Median (range) urinary DS levels (µg/mmolcreat)</th>
<th>Median (range) total uGAG levels (mg/mmolcreat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPS I-H</td>
<td>1348 (847-2796)</td>
<td>1893 (1009-2774)</td>
<td>31203 (19227-44185)</td>
<td>31614 (9959-44969)</td>
<td>151 (85-214)</td>
</tr>
<tr>
<td>MPS I-H/S</td>
<td>756 (680-837)</td>
<td>1157 (731-1239)</td>
<td>17839 (7411-22190)</td>
<td>22285 (14249-27849)</td>
<td>68 (53-74)</td>
</tr>
<tr>
<td>MPS I-S</td>
<td>575 (550-715)</td>
<td>833 (572-966)</td>
<td>8507 (1506-31647)</td>
<td>10598 (2391-15855)</td>
<td>37 (17-54)</td>
</tr>
</tbody>
</table>
single patient from the MPS I-H/S and MPS I-S patients. Age-corrected plasma DS levels were 1.1 to 4.0-fold higher in all but one of the MPS I-H patients compared to the MPS I-H/S group (Fig. 4). DS levels did not distinguish this MPS I-H patient from the MPS I-H/S patients. This patient is the same one for whom the plasma HS level overlapped with the MPS I-H/S and MPS I-S groups. Age-corrected plasma DS levels were almost equal to the level in this MPS I-H patient to 5.3-fold higher compared to the MPS I-S group.

Urinary HS and DS levels at baseline and in controls

The concentrations of urinary HS and DS in control subjects were found to decrease significantly during the first years of life and stabilize before 20 and 10 years of age, respectively [19]. All MPS I patients had significantly elevated urinary HS and DS
levels at baseline compared with the controls (Table 1). Urinary HS and DS levels were corrected for age by subtracting the upper value of the 95% prediction interval for age of the reference values (data not shown). Kruskal-Wallis analysis showed that there was a significant difference between the age-corrected levels of urinary HS and DS for all three groups ($p=0.038$). Mann-Whitney tests were used to follow-up on these findings. Age-corrected HS levels of the MPS I-H patients were significantly elevated compared to the MPS I-H/S patients ($p=0.027$) and the MPS I-S patients ($p=0.028$). However, the separation was not complete and less clear than in the data on plasma HS. No significant differences were detected between the age-corrected HS levels of the MPS I-H/S patients compared to the MPS I-S patients ($p=0.286$). Age-corrected DS levels of the MPS I-H patients were significantly elevated compared to the MPS I-S patients ($p=0.045$), as well as the age-corrected DS levels of the MPS I-H/S patients compared to the MPS I-S patients ($p=0.033$). However, as for urinary HS, separation was not complete. No significant
differences between the age-corrected DS levels of the MPS I-H patients compared to the MPS I-H/S patients were detected ($p=0.221$).

**Total urinary GAGs at baseline and in controls**
The concentrations of total uGAGs in control subjects have been found to decrease significantly during the first years of life and stabilize before 20 years of age [19]. The median concentrations of total urinary GAGs across patients are presented in Table 1. Concentrations of uGAGs at baseline were corrected for age by subtracting the upper value of the 95% prediction interval for age of the reference values, which was considered to be the upper limit of normal (Fig. 5). Kruskal-Wallis analysis showed that there was a significant difference between the age-corrected levels of total uGAGs between all the three groups ($p<0.001$). Mann-Whitney tests showed that there were significantly different total uGAG levels for the group of MPS I-H patients compared to the other groups (both $p<0.005$). In addition, significant differences were shown for MPS I-H/S compared to MPS I-S ($p=0.023$). One MPS I-H patient could not be discriminated from the MPS I-H/S group. This patient was not the same MPS I-H patient in whom HS and DS levels in plasma overlapped with the MPS I-H/S group. In addition, one MPS I-S patient showed overlap with the MPS I-H/S group. Apart from this overlap, the age-corrected total uGAG levels were up to 11.3-fold higher in the MPS I-H patients compared to the other patient groups and up to 3.7-fold higher in the MPS I-H/S group compared to the group of MPS I-S patients.
Plasma and urinary levels of NRE disaccharides at baseline

Digestion of heparan sulfate in the patient samples yielded NRE disaccharides consistent with the structures I0A0 and I0S6 [20] based on their mass spectra. The identity of these structures was confirmed by treatment of the samples with recombinant IDUA, leading to loss of the NRE disaccharides. We were not able to detect dermatan sulfate derived NRE disaccharides reliably in the plasma and urine samples of the patients. Heparan sulfate derived NRE disaccharides were not observed in batched control plasma. Fig. 6 shows the I0S6 ratios in plasma by phenotype. Kruskal-Wallis analysis showed a significant difference in the I0S6 ratio across the three groups ($p=0.004$). Mann-Whitney tests showed a significant difference between the MPS I-H patients compared with the MPS I-S patients ($p=0.003$), and between the MPS I-H patients compared with the MPS I-H/S.

![Figure 6. I0S6-ratios in plasma by phenotype](image1)

![Figure 7. Correlation between plasma heparan sulfate derived disaccharides and the I0S6-ratio in plasma](image2)
patients ($p=0.041$). No significant difference between the MPS I-H/S and MPS I-S patients was observed. The plasma I0A0 ratios did not significantly differ between the three phenotypic subgroups. The same was observed for the urinary I0S6 and I0A0 ratios. There was a strong correlation between the plasma HS levels and the I0S6 ratios using Pearson correlation: $r=0.92$, $p<0.001$ (Fig. 7). Plasma HS levels were also significantly correlated with plasma I0A0 ratios, $r=0.84$, $p<0.001$. In addition, urinary HS levels were significantly related to the urinary I0S6 ratios ($r=0.89$, $p<0.001$) and to the urinary I0A0 ratios ($r=0.94$, $p<0.001$; data not shown).

**DISCUSSION**

We have demonstrated that the concentrations of HS and DS in plasma and urine as well as total uGAGs are all significantly elevated in untreated MPS I patients across all phenotypes compared to control subjects.

In this study, we investigated whether HS and DS levels in plasma and urine correlated with the clinical severity of individual MPS I patients. HS and DS levels were also compared with total uGAGs, which is a widely available diagnostic marker for MPS I. If a new potential marker is detected, further studies would be needed to assess its value for prognostication in early diagnosed patients.

Our results suggest that in MPS I patients the total levels of uGAGs may differentiate different phenotypic groups (Figure 5). UGAGs seem to have even greater predictive value than do plasma HS and DS levels. This finding is remarkable, as measurement of total uGAGs by the DMB test is generally considered, due to its nonspecific nature, to have limited value other than as an efficient screening test for MPS I. The DMB test detects not only heparan and dermatan sulfate, but also chondroitin sulfate and keratan sulfate, and it is thought that the relatively high levels of chondroitin sulfate normally excreted by all individuals may obscure detection of more subtle changes in heparan and dermatan sulfate.

Our data indicate a significant difference between the age-corrected plasma HS and DS levels of MPS I-H patients and the levels of MPS I-H/S and MPS I-S patients. However, the different phenotypes were not completely distinguished using these measures. This outcome was mainly caused by the HS and DS levels of one MPS I-H patient, who was diagnosed with MPS I at the age of 11 months on the base of significant somatic signs and symptoms, as well as developmental delay. These data overlapped with the levels observed in MPS I-H/S and MPS I-S patients. Total uGAG levels of this patient were, in contrast, clearly elevated and comparable with the levels observed in the other patients with MPS I-H. These observations suggest that HS and DS may be less sensitive than total uGAGs as a biomarker for the severe Hurler phenotype. Our data on urinary HS and DS levels show even less distinction between different phenotypes than the plasma sample
data. Further studies in a larger group of newly diagnosed MPS I patients are needed to determine the value of uGAGs and HS and DS levels in plasma and urine for predicting specific disease phenotypes.

We were also able to detect two out of the four possible heparan sulfate derived NRE disaccharides, 1OA0 and 1OS6 [20], in the plasma and urine samples of MPS I patients. These levels, expressed as ratios, correlate well with concentrations of plasma and urinary HS. However, determination of the levels of heparan sulfate derived NRE disaccharides do not appear to result in improved separation between different phenotypes.

With the prospects of NBS, the need for reliable prediction of the MPS I phenotype at a very young age becomes even more pressing, as decisions concerning the optimal treatment strategy will depend on the predicted phenotype. Total uGAG levels and plasma HS and DS levels might help to predict severity in these early diagnosed patients, especially in the absence of data on their predictive genotypes. However, we recently showed that HS and DS levels in newborn blood spots of MPS patients do not seem to correlate with disease severity [22]. This finding should be further investigated. In addition, studies in urine of newborn or very young MPS I patients will be required to determine if uGAGs can be used as a predictive biomarker. However, such studies can only be conducted on a sufficiently large scale within the scope of (a pilot study on) NBS for MPS I.

Our study has several limitations. First, HS and DS in plasma and urine and total uGAGs in control subjects are relatively high during the newborn period, before declining with age (Fig. 1 and 2) [19]. However, little is known about levels of HS, DS and total uGAGs in untreated MPS I patients at different ages. Tomatsu et al. reported that urinary HS and DS levels and total uGAGs in untreated MPS patients may be age-dependent, a trend that was not observed for plasma levels of HS and DS [23]. We were not able to draw reliable conclusions about the age-dependency of HS and DS in plasma and/or urine and total uGAGs in our group of MPS I patients due to the relatively small number of patients and the unequal age distribution within and between the different phenotypic subgroups. In an attempt to minimize an effect of age on our data, we corrected all levels for age by subtracting the upper limit of normal values. However, we cannot rule out the possible influence of unequal age distributions among the different subgroups on our results, with median ages at sampling of 1.21 years among MPS I-H patients and 20.9 years among MPS I-S patients.

Second, we were neither able to identify all theoretically possible NRE disaccharides, nor any NRE disaccharides derived from dermatan sulfate [20]. This result is most likely observed because we did not derivatize the liberated disaccharides with aniline [20], which could be responsible for the less sensitive detection and quantitation of the digestion products. Therefore, our diagnostic approach to detect and analyze these disaccharides should be optimized, and a more thorough investigation of the quantity of NRE disaccharides in samples from unaffected controls should be undertaken.
In conclusion, we show that while plasma HS and DS levels seem to correlate with disease severity in MPS I, they do not separate all MPS I-H patients from those patients with more attenuated phenotypes. Surprisingly, total uGAGs, as measured by the DMB test, showed a better correlation with disease severity and an improved separation of phenotypes. Prospective studies on the predictive values of biomarkers in MPS I are needed, but they should only be performed using cases that have been detected using NBS.
REFERENCES


