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Residual α-L-iduronidase activity in fibroblasts of mild to severe Mucopolysaccharidosis type I patients

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ABSTRACT

Three major clinical subgroups are usually distinguished in Mucopolysaccharidosis type I: Hurler (MPS IH, severe presentation), Hurler–Scheie (MPS IH/S, intermediate) and Scheie (MPS IS, mild). To facilitate treatment with hematopoietic stem-cell transplantation, early diagnosis is important for MPS IH patients. Although screening for MPS I in newborns would allow detection at an early age, it may be difficult to predict the phenotype on the basis of the genotype in these infants. Extra diagnostic tools are thus required. Based on the hypothesis that distinct MPS I phenotypes may result from differences in residual α-L-iduronidase (IDUA) activity, we modified the common IDUA assay using the substrate 4-methylumbelliferyl-α-L-iduronide to allow quantification of low IDUA activity in MPS I fibroblasts. Enzyme incubation was performed with high protein concentrations at different time points up to 8 h. Mean residual IDUA activity was 0.18% (range 0–0.6) of the control value in MPS IH fibroblasts (n = 5); against 0.27% (range 0.2–0.3) in MPS IH/S cells (n = 3); and 0.79% (range 0.3–1.8) in MPS IS fibroblasts (n = 5). These results suggest that residual IDUA activity and severity of the MPS I phenotype are correlated. Two MPS IS patients with rare (E276K/E276K) or indefinite (A327P/unknown) IDUA genotypes had residual IDUA activity in the MPS IS range, illustrating the usefulness of our approach. IDUA^{E276K} was very unstable at 37 °C, but more stable at 23 °C, suggesting thermal instability. We conclude that this procedure for determining residual IDUA activity in fibroblasts of MPS I patients may be helpful to predict MPS I phenotype.

1. Introduction

Mucopolysaccharidosis type I (MPS I; OMIM #252800) is an autosomal recessively inherited lysosomal storage disorder caused by the lack of α-L-iduronidase (IDUA, E.C. 3.2.1.76), a glycosidase involved in the degradation of the glycosaminoglycans (GAG) dermatan sulfate and heparan sulfate [1,2]. The partly degraded GAG accumulate in the lysosomes, causing a wide range of clinical manifestations in MPS I patients.

Three major clinical subgroups are commonly distinguished: Hurler syndrome (MPS IH, OMIM #607014; severe presentation), Hurler–Scheie syndrome (MPS IH/S, OMIM #607015; intermediate); and Scheie syndrome (MPS IS, OMIM #607016; mild). However, this classification in subgroups is not precisely delineated, either clinically or biochemically [1,2].

MPS IH is characterized by the early onset of clinical signs and symptoms, including organomegaly, dysmorphic cardiac valves, restrictive pulmonary and obstructive airway disease, skeletal deformities, growth retardation, neurological complications, and severe mental retardation. Patients' life expectancy is short, with death usually occurring in childhood. In MPS IS, clinical problems develop later in life and are relatively mild compared to those in MPS IH; patients' intelligence is normal. MPS IH/S patients have an intermediate phenotype [1] with variable neurological involvement. Later in life (early to mid teens), they develop the clinical symptoms described for MPS IH patients. It is sometimes difficult to distinguish MPS IH/S patients from the severe or the mild forms of MPS I [3–6].

The clinical diagnosis of MPS I is confirmed on the basis of elevated levels of dermatan sufate and heparan sulfate in urine, and of deficient IDUA enzyme activity in leukocytes or fibroblasts [1].

The gene encoding IDUA is located on chromosome 4 (locus 4p16.3) spanning 19 kb and contains 14 exons [7]. Over 100 different mutations in the IDUA gene have been reported (Human Gene Mutation Database, http://www.hgmd.org). As well as the large number of disease-causing mutations, the attenuating polymorphisms, rare sequence variants in

Abbreviations: 4MU, 4-methylumbelliferone; ERT, enzyme-replacement therapy; HSCT, hematopoietic stem cell transplantation; IDUA, α-L-iduronidase; MPS, mucopolysaccharidosis; MPS IH, Hurler syndrome; MPS IH/S, Hurler–Scheie syndrome; MPS IS, Scheie syndrome.

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the *IDUA* gene, and variation in genetic backgrounds all make it hard to predict the clinical phenotype of MPS I patients through analysis of the *IDUA* gene [4,8].

Currently, the two main therapies for MPS I patients are hematopoietic stem-cell transplantation (HSCT) for MPS IH patients, and enzyme-replacement therapy (ERT) for less severely affected MPS I patients (MPS I H/S and S). Ideally, HSCT is performed before 18 months of age (maximum age of 2 1/2 years). If this is possible, it may largely prevent the disease from progressing in the central nervous system [9,10]. As the protection of the brain by the blood–brain barrier does not allow ERT to access sites of brain pathology, ERT does not affect or prevent mental retardation, although it effectively reduces storage in other tissues, such as liver and spleen [11,12].

Early recognition of the phenotype of MPS I patients is essential to timely initiate the most appropriate therapeutic strategy [9,10]. One option for identifying patients at a very early age before clinical features have become evident is newborn-screening for MPS I [13]. To confirm diagnosis, a positive newborn-screening result would be followed by determination of IDUA activity in leukocytes, and mutation analysis of the *IDUA* gene. However, if the latter reveals novel mutations with unknown effects on IDUA function, the phenotype cannot be predicted, and selection of the right therapy is precluded. Therefore, extra diagnostic tools are required. Based on the assumption that differences in severity of the MPS I phenotype result from differences in residual IDUA activity [3,14], one such tool may be accurate determination of residual IDUA activity.

To correlate residual IDUA activity in MPS I patients with phenotype and genotype, we adapted the commonly used IDUA assay described by Stirling et al. [15] in order to allow discrimination between MPS IH and MPS IS. Residual activity was measured in fibroblasts of MPS I patients with different mutations in the *IDUA* gene. Such enzymatic investigations in cells of young patients with previously undetected mutations may help to predict clinical outcome, thereby assisting decision-making on the best therapeutic strategy for individual patients.

2. Materials and methods

2.1. Cell lines and sample preparation

Fibroblast cultures of MPS I patients and cultures from healthy controls were all provided by the European Cell Bank, Rotterdam, the Netherlands. Classification of MPS I patients to one of the three groups commonly distinguished (H, H/S and S, [1]) was performed by reviewing patient files in combination with literature data on the selected genotypes. Fibroblasts were cultured under standard conditions in Ham's F10 medium supplemented with 10% fetal calf serum. They were then harvested by trypsinization and stored as cell pellets at −80 °C. One cell pellet was prepared from one 75 cm^2 flask. This study was approved by the Erasmus MC Institutional Review Board.

2.2. Enzyme assays

Fibroblast homogenates were prepared by resuspending one cell pellet in 100 μL water followed by sonication for 10 s at 130 W. Ten μL of homogenate was taken to determine protein concentration using the Pierce BCA Protein Assay kit (Thermo Scientific) according to the manufacturer's instructions.

Protein concentration in homogenates from MPS I cells was adjusted to 2 mg/mL, while homogenates from control cells were diluted with 0.2% BSA to 0.02 mg/mL homogenate protein. α-L-Iduronidase activity was measured essentially as described by Stirling et al. [15]. Enzyme reactions were started by the addition of 20 μL substrate solution, containing 2 mmol/L 4MU-α-L-iduronide (Glycosynth), 0.1 mol/L sodium formate (pH 3.5) and 37.5 mmol/L NaCl, to 10 μL fibroblast homogenate. In some experiments the protease inhibitor Pefabloc (Sigma) was added to the homogenate (final concentration of 0.45 mg/mL). The reactions were terminated after various incubation times (0 to 8 h) by adding 200 μL 0.5 mol/L sodium carbonate (pH 10.7) containing 0.25% (w/v) Triton-X-100. Substrate blanks were prepared at each time point and contained 10 μL water rather than fibroblast homogenate.

Fluorescence intensity was measured with a fluorimeter (Varioskan, Thermo Electron Corporation/Thermo Fisher Scientific Inc., Waltham, MA, US) at an excitation wavelength of 365 nm and an emission wavelength of 448 nm. A calibrator was prepared containing 750 nmol/L 4-methylumbelliferone (4MU). α-L-Iduronidase activities were calculated by subtracting corresponding substrate blanks and converting fluorescence readings to nmol product released per mg protein. The limit of detection (signal-to-noise ratio 3) was 3.5 pmol 4MU, which is equivalent to an activity of 0.2 nmol/mg protein for MPS I cell homogenates. Typical fluorescence readings after 8 h of incubation corresponded to 20–30 pmol for substrate blanks, 30–40 pmol for MPS IH cell lines, 100–200 pmol for MPS IS cell lines and 400 pmol for controls.

Half-life of IDUA activity was defined as the incubation time that led to a 50% reduction in activity relative to the value at the start of the incubation.

Total ß-hexosaminidase was used as a control enzyme and was measured according to O'Brien et al. [16] using 4MU-2-acetamido-2-deoxy-ß-D-glucopyranoside (Melford).

2.3. Statistical analysis

Statistical analysis was performed with SPSS, software version 17. A Mann–Whitney *U* test was used to test the significance of differences in IDUA activity between MPS IH and MPS IS. Statistical significance was defined as $P < 0.05$ (two-tailed).

3. Results

The residual activity of IDUA was measured in fibroblast cell lines of five MPS IH patients, three MPS IH/S patients and seven MPS IS patients (Table 1, Fig. 1). Barely detectable activities of maximal 0.2 nmol/mg protein/h at 37 °C were found in three of the MPS IH patients who were homozygous for Q70X, W402X and 134del12. The very low activities (0.1 to 0.2% of the controls) were in accordance with the fact that these mutations are essentially null alleles. Total ß-hexosaminidase was used as a control enzyme and was within normal range for all cell lines.

In the first 30 min of the incubation, residual activity in the MPS IH fibroblast cell line homozygous for A327P was consistently about 0.5 nmol/mg protein/h, then decreasing to an activity comparable to Q70X and W402X (Fig. 1B). In the MPS IH cell line homozygous for L218P, residual IDUA activity was initially higher, with a maximal activity of 1.6 nmol/mg protein/h (0.6% of the controls) after 1 h of incubation. During longer incubation, however, the enzyme appeared to be unstable, the activity half-life being 2–4 h (Fig. 1B).

Two of the MPS IH/S cell lines we analyzed were homozygous for P533R and L490P (Table 1, Fig. 1C). The activity of IDUA in the P533R cell line was 0.8 nmol/mg protein/h and that in the L490P cell line was 0.9 nmol/mg protein/h (0.3% of the controls). In a third MPS IH/S cell line, genotype Q70X/R383H, IDUA activity was 0.5 nmol/mg protein/h (0.2% of the controls). IDUA activities in these three MPS IH/S cell lines were slightly higher than those in the Q70X and W402X MPS IH cell lines.

We analyzed seven MPS IS cell lines with five different genotypes. A cell line homozygous for R383H had a residual IDUA activity of 1.9 nmol/mg protein/h (0.7% of the controls; Table 1, Fig. 1C). As expected on the basis of the combination of a milder Scheie mutation (R383H) with a severe Hurler mutation (A327P), the activity of IDUA in an MPS IS cell line with genotype A327P/R383H was lower than the activity in the cell line homozygous for R383H. The residual activity of an MPS IS cell line with genotype R621X/974ins12 was 2.5 nmol/mg protein/h (0.9% relative to the controls; Fig. 1C). The IDUA half-life at