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Plasmatic kinetics of dermatan sulfate during enzyme replacement therapy with iduronate-2-sulfatase in a mucopolysaccharidosis II Patient

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Abstract Enzyme replacement therapy (ERT) is the worldwide standard of care for a number of mucopolysaccharidosis (MPS) diseases. We report a kinetic study of plasmatic dermatan sulfate (DS) in a 3-year-old subject affected by a severe form of MPS II during the first 10 months of ERT with Idursulfase. A strong increase in the DS plasmatic concentration was measured immediately after the first enzyme infusion, with a maximum after 3 h, followed by a continuous decrease in the 8–15 days following the beginning of treatment. After this, a constant plasmatic content of DS concentration was observed. Overall, during the 10-month treatment period, ERT reduced the plasmatic concentration of DS up to ~80–85 %, but it was unable to totally remove it from the blood. We can suppose that immediately after the first enzyme administrations, a large amount of abnormal DS is removed from tissues reaching the blood compartment and eliminated *via* the urine, and thereafter only minimal changes are observed. The persistency of the residual amounts of DS with the actually recommended dosage in our Patient may suggest the opportunity to promote further studies with increased enzyme dosages to completely remove the accumulation of lysosomal DS.

Keywords Dermatan sulfate · Enzyme-replacement therapy · Glycosaminoglycans · Mucopolysaccharidoses · Mucopolysaccharidosis type II

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Abbreviations

DS	Dermatan sulfate
ERT	Enzyme-replacement therapy
GAG	Glycosaminoglycan
GlcA	Glucuronic acid
HS	Heparan sulfate
IdoA	Iduronic acid
MPS	Mucopolysaccharidoses
ΔDi4s	ΔUA-(1→3)-GalNAc-4*S*
ΔDi6s	ΔUA-(1→3)-GalNAc-6*S*

Introduction

Mucopolysaccharidoses (MPS) are rare genetic disorders characterized by an accumulation of intact and partially degraded glycosaminoglycans (GAGs) within lysosomes and in biological fluids caused by a deficiency in the activity of a specific lysosomal enzyme required for their degradation [1–4] producing progressive cellular damage leading to organ failure, cognitive impairment, and reduced life expectancy. MPS II, Hunter syndrome, is an X-linked recessive disorder caused by the deficiency of the lysosomal enzyme iduronate-2-sulfatase able to catalyze the hydrolysis of *O*-sulfate esters from iduronate-2-sulfate belonging to heparan sulfate (HS) and dermatan sulfate (DS) [5, 6]. The resulting accumulation of DS/HS leads to common features including facial dysmorphism, organomegaly, joint stiffness and contractures, pulmonary dysfunction, myocardial enlargement and valvular dysfunction, and neurological involvement [7].

Enzyme replacement therapy (ERT), providing the patient with exogenous active enzyme, is the worldwide standard of care for a number of MPS diseases, including Hunter syndrome [8], even if no improvement of the neuropsychological symptoms is expected due to the incapacity of the enzyme

molecule to cross the hemato-liquoral barrier [1, 4]. Idursulfase (Elaprase®, Shire Human Genetic Therapies, Inc., Cambridge, MA) is a recombinant form of human iduronate-2-sulfatase commercially available since 2006 [9–11]. However, to date, no detailed analysis of the plasmatic kinetics of this enzyme treatment is available in the literature. In this study, we report a kinetic study of plasmatic DS in a 3-year-old Subject affected by a severe form of MPS II during the first 10 months of ERT with the prospect of gaining a better understanding of the effect of therapy on the metabolic fate of this complex macromolecule.

Materials and methods

MPS II Patient

Our patient was first referred to the Pediatric Division of the Polytechnic University of the Marche, at the age of 2 years and 9 months due to his speech delay, sleep disorders and hyperactivity, macrocrania and a slightly coarse face. Urinary GAGs excretion was 1018 μg/mg creatinine (*versus* a normal value for age of 14–90 μg/mg) and electrophoretic urinary GAGs characterization showed the presence of bands corresponding to DS and HS. Plasma iduronate sulfatase activity was 0.8 nM/ml/4 h (*versus* a normal value of 12.9–28.0). Molecular analysis revealed the P120R mutation on Xq28. The diagnosis of MPS type II (Hunter syndrome), severe form, was made on the basis of biochemical and clinical evaluation. After 10 months of ERT, cardiovascular abnormalities and hearing loss had stabilized, hepatomegaly had been resolved and joint stiffness had improved. On the contrary, the neuropsychological evaluation did not show any significant improvement.

Healthy subjects and patient samples collection

This study was approved by an institutional review board of the Department of Life Sciences, University of Modena and Reggio Emilia, Italy, and parents of the patient and healthy controls gave informed consent for the collection of plasma and urine samples. Blood samples were withdrawn immediately before, and at 1 and 3 h after enzyme infusion, and at different times and days after treatment, over ten consecutive months of ERT, collected in tubes containing citrate as an anticoagulant, and recovered plasma stored at −80 °C for biochemical investigation.

71 normal pediatric and adult subjects, age from 0.04 to 66 years with a mean of 8.8±12.9 years, 28 females and 43 males, were registered in the Pediatric Division, Department of Clinical Sciences, Polytechnic University of Marche, Presidio Salesi, Ancona, Italy. Blood samples collected from healthy human subjects were immediately collected in tubes containing citrate as an anticoagulant and the plasma obtained was stored at −80 °C for analytical investigation.

Urine samples were collected at the Department of Clinical Sciences, Polytechnic University of the Marche, Presidio Salesi, Ancona, Italy, and frozen at −20 °C for analytical investigation.

Structural characterization of plasma

After extraction and purification from 500 μl of plasma (see [12] for details), the crude retained GAG fraction was dissolved in 50 μl distilled water. Aliquots of 10 μL were freeze-dried, dissolved in 30 μL Tris-Cl 100 mM pH 8.0 and treated with 20 μL of chondroitinase B (from *Flavobacterium heparinum*, Sigma-Aldrich, St. Louis, MO, USA). Unsaturated disaccharides were separated and quantified by HPLC and postcolumn derivatization with 2-cyanoacetamide and fluorimetric detection [13, 14]. The percentage determination of each disaccharide was obtained by means of specific calibration curves performed with authentic standards manufactured by Seikagaku Corporation (Tokyo City, Japan).

Urinary GAGs assay

For the quantitation of total urinary GAGs, 100 μL of urine were subjected to DMB assay performed according to Coppa *et al*. [12, 15]. The final quantitative data were normalized for mg creatinine and expressed as μg GAGs/mg creatinine.

Results

Normal human plasma contains low amounts of a major undersulfated chondroitin sulfate [16] covalently bound to an inter-trypsin inhibitor (bikunin) with a chain molecular mass of approx. 6–8 kDa [17] totally composed of glucuronic acid (GlcA) with no trace of iduronic acid (IdoA) typical of DS. As a consequence, for an accurate evaluation of plasmatic DS species, GAGs were subjected to treatment with chondroitinases B for the determination of disaccharide species and presence of IdoA.

After treatment with chondroitinase B, plasmatic DS before any ERT was found to be mainly composed of a high content of ~93 % disaccharide 4-sulfated (ΔDi4*S*) and ~7 % disaccharide 6-sulfated (ΔDi6*S*) (Fig. 1A) for a total content of DS of ~12–14 μg/mL (Fig. 2). After 300 days of continuous ERT, plasmatic DS was found to be composed of 100 % ΔDi4s (Fig. 1B) for a content of ~3–4 μg/mL (Fig. 2). Moreover, a strong decrease in plasmatic DS was observed during ERT up to 10 months of therapy (Fig. 2). It is worth mentioning that a strong increase in DS plasmatic concentration was measured immediately after the first enzyme infusion, after 1 h, with a maximum after 3 h, followed by a continuous decrease in the

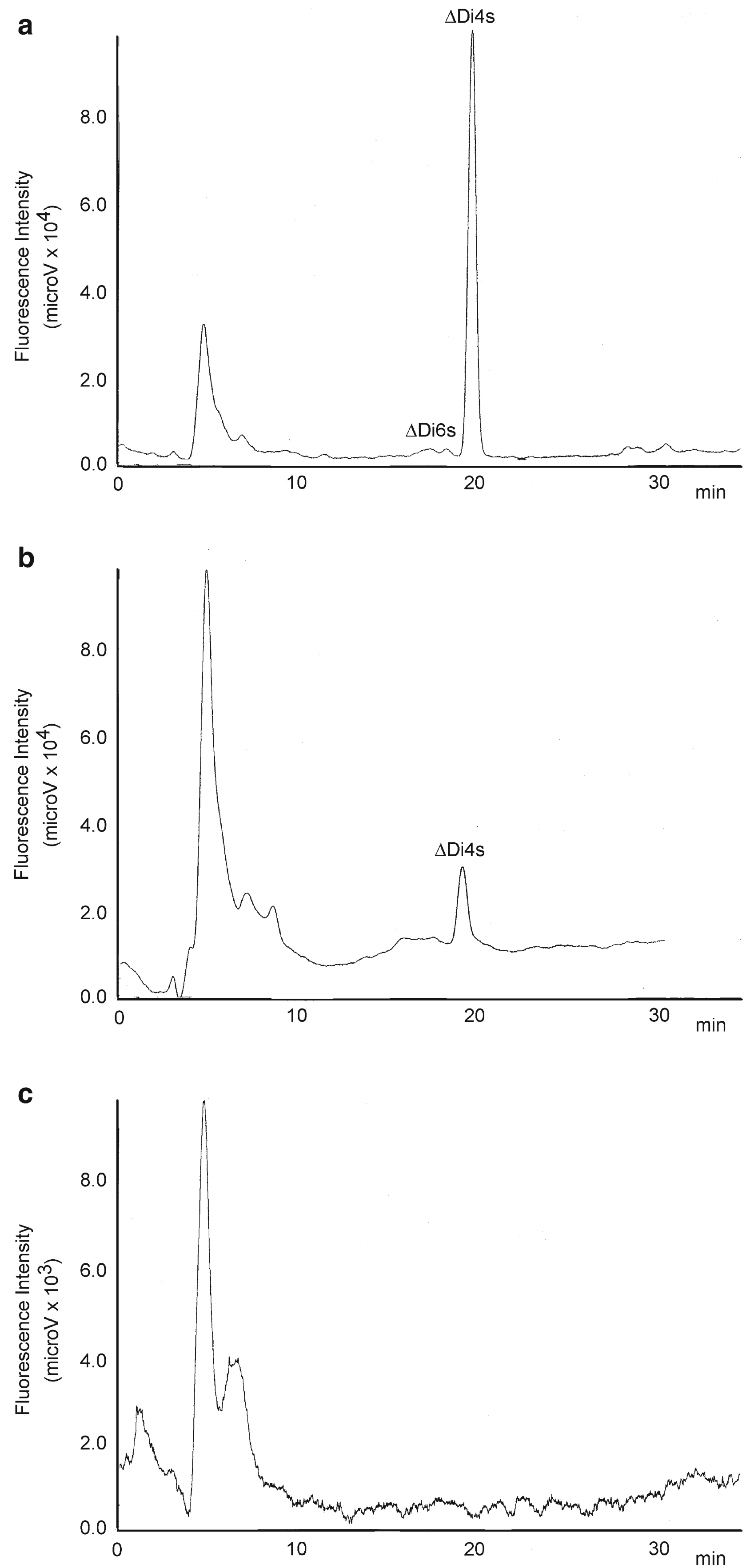

Fig. 1 HPLC separation and post-column derivatization with fluorescence detection of dermatan sulfate disaccharides from the plasma **a** of the MPS II Patient before any enzyme-replacement therapy (day −5), **b** of the MPS II Patient after 300 days of continuous treatment (day 300) and **c** of a normal pediatric subject. ΔDi4*S*, ΔUA-(1→3)-GalNAc-4*S*. ΔDi6*S*, ΔUA-(1→3)-GalNAc-6*S*

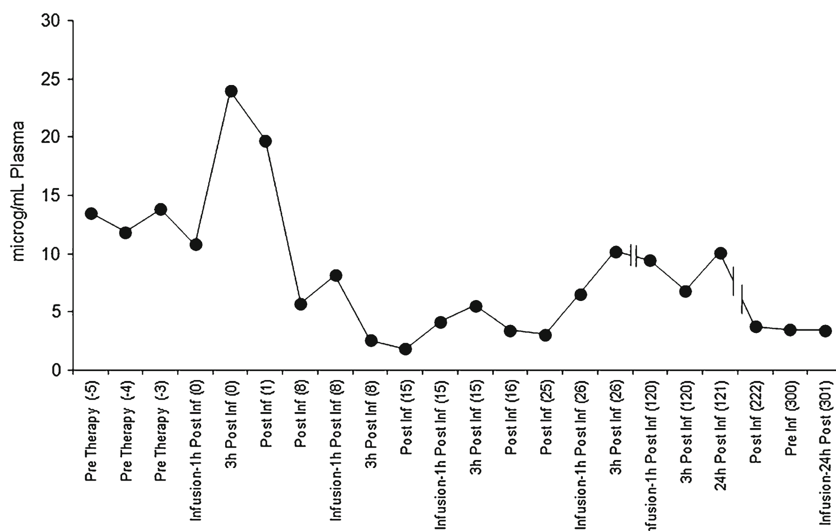

Fig. 2 μg/mL of plasmatic dermatan sulfate before any enzyme-replacement therapy (−5, −4, −3) and on different days (in brackets) of treatment, also with the indication of the moment of enzyme infusion up to 301 days

8–15 days following beginning of treatment (Fig. 2). After this, a constant plasmatic content of DS concentration was observed. Overall, during the 10-month treatment period, ERT reduced the plasmatic concentration of DS up to ~80–85 %, but it was unable to totally remove it from the blood. In fact, after 10 months of treatment, ~15–20 % DS was still detected. Finally, under our experimental conditions, no DS is detectable in healthy subjects' blood (see Fig. 1C as an example).

Total GAGs were determined directly in urine samples by a standard colorimetric assay accepted by the Guidelines for the management of MPS [4] by using DMB (normal range of DMB values for ages ranging from 22–86 μg/mg creatinine) (Fig. 3). A strong increase in the total urinary GAGs was measured immediately after the first infusion of enzyme (after 1–3 h, ~+70 %) followed by lower values for the 8 days following treatment. Fairly similar behaviour was observed after the second infusion with a strong increase in urinary GAGs during the first few hours immediately after administration followed by a constant decrease (Fig. 3). Further infusions after 16, 26, 120 and 300 days from the first treatment produced less important modifications in the urinary GAGs levels. Furthermore, after 10 months of therapy, the total content of urinary GAGs still produced higher values than normal (normal range of 22–86 μg/mg creatinine) according to other studies showing a reduction but not a normalization of urinary DMB values after many months of ERT [18, 19].

Discussion

ERT with idursulfase has been commercially available since 2006 and early access programs have been established since 2005. However, limited information on the effects of ERT on the principal accumulated macromolecules, DS, responsible for the pathological conditions is available to date. Furthermore, no information is available on the plasmatic kinetic effect of this enzyme on DS.

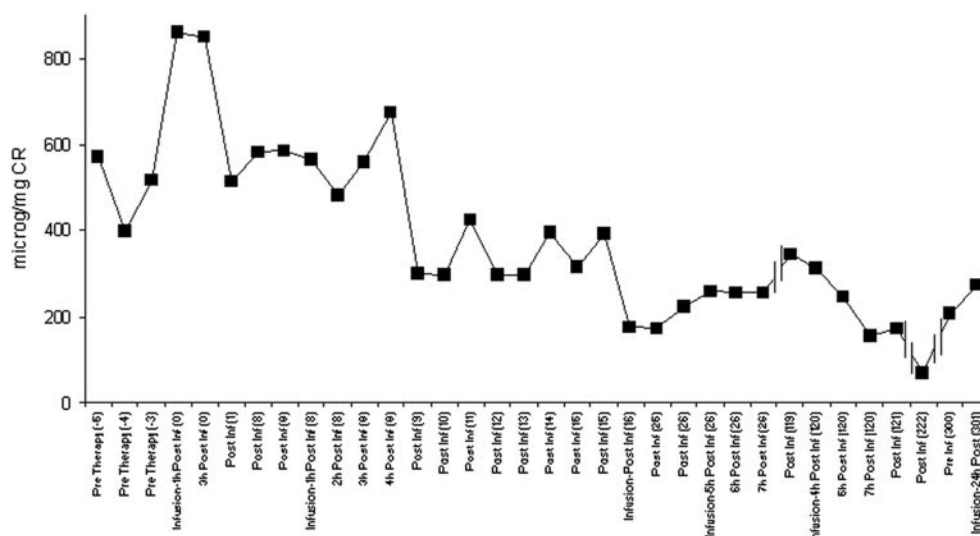

Fig. 3 Total amounts of GAGs expressed as μg/mg creatinine in the urine of the MPS II Patient before any enzyme-replacement therapy (−5, −4, −3) and on different days and times during enzyme-replacement treatment determined by DMB assay

As the main GAG accumulated in MPS II is DS, we used chondroitin B lyase specific for DS and the percentage of the main disaccharide generated by the activity of this enzyme to calculate the total plasmatic DS concentration to monitor the efficacy of ERT immediately after the first enzyme infusion and over the 10-month treatment period. A strong increase in the plasmatic DS content was measured immediately after the first enzyme infusion, followed by a continuous decrease in the 7–8 days following the beginning of treatment. In the urine, an increase in total GAGs excretion was measured after the first and second enzyme infusion followed by a continuous progressive decrease. After this, an almost constant plasmatic (and urinary) content of DS concentration was observed. On the basis of such data, we can suppose that immediately after the first enzyme administrations, a large amount of abnormal DS is removed from tissues reaching the blood compartment and eliminated *via* the urine. Thereafter, the concentration of DS present in plasma remains fairly constant over the following months of treatment, at ~15–20 %, and ~40 % of the pre-treatment levels.was still detected in the urine

A rather similar trend was observed in the urinary GAGs of two MPS I patients subjected to ERT in which a high concentration of DS was still detected in urine after 6 years of treatment [12]. However, in the previous study [12] we were unable to detect any pathological DS in human plasma during ERT. This difference may be due to several reasons, such as different periods of treatment (10 months *versus* 6 years), or possible variable effects depending on the Subjects.

To our knowledge, these are the first data regarding the plasmatic kinetics directly measured on products released by the activity of the recombinant enzyme. (Idursulfase, iduronate-2-sulfatase). The constant presence of a discrete amount of DS after 10 months of treatment, if confirmed in other MPS II patents under ERT, suggests the possibility that the present dosage might not be sufficient to totally remove the accumulation of lysosomal DS. Therefore, the present specific and sensitive measurements of human plasma DS would be extremely helpful for an accurate evaluation of the kinetics of catabolic products.

Contributors N.V. developed the applied methodologies. L.Z., F.M., L.S., F.G., and T.G. performed the experimental procedures and analyses. N.V., G.V.C. and O.G. designed and developed the experimental design, performed data analysis and wrote the manuscript. All authors reviewed and approved the study.

Conflict of interest We declare that we have no conflict of interest.

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