Cell Biol Toxicol (2014) 30:189–193
DOI 10.1007/s10565-014-9284-3

BRIEF REPORT

Glycosaminoglycans can be associated with oxidative damage in mucopolysaccharidosis II patients submitted to enzyme replacement therapy

G. W. Negretto · M. Deon · G. B. Biancini · M. G. Burin · R. Giugliani · C. R. Vargas

Received: 28 February 2014 / Accepted: 3 July 2014 / Published online: 15 July 2014

Introduction

The mucopolysaccharidoses (MPS) are a group of inherited metabolic diseases caused by the deficiency of specific lysosomal enzymes responsible for the degradation of glycosaminoglycans (GAG), leading to their abnormal storage (Neufeld and Muenzer 2001). As a consequence of the GAG accumulation, both architecture and function of cells and organs are compromised, and the clinical features presented by MPS patients are progressive and multisystemic (Berry 1987; Mabe et al. 2004; Neufeld and Muenzer 2001). Screening tests based on the identification and quantification of urinary GAG and clinical features are meaningful for MPS diagnosis, but the measurement of the deficient enzyme remains the gold standard (Coelho et al. 1997). The enzyme deficient in MPS type II, also known as Hunter syndrome, is iduronate-2-sulfatase (I2S; EC 3.1.6.13), one of those responsible for the catabolism of the GAG dermatan sulfate and heparin sulfate (Neufeld and Muenzer 2001). The enzyme replacement therapy (ERT) was approved in Brazil in 2008. The use of a recombinant human enzyme (idursulfase) as a therapy resulted in an increase in mobility, improvements in 6-min-walk time, functional outcomes and measures of pulmonary function, as well as reduction of liver and spleen volumes in MPS II patients (Germain 2005; Giugliani et al. 2009; Harmatz et al. 2008; Kakkis et al. 2001; Muenzer et al. 2007).

Oxygen and nitrogen reactive species can be produced by endogenous and exogenous sources. In order to minimize free radical damage, there are enzymatic and non-enzymatic antioxidant defense systems (Halliwell and Gutteridge 2007). The decrease of antioxidant defenses and/or the increase of intracellular reactive species concentration lead to a status known as oxidative stress, in which the excess of free radicals results in lipid, protein, and DNA damage and, consequently, tissue damage (Beckman and Ames 1998; Halliwell and Gutteridge 2007).

Studies in humans showed that oxidative stress occurs in inborn errors of metabolism, such as phenylketonuria, X-linked adrenoleukodystrophy, and some lysosomal storage disorders (Barschak et al. 2006; Biancini et al. 2012; Pereira et al. 2008; Roversi et al. 2006; Sitta et al. 2006; Vargas et al. 2004).

Literature suggests that oxidative stress can play an important role in the pathophysiology of MPS I, as it was demonstrated by a high level of lipid peroxidation in MPS I patients, and ERT induced an increase of

G. W. Negretto · C. R. Vargas
Programa de Pós-Graduação em Ciências Farmacêuticas, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Av. Ipiranga, 2752, 1° andar, Porto Alegre, RS 90610-000, Brazil

G. W. Negretto (✉) · M. Deon · G. B. Biancini · M. G. Burin · R. Giugliani · C. R. Vargas (✉)
Serviço de Genética Médica, Hospital de Clínicas de Porto Alegre (HCPA), Ramiro Barcelos 2350, Porto Alegre, RS 90035-903, Brazil
e-mail: gnegretto@gmail.com
e-mail: crvargas@hcpa.ufrgs.br

catalase and decrease of superoxide dismutase activities (Pereira et al. 2008). Considering this scenario, the aim of this study was to analyze urinary GAG of MPS II patients at the moment of diagnosis and during ERT, as well as oxidative stress parameters.

Materials and methods

Patients, controls, and biological samples

Twelve subjects with MPS II were recruited from the Medical Genetic Service of the Clinical Hospital of Porto Alegre, RS, Brazil, after confirmation of MPS II diagnosis by measurement of enzyme activity in leukocytes and by urinary GAG quantification (Table 1) (Jong et al. 1992; Voznyi et al. 2001). Samples of participants were divided in two groups: before ERT and after ERT. The treatment consisted of 6 months of idursulfase replacement (0.5 mg/kg) once a week by 3 h of intravenous infusion (Elaprase®, Shire Human Genetic Therapies Inc., Cambridge, MA, USA). The control group consisted of six healthy subjects with similar ages to the MPS II patients accompanied by Laboratory of Clinical Analysis of Pharmacy Faculty of Federal University of Rio Grande do Sul. This study was approved by the Ethics Committee of the Clinical Hospital of Porto Alegre, RS, Brazil. Informed consent was obtained according to the guidelines of this committee.

Blood samples were obtained from patients and controls by venous puncture in vials containing EDTA. Thereafter, blood samples were centrifuged at 1,000×*g*, and plasma was removed and frozen at −80 °C until biochemical analysis. Leukocytes were obtained from whole blood to do the alkaline comet assay. Occasional urine was obtained from the same patients and controls, and these samples were frozen at −80 °C until GAG analysis.

Oxidative stress biomarkers

The lipid peroxidation index was evaluated by measurement of malondialdehyde (MDA) by high performance liquid phase chromatography (HPLC) following the Esterbauer and Cheeseman modified method. MDA results were expressed in micromolar (μM) (Esterbauer and Cheeseman 1990). In order to evaluate DNA damage index, the alkaline comet assay was performed, as described by Singh et al. (Singh et al. 1988).

Urinary glycosaminoglycans determination

The quantification of urinary GAG followed the dimethylene blue method by Blau et al., which consists of the color reaction between the dye 1,9-dimethylmethylene blue chloride and glycosaminoglycans followed by spectrophotometry (Blau et al. 1996).

Table 1 Characteristics of Mucopolysaccharidosis II patients before ERT

Patient	Urinary GAG levels at diagnosis (μg/mL)	Age at the beginning of ERT	Coarse facial features	Short stature	Developmental delay	Joint contractures
1	648	1 year, 7 months	Absent	Absent	Absent	Absent
2	683	1 year, 7 months	Absent	Absent	Absent	Absent
3	525	3 years, 6 months	Absent	Absent	Absent	Mild
4	265	7 years, 6 months	Absent	Absent	Absent	Mild
5	430	4 years, 1 month	Mild	Absent	Mild	Mild
6	280	4 years, 4 months	Moderate	Moderate	Moderate	Mild
7	314	4 years, 1 month	Mild	Absent	Mild	Mild
8	335	5 years, 2 months	Moderate	Moderate	Severe	Moderate
9	481	4 years, 8 months	Mild	Absent	Moderate	Mild
10	399	5 years, 2 months	Moderate	Mild	Mild	Mild
11	428	6 years, 9 months	Mild	Mild	Absent	Mild
12	949	2 years, 2 months	Mild	Mild	Mild	Mild

Statistical analysis

Data were expressed as mean±standard deviation (mean±SD). For the statistical analysis, non-paired Student's *t* test was used to compare results from control and MPS II patients, and paired Student's *t* test was used to compare results from MPS II patients at diagnosis and after ERT. Correlations between biochemical parameters in patients at diagnosis and during ERT were carried out using the Pearson correlation coefficient. A *p* value of less than 0.05 was considered to be significant. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) software in a compatible computer.

Results

Comet assay was performed in peripheral blood leukocytes from MPS II patients, resulting in an increase of DNA damage levels in this group (mean±SD 66.20±1.87 arbitrary units) when compared to controls (mean±SD 17.20±2.68 arbitrary units), as well as a reduction of this damage after initiation of treatment (mean±SD 51.23±4.49 arbitrary units) ($p<0.01$). MDA results showed a higher lipid oxidative damage in diagnosis (mean±SD 149.19±7.12 μM) and after ERT (mean±SD 74.80±10.90 μM) when compared to controls (mean±SD 28.76±3.38 μM), suggesting that there is more lipid injury in MPS II patients when compared to healthy individuals ($p<0.01$). Moreover, there was a decrease in MDA levels in treated patients (mean±SD 74.80±10.90 μM) when compared to non-treated group (mean±SD 149.19±7.12 μM), showing that ERT is able to reduce lipid peroxidation ($p<0.01$). A strong significant positive correlation ($r=0.905$, $p<0.01$) was observed between lipid peroxidation and DNA damage, suggesting that DNA injury is probably oxidative.

Figure 1 represents urinary GAG quantification, which showed that MPS II patients have very significant high levels of GAG at diagnosis when compared to controls ($p<0.01$). On the other hand, ERT induced a decrease in GAG storage when compared to non-treated patients ($p<0.01$), once the deficient enzyme is being replaced.

Discussion

Since it has been demonstrated that oxidative stress is presented in many LSD, researches should be encouraged to investigate oxidative pathways presented by MPS disorders, in order to better understand the pathophysiology of this LSD as well as to improve new treatments and patients' quality of life. There are reports showing a decrease in GAG storage and clinical benefits after ERT, such as an improvement in mobility and pulmonary function, as well as a reduction in hepatosplenomegaly (Germain 2005; Kakkis et al. 2001). Therefore, in this study, we analyzed GAG storage in MPS II patients at the moment of diagnosis and after ERT, as well as oxidative stress in biomolecules, such as DNA and lipids.

Our results showed increased lipid peroxidation and DNA damage at diagnosis in MPS II patients, and ERT induced a decrease on this process. Moreover, ERT

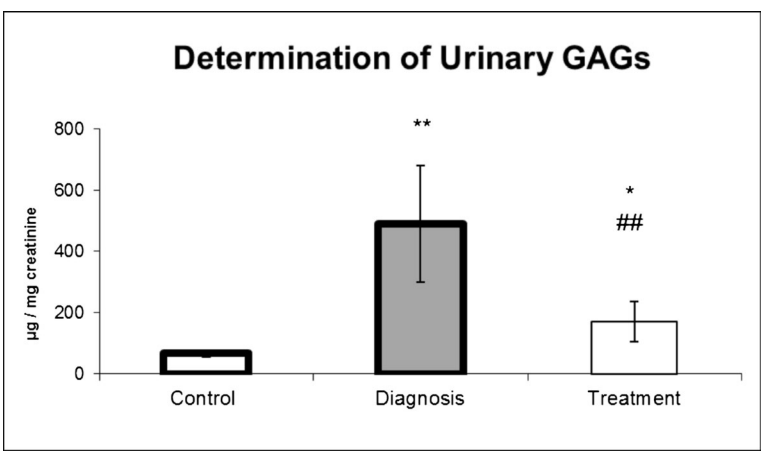

Fig. 1 Determination of glycosaminoglycans (GAG) in urine from MPS II patients at diagnosis and after ERT, as well as control individuals. Data represent the mean±standard deviation. Difference from control, *$p<0.05$, **$p<0.01$ (Student's *t* test for unpaired samples). Difference between patients at diagnosis and after ERT groups, $^{\#\#}p<0.01$ (Student's *t* test for paired samples)

induces a decrease in GAG urinary levels. These results demonstrated that the enzyme replacement can decrease GAG storage and that probably GAG abnormal accumulation can be associated with oxidative damage in lipids, such as cell membrane lipids, after cell swelling. Furthermore, it is possible to speculate that the high levels of GAG in lysosomes can also be associated with DNA damage. Besides, the positive correlation between lipid peroxidation and DNA damage index suggested that DNA injury is oxidative.

Di Domenico et al. (2009) showed in a MPS IIIB murine model, using the lentiviral-alpha-N-acetylglucosaminidase (NAGLU) vector to intracranial deliver of the functional human NAGLU gene into the brain of young adult mice, a significant decrease in the expression of the cytokine MIP1-alpha (Ccl3), at 6 months from treatment, when compared to untreated mice. Ccl3 is an inflammatory chemokine responsible for macrophage recruitment. Not only inflammation-related genes but also oxidative stress-related genes, such as gp91phox, a component of the enzyme complex NADPH-dependent oxidase and also a source of ROS during inflammation, were studied, leading to a decrease in expression at 6 months from treatment comparing to untreated brains, suggesting the possibility of its use as biomarkers for the follow-up of therapy (Di Domenico et al. 2009).

Different oxidative stress parameters have been used to analyze oxidative damage in patients affected by LSD, and an increase in oxidative injury to biomolecules in several of these disorders, such as Fabry disease, Niemann-Pick type C (NPC), different types of MPS, and Gaucher disease was demonstrated. Pereira et al. (2008) observed high levels of lipid peroxidation in MPS I patients and also verified that ERT in MPS I resulted in an increase of catalase activity as well as a decrease in superoxide dismutase activity. Besides, there are studies regarding oxidative stress before and after the treatments with ERT that are currently recommended for LSD, as for example MPS I, Fabry disease, NPC, and Gaucher disease, showing the benefits of ERT upon oxidative damage (Biancini et al. 2012; Deganuto et al. 2007; Pereira et al. 2008; Ribas et al. 2012; Roversi et al. 2006; Shen et al. 2008).

According to our results, oxidative stress process is present at diagnosis in MPS II patients, and ERT protects against lipid and DNA damage. Besides, ERT can lead to GAG reduction, probably influencing positively against oxidative damage to biomolecules, such as lipids and DNA in this disease.

Acknowledgments This study was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundo de Incentivo à Pesquisa e Eventos (FIPE/HCPA). We also thank the patients who participated in this study for all the support during the research.

Conflict of interest All authors declare that there are no financial or commercial conflicts of interest.

References

Barschak AG, Sitta A, Deon M, et al. Evidence that oxidative stress is increased in plasma from patients with maple syrup urine disease. Metab Brain Dis. 2006;21(4):279–86.

Beckman KB, Ames BN. The free radical theory of aging matures. Physiol Rev. 1998;78(2):547–81.

Berry HK. Screening for mucopolysaccharide disorders with the Berry spot test. Clin Biochem. 1987;20:365–71.

Biancini GB, Vanzin CS, Rodrigues DB, et al. Globotriaosylceramide is correlated with oxidative stress and inflammation in Fabry patients treated with enzyme replacement therapy. Biochim Biophys Acta. 2012;1822:226–32.

Blau N, Duran M, Blaskovics ME, et al. Approach to diagnosis. Simple tests in urine and blood. In: Blau N et al., editors. Physician's guide to the laboratory diagnosis of metabolic diseases. New York: Chapman & Hall Medical; 1996. p. 3–12.

Coelho JC, Wajner M, Burin MG, et al. Selective screening of 10000 high-risk Brazilian patients for the detection of inborn errors of metabolism. Eur J Pediatr. 1997;156:650–4.

Deganuto M, Pittis MG, Pines A, et al. Altered intracellular redox status in Gaucher disease fibroblasts and impairment of adaptive response against oxidative stress. J Cell Physiol. 2007;212(1):223–35.

Di Domenico C, Villani GR, Di Napoli D, et al. Intracranial gene delivery of LV-NAGLU vector corrects neuropathology in murine MPS IIIB. Am J Med Genet A. 2009;149A(6):1209–18.

Esterbauer, Cheeseman KH. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Methods Enzymol. 1990;186:407–21.

Germain DP. Enzyme replacement therapies for lysosomal storage disorders. Med Sci. 2005;21(11):77–83.

Giugliani R, Rojas VM, Martins AM, et al. A dose-optimization trial of laronidase (Aldurazyme) in patients with mucopolysaccharidosis I. Mol Genet Metab. 2009;96(1):13–9.

Halliwell B, Gutteridge JMC, editors. Free radicals in biology and medicine. Oxford: Oxford University Press; 2007.

Harmatz P, Giugliani R, Schwartz IV, et al. Long-term follow-up of endurance and safety outcomes during enzyme replacement therapy for mucopolysaccharidosis VI: final results of three clinical studies of recombinant human N-acetylgalactosamine 4-sulfatase. Mol Genet Metab. 2008;94(4):469–75.

Jong JG, Wevers RA, Liebrand-van Sambeek R. Measuring urinary glycosaminoglycans in the presence of protein: an improved screening procedure for mucopolysaccharidoses

based on dimethylmethylene blue. Clin Chem. 1992;38(6): 803–7.

Kakkis ED, Muenzer J, Tiller GE, et al. Enzyme replacement therapy in mucopolysaccharidosis I. N Eng J Med. 2001;344:182–8.

Mabe P, Valiente A, Soto V, et al. Evaluation of reliability for urine mucopolysaccharidosis screening by dimethylmethylene blue and Berry spot tests. Clin Chim Acta. 2004;345:135–40.

Muenzer J, Gucsavas-Calikoglu M, McCandless SE, et al. A phase I/II clinical trial of enzyme replacement therapy in mucopolysaccharidosis II (Hunter syndrome). Mol Genet Metab. 2007;90(3):329–37.

Neufeld EF, Muenzer J. The mucopolysaccharidoses. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. The metabolic basis of inherited disease. New York: McGraw Hill; 2001.

Pereira VG, Martins AM, Micheletti C, D'Almeida V. Mutational and oxidative stress analysis in patients with mucopolysaccharidosis type I undergoing enzyme replacement therapy. Clin Chim Acta. 2008;387(1–2):75–9.

Ribas GS, Pires R, Coelho JC, et al. Oxidative stress in Niemann-Pick type C patients: a protective role of N-butyl-deoxynojirimycin therapy. Int J Dev Neurosci. 2012;30(6): 439–44.

Roversi FM, Galdieri LC, Grego BHC, Souza FG, Micheletti C, Martins AM, et al. Blood oxidative stress markers in Gaucher disease patients. Clin Chim Acta. 2006;364(1–2):316–20.

Shen JS, Meng XL, Moore DF, et al. Globotriaosylceramide induces oxidative stress and up-regulates cell adhesion molecule expression in Fabry disease endothelial cells. Mol Genet Metab. 2008;95(3):163–8.

Singh N, McCoy M, Tice R, Schneider E. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res. 1988;175(1):184–91.

Sitta A, Barschak AG, Deon M, et al. Investigation of oxidative stress parameters in treated phenylketonuric patients. Metab Brain Dis. 2006;21(4):287–96.

Vargas CR, Wajner M, Sirtori LR, et al. Evidence that oxidative stress is increased in patients with X-linked adrenoleukodistrophy. Biochim Biophys Acta. 2004;1688(1):26–32.

Voznyi YV, Keulemans JL, van Diggelen OP. A fluorimetric enzyme assay for the diagnosis of MPS II (Hunter disease). J Inherit Metab Dis. 2001;24(6):675–80.