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Oxidative stress and inflammation in mucopolysaccharidosis type IVA patients treated with enzyme replacement therapy

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ABSTRACT

Mucopolysaccharidosis type IVA (MPS IVA) is an inborn error of glycosaminoglycan (GAG) catabolism due to the deficient activity of N-acetylgalactosamine-6-sulfate sulfatase that leads to accumulation of the keratan sulfate and chondroitin 6-sulfate in body fluids and in lysosomes. The pathophysiology of this lysosomal storage disorder is not completely understood. The aim of this study was to investigate oxidative stress parameters, pro-inflammatory cytokine and GAG levels in MPS IVA patients. We analyzed urine and blood samples from patients under ERT ($n = 17$) and healthy age-matched controls ($n = 10$–15). Patients presented a reduction of antioxidant defense levels, assessed by a decrease in glutathione content and by an increase in superoxide dismutase activity in erythrocytes. Concerning lipid and protein damage, it was verified increased urine isoprostanes and di-tyrosine levels and decreased plasma sulfhydryl groups in MPS IVA patients compared to controls. MPS IVA patients showed higher DNA damage than control group and this damage had an oxidative origin in both pyrimidine and purine bases. Interleukin 6 was increased in patients and presented an inverse correlation with GSH levels, showing a possible link between inflammation and oxidative stress in MPS IVA disease. The data presented suggest that pro-inflammatory and pro-oxidant states occur in MPS IVA patients even under ERT. Taking these results into account, supplementation of antioxidants in combination with ERT can be a tentative therapeutic approach with the purpose of improving the patient's quality of life. To the best of our knowledge, this is the first study relating MPS IVA patients with oxidative stress.

1. Introduction

Mucopolysaccharidoses (MPSs) are a heterogeneous group of inherited lysosomal storage disorders (LSDs) caused by deficiency of the enzymes involved in the degradation of glycosaminoglycans (GAGs). MPSs are classified into 11 syndromes according to the specific deficient enzyme [1]. MPS IVA (or Morquio A Syndrome) is a rare disorder with an incidence in the general population estimated in 1:201,000 [2]. However, this incidence ranges among different populations from 1 in 76,000 live births in Northern Ireland to 1 in 640,000 live births in Western Australia [2]. This disorder is caused by a defect on N-acetylgalactosamine-6-sulfate sulfatase enzyme (GALNS; E.C.3.1.6.4),

Abbreviations: 8-OHdG, 8-hydroxy-2′-deoxyguanosine; CAT, catalase; Cr, creatinine; DI, damage index; Di-Tyr, di-tyrosine; DMB, 1,9-dimethylmethylene blue; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); Endo III, endonuclease III; ELISA, enzyme-linked immunoassay; ERT, enzyme replacement therapy; FU, fluorescence units; GAGs, glycosaminoglycans; GALNS, N-acetylgalactosamine-6-sulfate sulfatase; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, glutathione oxidized; H_2O_2, hydrogen peroxide; IEM, inborn errors of metabolism; IL-6, interleukin 6; LSDs, lysosomal storage diseases; MDA, malondialdehyde; MPS, mucopolysaccharidoses; $OH^{\bullet}$, hydroxyl radical; RNA, ribonucleic acid; SEM, standard error of the mean; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; TNB, tionitrobenzoic acid

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responsible for chondroitin 6-sulfate and keratan sulfate degradation. The GALNS enzyme deficiency leads to the accumulation of GAGs and subsequent cellular damage, mainly in connective tissues where keratan sulfate is abundant, specially cartilage, cornea and heart valve [3,4]. The main clinical manifestations of MPS IVA patients are: skeletal dysplasia, restricted growth and short stature, joint hypermobility, valvular heart disease, pulmonary disease, corneal clouding, hearing loss, and poorly formed teeth [5]. It was already described about 277 mutations in the *GALNS* gene, and this extensive allelic heterogeneity is consistent with the broad spectrum of phenotypes observed in MPS IVA [6,7]. The characteristic signs and symptoms of this disease appear at around two or three years of age. Phenotypic spectrum may range from a more attenuated phenotype, with patients reaching the age of 70 years, to a more severe phenotype, where patients survive no more than 20–30 years [5,8–10]. MPS IVA patients have no neurological impairment, differently of many other MPSs and inborn errors of metabolism (IEM) [11].

Diagnosis of MPS IVA is typically based on clinical examination, skeletal radiographs, evaluation of the pattern of urinary GAGs and measurement of the enzymatic activity of GALNS in white blood cells or fibroblasts [5,10,12]. Until recently, there was no effective therapy for MPS IVA and treatment was palliative, focusing on management of the multiple clinical symptoms [10,13]. The recent introduction of ERT with recombinant human enzyme (elosulfase alfa), however, has raised the possibility that the burden of GAGs storage can be reduced and that the progressive tissue and organ damage associated with MPS IVA can be slowed or even prevented. In a recent study, MPS IVA patients undergoing ERT showed improvements in 6-minute-walk test and a decrease in keratan sulfate levels [14].

Oxidative stress may play an important role in the pathophysiology of some IEM. Even though this relationship is not well elucidated, the accumulation of toxic metabolites is considered the main responsible for the increased generation of reactive species, which can react with lipids, proteins, DNA and RNA [15–18]. Most studies linking oxidative stress and MPS were performed in animal models. In this context, we can mention the study developed by Villani et al. [19] that showed the presence of oxidative stress even in the early stages of MPS IIIB, and another work demonstrating an oxidative imbalance in an animal model of MPS I [20]. Furthermore, a possible link between inflammation and oxidative stress in MPS IIIA mice was reported by Arfi et al. [21]. In animal models of MPS VI and VII, it was verified an inflammation process caused by intralysosomal accumulation of GAGs, which could trigger the release of cytokines, chemokines, proteases and nitric oxide, culminating in apoptosis and consequent destruction of connective tissue [22]. Ohmi et al. [23] showed microglia activation in mouse models of MPS I and IIIB, and this activation is one possible explanation for the involvement and occurrence of inflammatory processes along with oxidative damage in various diseases. In mice with MPS IIIB, Di Domenico et al. [24] demonstrated a significant reduction in the expression of several genes related to oxidative stress and inflammation, after six months of gene therapy, corroborating with the hypothesis that both processes are involved in the pathogenesis of MPS IIIB.

With regard to research in patients, there are few reports relating MPS with oxidative stress and inflammation. Pereira et al. [25] found high lipid peroxidation levels in MPS I patients. Moreover, they showed that ERT was able to induce an increase of catalase (CAT) and a decrease of superoxide dismutase (SOD) activities at specific times of treatment. However, these enzymatic changes were not sufficient to reduce lipid peroxidation levels, suggesting an important role of oxidative stress in the pathophysiology of MPS I, even during treatment. In a study with MPS II patients, it was observed oxidative damage to proteins and lipids, as well as increased CAT activity and decreased total antioxidant status before treatment when compared to controls. In these patients, until the third month of the ERT, there was a decrease in lipid peroxidation levels and in protein damage, suggesting a possible involvement of oxidative stress in the pathophysiology of MPS II and a protective role of therapy [26]. In addition, ERT was capable to decrease DNA damage [27] and also GAG levels in MPS II patients [28], what suggests that the decrease of metabolites accumulation probably may be related to the improvement of oxidative stress parameters. Nevertheless, patients with Fabry disease, another type of LSD, under ERT, presented high levels of oxidative damage to proteins and lipids, accompanied by low antioxidant defenses and high pro-inflammatory cytokines [29]. This study also showed, in treated Fabry patients, a positive correlation between oxidative stress, inflammation and the metabolites accumulated in this disease.

Considering the involvement of oxidative stress and inflammation in other MPSs and the lack of reports in this issue in MPS IVA, the aim of this study was to evaluate oxidative damage to biomolecules, antioxidant defenses, pro-inflammatory cytokine and GAG levels in patients with Morquio A disease.

2. Materials and methods

2.1. Subjects

For this study it was collected blood and occasional urine samples from 17 MPS IVA patients with ages varying between 6 and 36 years (15.3 ± 8.9 years old, mean ± standard deviation). For the control group we collected blood from 14 healthy individuals with ages ranging between 10 and 28 years (24.0 ± 4.8 years old, mean ± standard deviation) and for comet assay from 10 healthy individuals with ages varying between 19 and 23 years (20.8 ± 1.5 years old, mean ± standard deviation). Urine was collected from 15 healthy individuals with ages between 7 and 32 years (19.7 ± 7.9 years old, mean ± standard deviation). All MPS IV A patients were receiving ERT treatment (elosulfase alfa—Vimizim® 2 mg/kg) every week by intravenous infusion, for about 32 weeks. At the moment of diagnosis, patients presented the classic symptoms, usually including short stature, skeletal deformities (pectus carinatum and genu valgum almost always present), limited ambulation, restrictive airway disease, heart valves problems and corneal clouding. Patients' data were described in Table 1. Diagnosis was confirmed by evaluation of GAGs in urine (increased total content and presence of increased amounts of keratan sulfate) and measurement of GALNS in leucocytes (deficient activity) [10,30].

Informed consent was obtained from all participants. The study was approved (number 13-0246) by The Ethics Committee of the *Hospital de Clínicas de Porto Alegre* (HCPA), RS, Brazil.

2.2. Sample collection and preparation

Occasional urine and heparinized blood samples were obtained from patients immediately before the session of ERT. Urine samples were collected in sterile flask, aliquoted and frozen at −80 °C until analysis. Samples were obtained from controls concomitantly. Whole blood was centrifuged at 1000 ×*g* for 10 min and plasma was removed by aspiration, aliquoted and frozen at −80 °C until biochemical determinations. An aliquot of whole blood was separated for comet assay. Erythrocytes were washed three times with cold saline solution (0.153 mol/L sodium chloride) and the lysates were prepared by addition of 1 mL of distilled water to 100 μL of washed erythrocytes. The lysates were frozen at −80 °C until determination of GSH and antioxidant enzymes activities. For these determinations, the supernatant (after centrifugation at 13,500 ×*g* for 10 min) was diluted in order to contain approximately 0.5 mg/mL of protein.

2.3. Reduced glutathione (GSH) content in erythrocytes

In order to measure GSH levels, the main intracellular antioxidant, lysates of erythrocytes were processed as described by Browne and Armstrong [31] and the fluorescence measured (λexcitation = 350 nm,