A-Glucosidase Deficiency Promotes Increasing Protein Oxidative Damage in Pompe Disease Patients

Alexandre Silva de Mello1,*, Marina Rocha Frusciante1, Luciana Gonçalves Kneib1, Gabrielli Bortolato1, Jaqueline Cé2, Caroline Dani1, Cláudia Funchal1 and Janice Carneiro Coelho2

1Graduate Program in Bioscience and Rehabilitation, Methodist University, IPA, Porto Alegre, RS, Brazil
2Undergraduate Program in Biological Sciences: Biochemistry, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil

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Introduction

Pompe disease (MIM 232300) is an autosomal recessive disorder of lysosomal storage caused by the deficiency of α-glucosidase lysosomal enzymes. Several studies have demonstrated the involvement of oxidative stress in numerous pathophysiological changes observed [4-9]. Oxidative stress is characterized by an imbalance in oxidant and antioxidant molecules with this imbalance causing possible cellular damage [10]. This condition is caused either by an overproduction of pro-oxidants or by a deficiency in the antioxidant system which may cause damage to lipids, proteins and deoxyribonucleic acid (DNA) [11,12].

The deleterious effects of reactive species over biomolecules results in the manifestation of potential oxidative damage [13-15]. Biomarkers based on the oxidative damage of proteins are very important due to their implications, which are the functional alteration of enzymes, transport of proteins and others. The products resulting from the oxidative damage of proteins may contribute to the generation of secondary damage to other biomolecules. Damage to the DNA, for example, may be irreversible, due to the functional alteration of repair enzymes [14]. Although controversial the precursor protein-mediated accumulation of amyloid-β-peptide (Aβ) is an important phenomenon in the pathology of Alzheimer’s disease. If the production and clearance of Aβ is deregulated, the blood-brain barrier is compromised [16].

In this sense, the aim of this study was to analyze the levels of oxidative stress markers, as well as the levels of antioxidant defense markers, in order to seek a better understanding of the cellular changes that occur to patients with PD compared to healthy controls.

Materials and Methods

Patients and controls

This study was characterized by a prospective study. The diagnosis of PD in patients happened at the Federal University of Rio Grande do Sul (Porto Alegre, RS, Brazil).

Blood samples of 9 mL. were collected directly (by one of the researchers of this study) from 10 patients (4 women and 6 men) previously diagnosed with PD and from 10 controls (6 women and 4 men) (Table 1). For donors over 18 years old or those responsible for

*Corresponding author: Alexandre Silva de Mello, Graduate Program in Bioscience and Rehabilitation of the Methodist University Center IPA, Rua Coronel Joaquim Pedro Salgado, 80, Rio Branco, Porto Alegre, RS, CEP 90420-060, Brazil, Tel: +555196622626; E-mail: melloas@gmail.com

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underage donors, an informed consent was obtained according to the committee guidelines. All samples were identified with numbers, preserving the identity of donors, who were not informed of the results nor had their identities revealed at any stage of the procedure. As a criterion for inclusion of the samples, individuals had to be at least 7 years old, weigh more than 18 kg and be over 90 cm tall.

The blood was processed for plasma separation and leukocyte isolation according to the Skoog and Beck [17] method.

Lipid peroxidation (TBARS), protein damage (Carbonyl assay), contents of non-enzymatic antioxidant defenses (SH) and antioxidant defenses enzymatic activity (CAT and SOD1) were analyzed by plasma samples.

We carried out the sample size calculation for comparing averages with different variances establishing the level of significance at 5% and power by 90%. For this we used the MiniTab® 15 statistical software. The calculation indicated 10 subjects per group for a total of 20 samples.

The Research and Ethical Committee of Centro Universitário Metodista - IPA (no. 47608615.5.0000.5308), ethically and scientifically approved of the research protocols and consent forms, as well as the investigation.

Activity of α-glucosidase enzyme

The activity of the α-glucosidase enzyme was evaluated according to another method [18], which uses 4-methylumbelliferyl- α-D-glucoside (Sigma) as the synthetic substrate, together with acetate (Sigma) in standard conditions (pH 4.0 and 37°C). Data are expressed as mmol/h/mg of protein [19].

Thiobarbituric reactive substances (TBARS)

Substances reactive to thiobarbituric acid (TBARS) are used as a measure of lipid peroxidation and in this case were dosed according to Wills [20]. The TBARS reacts with the liperoxidation products, forming a compound of pink color, which was measured in a spectrophotometer (535 nm). The results were expressed in nmol TBARS/mg protein.

Measuring oxidatively modified proteins levels

Oxidative damage of proteins was measured by determining the carbonyl groups and is based on the reaction with dinitrophenylhydrazine (DNPH) according to Levine et al. [21]. The DNPH reacts with the protein carbonyls to form hydrazones, which can be measured spectrophotometrically at 370 nm. The results were expressed in nmol per mg protein.

Total measurement of sulfhydryl groups (SH)

The quantitation of the total concentration of sulfhydryl groups provides a view of the oxidative damage level of plasma protein that is based on the reaction with 5,5’-dithiobis-2-nitrobenzoic acid (DTNB) and Portion according to Aksenov and Marksbery [22]. Results were expressed as nmol/mg protein.

Measurement of antioxidant enzymes (SOD1 and CAT)

Catalase (CAT) activity was determined by absorbance decay of the hydrogen peroxide (H₂O₂) at 240 nm at 25°C according to Aebi [23]. The result was expressed as unit per mg of CAT protein. Superoxide dismutase 1 (SOD1) activity was determined spectrophotometrically by measuring the inhibition of adrenochrome formation rate at 480 nm [24]. Enzymatic activity was expressed as unit per mg of SOD protein.

Protein dosage

Proteins were quantified by the Lowry method [19] using bovine serum albumin at a concentration of 1 mg/ml as standard.

Statistical Analysis

Data from carbonyl, SH and SOD1 and the enzymatic activity were statistically analyzed by Student's t test, followed by Levene’s test. The results were reported as mean ± standard deviation. As to the TBARS and CAT, they were expressed as median (interquartile range) and compared using the Mann-Whitney U test. P values <0.05 were considered significant. All analyzes were performed using SPSS, version 17.

Results and Discussion

Once confirmed the enzyme deficiency, we observed a decrease of the enzymatic activity of α-glucosidase in the leukocytes samples from PD patients compared to samples of normal individuals (Table 1).

Enzyme deficiencies are characteristic of DLDs and can induce a cascade of events that result(s) in side effects such as the production of reactive oxygen species (ROS) [25–27]. In contrast, production and degradation of ROS are generally balanced in the bodies of healthy individuals [25].

Regarding the oxidative stress parameters, there is an increase in protein oxidation levels (carbonyl groups) in PD patients plasma compared to controls, thus suggesting oxidative damage to proteins (Figure 1). Such damage can be harmful in vivo by direct effects (change or loss of function of enzymes or transporters and indirect contribution to DNA damage). Furthermore, the direct alteration of the protein structure by oxidation can trigger an autoimmune response, once modifier proteins are recognized as non-self by the immune system.
system [14]. In relation to the protein oxidative damage biomarkers, the carbonyl groups are the most relevant [28], these are generated as a result of direct action of the reactive species on the amino acid side chains or indirectly upon glucose binding (Glico oxidation process) or aldehydes (including those formed during lipid peroxidation). The increase in the protein carbonyl group is associated with numerous pathological disorders, including rheumatoid arthritis, Alzheimer's disease (AD), respiratory distress syndrome, Parkinson's disease and atherosclerosis [29]. Particularly in the AD, Aβ leads to senile plaques, which, together with hyperphosphorylated tau-based neurofibrillary tangles and synapse loss, are the principal pathological hallmarks of AD. Aβ is associated with the formation of ROS and nitrogen (RNS) species, and induces calcium-dependent excitotoxicity, impairment of cellular respiration, and alteration of synaptic functions associated with learning and memory [30].

As for the levels of lipid peroxidation (TBARS), we did not observe any significant difference among individuals with PD and healthy controls (Figure 1). The data found in TBARS, corroborated by the literature, shows the difference in the values between the groups studied [26]. These results confirm the findings obtained by this group in a previous study [31], which also showed no significant difference in the parameter between patients with Gaucher's Disease and healthy controls [26]. These results confirm the findings obtained by this group [31], which also showed no significant difference in the values between the groups studied [26].

In conclusion, the results show that the enzymatic deficiency of α-glucosidase, a characteristic of PD, alters the oxidation system associated to proteins. This is involved in the structures that move the body, affecting the skeletal muscles of individuals.

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