Research in Developmental Disabilities 35 (2014) 1228–1236

Contents lists available at ScienceDirect

Research in Developmental Disabilities

Antioxidant intervention attenuates oxidative stress in children and teenagers with Down syndrome

Eduardo Benedetti Parisotto [a], Thais Regina Garlet [a], Vera Lúcia de Liz Oliveira Cavalli [b], Ariane Zamoner [b], Julia Salvan da Rosa [c], Juliana Bastos [d], Gustavo Amadeu Micke [d], Tania Silvia Fröde [c], Rozangela Curi Pedrosa [b], Danilo Wilhelm Filho [a,*]

[a] Department of Ecology and Zoology, Federal University of Santa Catarina, Florianópolis, Brazil
[b] Department of Biochemistry, Federal University of Santa Catarina, Florianópolis, Brazil
[c] Department of Clinical Analysis, Federal University of Santa Catarina, Florianópolis, Brazil
[d] Department of Chemistry, Federal University of Santa Catarina, Florianópolis, Brazil

ARTICLE INFO

Article history:
Received 9 January 2014
Received in revised form 7 March 2014
Accepted 7 March 2014
Available online 28 March 2014

Keywords:
Down syndrome
Reactive oxygen species
Oxidative stress
Antioxidant therapy

ABSTRACT

We previously demonstrated that systemic oxidative stress is present in Down syndrome (DS) patients. In the present study we investigated the antioxidant *status* in the peripheral blood of DS children and teenagers comparing such *status* before and after an antioxidant supplementation. Oxidative stress biomarkers were evaluated in the blood of DS patients ($n = 21$) before and after a daily antioxidant intervention (vitamin E 400 mg, C 500 mg) during 6 months. Healthy children ($n = 18$) without DS were recruited as control group. The activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), gamma-glutamyltransferase (GGT), glucose-6-phosphate dehydrogenase (G6PD) and myeloperoxidase (MPO), as well as the contents of reduced glutathione (GSH), uric acid, vitamin E, thiobarbituric acid reactive substances (TBARS), and protein carbonyls (PC) were measured. Before the antioxidant therapy, DS patients presented decreased GST activity and GSH depletion; elevated SOD, CAT, GR, GGT and MPO activities; increased uric acid levels; while GPx and G6PD activities as well as vitamin E and TBARS levels were unaltered. After the antioxidant supplementation, SOD, CAT, GPx, GR, GGT and MPO activities were downregulated, while TBARS contents were strongly decreased in DS. Also, the antioxidant therapy did not change G6PD and GST activities as well as uric acid and PC levels, while it significantly increased GSH and vitamin E levels in DS patients. Our results clearly demonstrate that the antioxidant intervention with vitamins E and C attenuated the systemic oxidative damage present in DS patients.

1. Introduction

Down syndrome (DS), the most frequent genetic disorder occurring in 1 out of every 700 to 1000 live births, is caused by trisomy of all or part of human chromosome 21 (HSA21). The syndrome is characterized by chronicity and severity of abnormalities including intellectual disability, dysmorphic features, as well as immunological, hematological and endocrine

* Corresponding author. Tel.: +55 4837216917; fax: +55 483315156.
E-mail address: dawifi@ccb.ufsc.br (D. Wilhelm Filho).

http://dx.doi.org/10.1016/j.ridd.2014.03.013
0891-4222/

defects (Pueschel, 1990). The phenotypic expression of human trisomy 21 is presumed to result from overexpression of certain genes residing on chromosome 21 at the segment 21q22-the Down locus (Groner et al., 1994; Pagano & Castello, 2012). Moreover, chromosome 21 contains several genes that have been implicated in oxidative stress associated with neurodegeneration, including Cu/Zn superoxide dismutase (SOD) (Lott, 2012).

Oxidative stress is part of the fundamental biology of DS. It has been suggested that the main source of reactive oxygen species (ROS) in DS patients is the excessive production of hydrogen peroxide (H_2O_2) through the action of Cu,Zn-superoxide dismutase (Cu,Zn-SOD) (Garlet et al., 2013; Lott, 2012). As a result of the overexpression of Cu,Zn-SOD in DS patients, an imbalance between Cu,Zn-SOD and other antioxidant enzymes occurs, such as catalase and glutathione peroxidase, inducing a systemic oxidative damage (Garlet et al., 2013). SOD1 promotes the production of H_2O_2, an important precursor of hydroxyl radical ($^{\bullet}OH$), the most reactive and deleterious ROS, therefore it is able to react with important cellular components, oxidizing biomolecules such as amino acid residues, proteins, lipids and DNA (Halliwell & Gutteridge, 2006).

Many epidemiological studies suggest the importance of antioxidants in the prevention of various diseases. In this sense, antioxidant therapy has become the target of interest in several studies involving both natural antioxidants and synthesized molecules (Halliwell & Gutteridge, 2006). The efficacy of antioxidant supplementation in attenuating biomarkers of oxidative stress and its deleterious effects was already been demonstrated in several studies (Halliwell & Gutteridge, 2006), including those from our laboratory (Farias et al., 2012; Halliwell & Gutteridge, 2006; Maçao et al., 2007).

The aim of the present study was to compare the antioxidant *status* in the blood of DS children, before and after 6 months of daily antioxidant supplementation with vitamins E and C.

2. Subjects and methods

2.1. Subjects

The control group was constituted of 18 healthy children (average age of 6.7 ± 3.0 years showing inflammatory markers within normal ranges) without DS (10 males and 8 females; 3–12 years) recruited from the Joana de Gusmão Children's Hospital in Florianópolis, State of Santa Catarina, South Brazil. The DS participants are between 3 and 14 years old, with an average age of 7.7 ± 3.18 years ($n = 21$; 12 males and 9 females). Most of the individuals are concentrated in the age group 6–10 years (65%). The inclusion criteria were age between 3 and 14 years without illnesses associated with systemic diseases and not participating in other studies. Patients who have taken medication or food supplements were excluded. The 21 DS individuals were recruited from two local organizations for DS children (''Associação Amigos Down'' and ''Associação de Pais e Amigos dos Excepcionais – APAE''). The study protocol was previously approved by the Ethics Committee of UFSC, according to the national and international guidelines for research involving human subjects (Resolution No. 1996 of the National Health Council), which regulate experiments involving human subjects (local Protocol CEP N. 2112/2011). All patients received information about the study and their parents signed the free and informed consent form.

2.2. Study design

In a recent accompanied paper by Garlet et al. (2013), biomarkers of oxidative stress were evaluated in blood samples collected from children and teenagers with DS. After the first sample collection of blood from subjects involved in that study, vitamin C (500 mg/day) and vitamin E (400 mg/day) were administered daily to the same subjects during 6 months. At the end of antioxidant supplementation period, a second sample collection was performed and the same oxidative stress biomarkers were evaluated to verify the possible beneficial effect of vitamins C and E (E-TABS® and Energil C®, respectively) supplementation.

2.3. Sample preparation

The whole blood was obtained from the antecubital vein in chilled tubes containing EDTA as anticoagulant, or without EDTA to obtain serum. Immediately after blood collection a blood fraction (200 μL) was precipitated in trichloroacetic acid (TCA 12%, 1:4, v/v) for reduced glutathione (GSH) assays. The remaining blood was centrifuged at $1500 \times g$ for 10 min to separate red cells from plasma. For enzymatic assays, red cells were diluted in distilled water (1:4) and hemolysis was carried out by freezing and thaw procedure. After this, plasma, serum and the acid extracts were stored in liquid nitrogen ($-170\,^{\circ}C$) until analysis of the parameters. Enzymatic evaluations were carried out in hemolysates, while the contents of GSH were obtained in whole blood acid extracts. Thiobarbituric acid reactive substances (TBARS), protein carbonyl and vitamin E contents were examined in plasma. The myeloperoxidase (MPO) activity and the uric acid levels were analyzed in serum.

2.4. Antioxidant enzyme assays

Catalase (CAT) activity was determined by measuring the decrease in a freshly prepared 10 mM hydrogen peroxide solution at 240 nm (Aebi, 1984). Superoxide dismutase activity was measured at 480 nm according to the method of epinephrine autoxidation, with modifications (Boveris, Fraga, Varsavsky, & Koch, 1983; Misra & Fridovich, 1972).