Systemic oxidative stress in children and teenagers with Down syndrome

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A B S T R A C T
Aims: The aim of this study was to evaluate the antioxidant status and oxidative stress biomarkers in the blood of children and teenagers with Down syndrome.

Main methods: The analysis of enzymatic antioxidant defenses, such as the activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione transferase (GST), non-enzymatic antioxidants, such as levels of reduced glutathione (GSH), uric acid (UA) and vitamin E, as well as oxidative damage indicators, such as protein carbonyls (PC) levels and lipoperoxidation (TBARS), of DS individuals (n = 20) compared to healthy controls (n = 18). Except the vitamin E was measured by HPLC, all other markers were measured spectrophotometrically.

Key Findings: Antioxidant enzymes analysis showed significant increases in the SOD (47.2%), CAT (24.7%) and GR (49.6%) activities in DS subjects. No significant difference in GPx activity was detected while GST activity (61.2%) was decreased, and both responses may be consequence of the depletion of GSH (24.9%) levels. There were no significant differences in TBARS levels, while PC levels showed decreased (31.7%) levels compared to healthy controls, which may be related to the increase (16.1%) found in serum UA. Levels of vitamin E showed no significant differences between DS individuals compared to controls.

Significance: The results revealed a systemic pro-oxidant status in DS individuals, evidenced by the increased activity of some important antioxidant enzymes, together with decreased GSH levels in whole blood and elevated UA levels in plasma, probably as an antioxidant compensation related to the redox imbalance in DS individuals.

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Introduction

The incidence of Down syndrome (DS) is approximately 1/1,000 to 1/1,200 of live births (World Health Organization, 2007). DS is caused by a chromosomal aberration involving total or partial trisomy of chromosome 21 in humans. The presence of an extra copy of chromosome 21 affects several phenotypic and physiological features. Mental retardation associated with DS is accompanied by learning and memory deficits, a high rate of repetitive behaviors and impairments in adaptive behavior. In addition, the brain abnormalities observed in DS have been linked to an inherent oxidative stress, for example, characteristics of accelerated aging and high incidence of hyperactivity, with accompanying attention deficits (Ribeiro et al., 2003; Shichiri et al., 2011).

In a region of the distal part of chromosome 21 (21q22 band), which is tripled and known as the critical region of DS, the superoxide dismutase 1 (SOD1) gene is located (Antonarakis, 1998). SOD1 catalyzes the conversion of superoxide anion (O2−) into hydrogen peroxide (H2O2), two of the main reactive oxygen species (ROS) continuously generated mainly in the aerobic cells in the mitochondria (Halliwell and Gutteridge, 2006). H2O2 in turn is degraded by catalase (CAT) and glutathione peroxidase (GPx) into water and molecular oxygen. Hydrogen peroxide produced in excess is a result of increased SOD1 activity without the concomitant increase of complementary antioxidant defense mechanisms, such as CAT and GPx activity (Halliwell and Gutteridge, 2006). The accumulation of endogenous H2O2, which is highly diffusible and relatively stable, therefore is able to generate other deleterious ROS through the Haber–Weiss–Fenton reactions, in this way damaging important cellular components, oxidizing biomolecules such as amino acid residues, proteins, lipids and DNA (Halliwell and Gutteridge, 2006).

The objective of this study is to evaluate the antioxidant status through oxidative stress biomarkers present in the blood of children and adolescents with DS compared to healthy controls, taken into consideration the relatively paucity of information in the specific literature (Pallardó et al., 2006; Lott et al., 2011; Komatsu et al., 2013), especially regarding children with this syndrome.
Reduced glutathione (GSH) assay

Reduced glutathione (GSH) was measured at 412 nm (Beutler et al., 1963), using the reagent DTNB (2-dinitrothiobenzoic acid). After being centrifuged at 3000 g for 5 min, the supernatants from the acid extracts were added to 0.25 DTNB in 0.1 M sodium phosphate buffer pH 8.0, and the formation of the corresponding thiolate anion was immediately measured at 340 nm, by measuring the rate of NADPH oxidation through the glutathione/NADPH/glutathione reductase system, according to the method of epinephrine autoxidation (Misra and Fridovich, 1972).

Vitamin E assay

Vitamin E activity was determined at 340 nm using Analisa® kits through a colorimetric method, based on the oxidation of uric acid, uricase producing a red chromophore measured at 520 nm.

Lipid peroxidation assessment (TBARS levels)

Lipid peroxidation in plasma was determined spectrophotometrically at 535 nm in triplicate by the thiobarbituric acid method (TBARS) (Bird and Draper, 1984). Briefly, plasma was precipitated with trichloroacetic acid (TCA) followed by the incubation with TBA 0.76%, at 100 °C, for 60 min in a phosphate buffer at pH 7.4 (60 mM Tris-acetic acid (TCA) followed by the incubation with TBA 0.76%, at 100 °C, for 60 min in a phosphate buffer at pH 7.4 (60 mM Tris-HCl and 0.1 mM DPTA), at high (100 °C) temperature, the samples were then centrifuged (5 min, 1500 g) and the absorbance of the pink chromophore was measured, using ε = 1.56 × 10^5 M^-1 cm^-1. The values were expressed in nmol TBARS ml^-1.

Protein carbonyls (PC)

Oxidative damage promoted by protein carbonylation was determined using 100 μl of plasma, which were added to 600 μl DPH (2,4-dinitrophenyl-hydrazine). Ethanol/ethylic acetate (1:1; v:v) was used to remove hydrazine excess. After this, incubation with guanidine chloride (600 μl) was performed and the maximum carbonyl absorbance in the range of 360–370 nm was recorded (Levine et al., 1990). The total protein concentration was determined by the method of Lowry et al. (1951), using albumin as a standard. The concentration of carbonyl protein was expressed in nmol·mg^-1. All measurements were carried out in triplicate.

Chemicals

All the chemicals and solvents used were of analytical grade and purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Statistical analysis

Data were expressed as means ± SEM. Unpaired Student t test was used to compare the different groups. The coefficient of Pearson was used to evaluate correlations among GST and GPx activity and GSH concentrations. Minimal values of p < 0.05 were considered statistically significant.
Fig. 1. Enzymatic activity in hemolysates of (A) superoxide dismutase (SOD), (B) catalase (CAT), (C) glutathione peroxidase (GPx), (D) glutathione reductase (GR), and (E) glutathione S-transferase (GST). Contents of (F) reduced glutathione (GSH) in whole blood extracts, contents of (G) uric acid (UA) in serum, contents of (H) vitamin E (vit E), (I) substances that react with thiobarbituric acid (TBARS), and (J) protein carbonyls (PC) in plasma, in Down syndrome subjects (n = 20). Asterisks (*) (***) mean statistical differences for p < 0.05 and p < 0.001, compared to controls (n = 18), respectively.
Results

There was no significant difference between the body mass index (BMI) of patients with DS (17.9 ± 0.76) compared to controls (16.9 ± 0.61). The values of SOD activity in individuals with DS showed increased activity (47.2%; values around 120 U SOD·ml⁻¹) compared to the activity of SOD found in healthy individuals (−63 U SOD·ml⁻¹) (Fig. 1A). Catalase activity also showed a significant (24.7%) increase (−157 μmol·min⁻¹·ml⁻¹) in DS individuals compared to healthy controls (−118 μmol·min⁻¹·ml⁻¹) (Fig. 1B). GR also showed a significantly higher activity (49.6%; −1 μmol·min⁻¹·ml⁻¹) in DS patients compared to controls (−0.5 μmol·min⁻¹·ml⁻¹) (Fig. 1D). GPx activity in DS patients (−57 μmol·min⁻¹·ml⁻¹) showed no significant difference compared to controls (−59 μmol·min⁻¹·ml⁻¹) (Fig. 1C). Contrary to the behavior of the other enzymes examined, GST activity was significantly decreased (61.2%; −43 μmol·min⁻¹·ml⁻¹) compared to subjects of the control group (−111 μmol·min⁻¹·ml⁻¹) (Fig. 1E). The concentrations of TBARS and vitamin E in plasma also showed no significant difference between the groups. On the other hand, GSH and PC concentrations were significantly decreased (24.9%; −1.3 mmol·ml⁻¹ and 31.7%; −0.03 mmol·mg⁻¹, respectively) in DS subjects compared to controls (−1 mmol·ml⁻¹ and −0.02 mmol·mg⁻¹, respectively) (Fig. 1F, H, I, J). The measurement of uric acid levels in the blood of DS individuals (−4 mg·dl⁻¹) showed a significant increase of 16.1% compared to subjects from the control group (−3 mg·dl⁻¹) (Fig. 1G).

Discussion

The results related to the different biomarkers of oxidative stress obtained in the present study clearly revealed a systemic pro-oxidant condition in the blood of DS subjects. The main findings are the upregulation found in most of the activity of antioxidant enzymes in hemolysates as well as the GSH depletion found in whole blood together with the increased contents detected in serum urate values. Several studies carried out on other human pathologies have shown that the augmented activities found in distinct antioxidant enzymes are a physiological response to compensate the persistent oxidative insult, which is also present in DS subjects (Halliwell and Gutteridge, 2006).

The enhanced (47.2%) SOD1 activity detected in the present study is expected in DS patients and also in genetically modified laboratory animals that mimic the overexpression of this important antioxidant enzyme, which is located in the region 21q22 of the chromosome 21 (Brooksbank and Balazs, 1984; Epstein et al., 1987).

Nevertheless, the chronic SOD1 overexpression in all cells that characterize the trisomy 21 subjects and the consequent overgeneration of endogenous hydrogen peroxide apparently is not adequately compensated by the relatively modest upregulation of catalase. Therefore, this chronic imbalance between the levels of both important antioxidant enzymes (SOD/CAT) and their corresponding substrates (O₂⁻/H₂O₂), thereby favoring the generation of the most deleterious hydroxyl radical (•OH), is apparently the basis for DS disturbances (Ani et al., 2000; Vasconcelos et al., 2007), such as neurological and hematological abnormalities as well as the accelerated aging process (Aguilar-da-Silva et al., 2003).

Catalase also showed enhanced activity (24.7%) in DS subjects compared to healthy controls. This CAT upregulation is expected in DS subjects (Pastor et al., 1998), considering that beside the general intervention of glutathione peroxidase on hydroperoxide detoxification, catalase is specifically able to decompose normal levels of hydrogen peroxide generation into water and molecular oxygen (Halliwell and Gutteridge, 2006). However, as mentioned before, such increased activity seems not to be sufficient to avoid the excess of H₂O₂ generated by the overexpression of SOD1 in all cells of trisomy 21 subjects, in this way contributing to a persistent oxidative stress found in DS subjects.

The cellular response involving GPx activity in the erythrocytes of DS subjects is controversial (Kedziora and Bartosz, 1988). As found in the present work, other related studies also reported no alterations in this important antioxidant enzyme compared to healthy individuals (Brooksbank and Balazs, 1983; Vertongen et al., 1984). However, similar studies revealed enhanced GPx activity, both in humans and also in laboratory animals (Ceballos et al., 1988; Pastor et al., 1998). To detoxify hydroperoxides, GPx needs the participation of GSH as a co-factor leading to the formation of its oxidized form (GSSG), which is very toxic to cells (Chance et al., 1979). The erythrocytic GPx is the main enzyme in H₂O₂ detoxification in physiological conditions, while CAT upregulation occurs when higher levels of this ROS are generated, as found in all cells of DS subjects (Halliwell and Gutteridge, 2006).

GSH depletion is usually reported in DS subjects (Muchová et al., 2001; Pastore et al., 2003; Hamed et al., 2011), and it was also detected in the present study. Such depletion may also contribute to the relatively low GPx activity, which is a limiting factor to its function (Halliwell and Gutteridge, 2006). Under oxidative stress the ratio between the reduced and oxidized form of glutathione (GSH:GSSG) is critical for the loss of mitochondrial function and cellular viability (Meister and Anderson, 1983; Schulz et al., 2000). Beside its crucial role as a general antioxidant including HO• detoxification, the other main role of the GSH cycle is to support GPx and GST activities constantly supplying and maintaining a high concentration (approximately in the range of 1–10 mM) pool of this important tripeptide, apparently in all aerobic cells (Wilhelm Filho et al., 2000). Furthermore, GSH also participate in vitamins E and C regeneration, amino-acid transport, immunological functions, cellular signaling, among other important functions (Halliwell and Gutteridge, 2006), which are jeopardized in DS subjects, thereby contributing to the manifestation of this syndrome.

The results obtained in the present study showed enhanced activity of GR in DS subjects in accordance with a previous related work (Pastor et al., 1998). GR is a flavoprotein that allows the continuous conversion of GSSG to GSH, through the oxidation of NADPH to NADP (Meister and Anderson, 1983). The GR upregulation found in the blood of DS subjects probably reflects the necessity to counteract the oxidative stress present in their blood, trying to restore the high tissue concentrations (−99%) usually reported for normal cell metabolism in all aerobic organisms (Wilhelm Filho et al., 2000).

Similar to the relatively low GPx activity detected in the present study, the decreased activity found in erythrocytic GST from DS subjects may be attributable to the GSH depletion. Weak negative correlations measured through the coefficients of Pearson were found between GST activity (−0.30) and GPx activity (−0.15) GSH concentrations, probably indicating the use of GSH by both enzymes. The super-family of distinct isoforms of glutathione S-transferase, beyond their role in xenobiotic detoxification, also helps in the detoxification of endogenous hydroperoxides continuously generated through cellular liperoxidation processes (Danielson and Mannervik, 1985; Hayes and McCellan, 1999). In this regard, the relatively low GSH levels available for GST and GPx activities, which are probably limiting their responses to the persistent oxidative stress present in DS subjects, may be responsible for exacerbating such oxidative insult.

As found in other studies (Meguid et al., 2010; Campos et al., 2011), no alterations were found regarding lipid damage by ROS (TBARS levels), as well as protein damage (PC levels), which were decreased in DS subjects compared to controls. However, other studies revealed increased damage to proteins, lipids and DNA (Brooksbank and Balazs, 1984; Ani et al., 2000), including high in vitro lipid oxidation in the brain of fetuses (Le Pecheur et al., 2005), despite the fact that DS children possess higher levels of monounsaturated fatty acids compared to healthy children (Santos, 2006), an aspect that may predispose the latter children to potentially high oxidation processes. In the only other study that reported protein damage associated to DS, Campos et al. (2010) found higher oxidation levels in diTyr (dityrosine) in DS children when compared to their non-DS brothers.

The relatively high levels of urate, a well known and powerful antioxidant (Nagiová et al., 2000; Campos et al., 2011) found in the serum
and urine of apparently all DS children, young and adults (Pallardó et al. 2006; Campos et al. 2010, 2011), may reflect an antioxidant compensation for the persistent oxidative insult promoted by the disease. Urate is able to reduce the peroxy radical (ROO•) to hydroperoxide (ROOH), blocking its transformation to low-molecular aldehydes, including the toxic and major product of liperoxidation, malondialdehyde (MDA) (Halliwell and Gutteridge, 2006). As a consequence, lower levels of MDA/TBARS are expected in DS subjects (Ameis et al. 1981; Simão et al., 2008), as found in the present work and in other related studies.

Vitamin E levels did not show any difference between both studied groups, a result in accordance with other related study (Meguid et al., 2010). However, in DS patients who were also diagnosed with Alzheimer disease, plasma contents of vitamin E were lower compared with DS patients not showing this neurodegenerative disease (Jackson et al., 2008).Lockrow et al. (2009) reported that vitamin E supplemented Ts65Dn rats, a murine model of DS, oxidative stress was attenuated by this antioxidant intervention, strongly suggesting that an early antioxidant treatment could be useful (Zana et al., 2007). This liposoluble chain-breaker protects membranes of cells and organelles and also avoiding LDL oxidation (Halliwell and Gutteridge, 2006), and requires GSH, ascorbate and coenzyme Q10 (ubiquinol) as synergetic co-antioxidants (Halliwell and Gutteridge, 2006). Accordingly, some previous reports showed that the early supplementation of micronutrients such as vitamins E, C and B6, folic acid, zinc, selenium, α-lipoic acid and L-cysteine, is able to prevent or delay cognitive retardment in DS patients (Ani et al., 2000, Thiel and Fowkes, 2005). However, no cognitive improvement in DS patients after an antioxidant intervention was found (Lott 2011; Lott, 2012). On the other hand, preliminary results from our laboratory after six months of vitamins E and C intervention indicate a significant attenuation of the systemic oxidative stress in DS patients.

Conclusions

The present study shows that the presence of trisomy 21 in children and adolescents results in significant biochemical changes that contribute to a systemic and exacerbated oxidative stress in the blood of DS patients, in a way similar to that already demonstrated by our group in other chronic diseases where oxidative stress is highlighted (Maço et al. 2007; Farias et al. 2012).

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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