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Selenium inadequacy is not associated with oxidative stress in child and adolescent acute lymphocytic leukemia survivors

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ABSTRACT

Objective: Acute lymphocytic leukemia (ALL) and its subsequent treatment may provoke increased oxidative stress. The aim of this study was to investigate the antioxidant status of children and adolescents who had received ALL therapy, and to test the hypothesis that selenium (Se) inadequacy is correlated with reduced defenses against oxidative stress in this population.
Methods: This case–control study involved 24 patients between ages 5 and 13 y who had been treated successfully for ALL (ALL group) and 60 children of similar age and socioeconomic background with no clinical history of leukemia (control group). Dietary intake of Se was evaluated by the 24-h recall method, and the concentrations of Se in plasma, erythrocytes, and urine determined. Antioxidant status was assessed by analysis of the oxidative stress markers, namely, superoxide dismutase (SOD), glutathione peroxidase (GPx), malondialdehyde (MDA), α-tocopherol, and 8-oxo-deoxyguanosine (8-oxo-dG).
Results: There were no between-group differences with respect to plasma ($P = 0.122$), erythrocyte ($P = 0.202$), urinary ($P = 0.608$), or dietary ($P = 0.757$) levels of Se. GPx activity was significantly ($P < 0.001$) reduced in the ALL group compared with the control group, whereas SOD activity and MDA concentrations were similar. The concentrations of α-tocopherol and 8-oxo-dG were significantly increased in the ALL group compared with the control group ($P < 0.001$ and $P = 0.031$, respectively).
Conclusion: All participants were Se inadequate, but such inadequacy was not correlated with reduced defenses against oxidative stress. However, individuals of the ALL group were with increased oxidative stress compared with the control group, possibly due to previous disease and to intensive polychemotherapy.

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Introduction

Acute lymphocytic leukemia (ALL) is a hematologic disorder resulting from the malignant proliferation of lymphocytes that infiltrate bone marrow, peripheral blood, and other tissues. ALL is considered the most common type of cancer in pediatric patients [1], with the highest prevalence among children ages 2 to 5 y [2]. In Brazil, the incidence rate among individuals ages ≤15 y is 35.2/1 million [3].

The ALL treatment involves the administration of complex combinations of chemotherapies over periods of up to 3 y. Although modern therapy has led to a significant increase in the rate of survival (nearly 90%) [4], the disease and the applied treatments can promote serious consequences, including the generation of excess free radicals that may affect the antioxidant status of the patient [5,6].

Selenium (Se) is an essential micronutrient for humans and animals, and plays a central role in a number of enzyme-mediated pathways that form part of the antioxidant defense system [7]. An inadequate dietary intake of Se may result in suboptimal concentrations of selenoproteins and, therefore,

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diminished protection against oxidative stress [8,9], which has been linked to several types of cancer [7,9].

The aim of this study was to investigate the antioxidant status of children and adolescents who had received ALL therapy, and to determine whether Se inadequacy is correlated with reduced defenses against oxidative stress in this population.

Materials and methods

The study was approved by the Ethics Committee in Research of the Faculdade de Ciências Farmacêuticas da Universidade de São Paulo (USP), São Paulo, SP, Brazil (protocol number CAAE # 0012.0.018.174-09). The aims and objectives of the study were explained to all potential participants with emphasis placed on the voluntary nature of participation. Written informed consent was obtained from parents or legal guardians before the commencement of the study.

Study population

This case–control study was conducted between February and December 2010 and included 24 patients ages 5 to 13 y (ALL group) who had been treated for ALL at the Instituto de Oncologia Pediátrica, Universidade Federal de São Paulo (UNIFESP), São Paulo, Brazil, and 60 individuals of similar age range and socioeconomic background but with no clinical history of leukemia (control group) selected at the Escola de Aplicação (USP). Individuals belonging to the ALL group had completed treatment at least 6 mo before commencement of the study and were in complete clinical remission. All participants were free of comorbidities such as diabetes or renal and hepatic diseases.

Because no data were available concerning Se nutritional status in a population that had been treated for ALL, the total sample size required for such estimation was determined using the non-parametric test:

$$N = \frac{\left(z_\alpha + z_\beta\right)^2}{12c\cdot(1-c)\cdot\left(\S - \frac{1}{2}\right)^2} \quad (1)$$

where Z_α denotes the upper α-level significance point, Z_β is the critical value for $1-\beta$, c is the expected proportion of individuals of the control group, and § is the probability of the variable of interest (Se concentration in the blood) in one group being smaller than in the other group. In the present study, c and § were set at 70%, α at 0.5, and statistical power at 90%.

Participants' characteristics

Participant characteristics were evaluated in both groups by clinical examination and/or medical records. These included sex, age at diagnosis of ALL, age at study assessment, duration of treatment, and time since last ALL treatment.

Anthropometrical evaluation

Body weight and the height were measured as described previously [9]; body mass index (BMI) was calculated.

Dietary assessment

Food consumption of macronutrients and Se was evaluated using the 24-h recall method with three repetitions in nonconsecutive days: Two during the week and one on the weekend. These data was analyzed using NutWin software, version 1.5.2.11 developed in 2002 by Universidade Federal de São Paulo. Se contents of Brazilian foodstuffs that were not included in the NutWin data bank were added on the basis of values obtained from analyses performed in our own laboratory and those quoted previously [10] and in the U.S. Department of Agriculture's National Nutrient Database Food Search for Windows, Version 1.0, SR23. The ranges of cutoff points for Se intake were as follows: Prevention of Keshan disease (KD), cardiomyopathy associated with dietary deficiency of Se (20 μg/L), optimal selenoproteins activity (>30–50 μg/L), and prevention of cancer (120 μg/L) [11].

Collection and analysis of blood and urine

Blood samples (10 mL) were collected by venous puncture from 8-h fasted participants, transferred to polyethylene tubes containing ethylenediaminetetraacetic acid (EDTA) and maintained in the dark to avoid degradation of tocopherol. A sample of total blood was separated for hematologic analysis. Plasma, erythrocyte, and leukocyte fractions were separated and stored in polypropylene microcentrifuge tubes at –80°C until required for the analysis of Se and/or of the markers of oxidative stress, namely, superoxide dismutase (SOD), glutathione peroxidase (GPx), α-tocopherol, 8-oxo-deoxyguanosine (8-oxo-dG), and malondialdehyde (MDA). Aliquots of 24-h urine samples were transferred to polypropylene vials and stored at −80°C until required for the determination of Se excretion. All materials used in the biochemical assays were demineralized with 30% nitric acid (v/v) with the exception of those employed in the analyses of 8-oxo-dG, which were autoclaved before use.

Levels of Se in plasma, erythrocytes, and urine were determined by atomic absorption spectroscopy using the method described previously [9] with Seronorm Trace Elements Serum, Whole Blood and Urine (Sero AS, Billingstad, Norway) as reference standards. The ranges of cutoff points were as follows: Plasma Se: Prevention of KD (>21 μg/L), optimal selenoproteins activity (>65–95 μg/L), prevention of cancer (>115 μg/L) [11], and erythrocyte Se: 60 to 120 μg/L [12].

SOD activities in erythrocytes were determined at 37°C using a RANSOD (Randox Laboratories, Antrim, UK) reagent kit and an automatic biochemical analyzer (Lama 240, Tokyo Boeki, Tokyo, Japan) operated at a wavelength of 340 nm. Erythrocyte GPx activities were evaluated according to a method described previously [13] with the aid of a RANSEL (Randox Laboratories) reagent kit. Enzyme activities were expressed as U/g hemoglobin (U/g Hb). The hemoglobin used for this correction was assessed in pool of erythrocytes.

Plasma α-tocopherol was assayed by high-performance liquid chromatography (HPLC) according to an earlier method of [14] using a Shimadzu SCL-10 AVP system controller (Kyoto, Japan) equipped with a Rheodyne manual sample injector and a Phenomenex (Torrance, CA, USA) HyperClone ODS C18 (5 μm) analytical column. Chromatograms were integrated using Shimadzu Class VP software and calibration curves were constructed for the calculation of α-tocopherol concentrations.

For the purpose of analyzing 8-oxo-dG, DNA was extracted from samples of leukocytes with the aid of a DNA Gentra Puregene blood kit (Qiagen Sciences, Germantown, MD, USA) used according to the recommendations of the manufacturer. The concentration of DNA in the resulting extract was determined by measuring the absorbance at 260 nm (A_{260}) with a NanoPhotometer Pearl (Implen Inc, Westlake Village, CA, USA) spectrophotometer, whereas DNA purity was assessed from the A_{260}/A_{280} ratio. DNA samples were resuspended in 0.1 mM deferoxamine, an iron chelator that minimizes the oxidation of nucleotide bases [15]. The analysis of 8-oxo-dG was performed according to the method described previously [16]. Briefly, DNA samples (100 μg) were spiked with known amounts (2000 fmol) of isotopically labeled internal standard ($[^{15}N_5]$8-oxo-dG) and submitted to enzymatic hydrolysis. Aliquots (50 μg) of the hydrolyzed samples were analyzed by HPLC with electrospray ionization tandem mass spectrometric detection. Concomitantly, hydrolyzed DNA samples (6 μg) were submitted to HPLC with photodiode array detection for the quantification of dG.

Plasma MDA was determined by the thiobarbituric acid assay as previously described [17]. Samples were analyzed by HPLC on a Phenomenex reverse-phase C_{18} column (150 × 4.6 mm id), and the concentrations of MDA assessed by means of a standard calibration curve.

Hematologic indexes were assessed in total blood by automated system Sysmex XT 1800 (Sysmex Corp., Mundelein, IL, USA). The reference values for hematologic indexes were: ≥12 g/dL for hemoglobin; 40%–54% (male) and 37%–49% (female) for hematocrit; 4000–11 000/mm^3 for leukocytes; 1000–4000/mm^3 for lymphocytes; and 150–400 k/μL for platelet index [18].

Statistical analyses

Statistical analyses were performed using software R for Windows, version 2.12.2. In the descriptive analysis, data corresponding to the different variables were expressed as mean and SD. The normality and homogeneity of variables were assessed using the Kolmogorov-Smirnov and Levene's tests, respectively. Mean values were compared using the Student's *t* test for independent samples in the case of variables presenting normal and homogeneous distribution, whereas the Mann-Whitney U test was employed otherwise. The statistical level of significance was set at 5%.

In order to reduce the errors associated with dietary measurements, the values were adjusted according to the total energy intake using a previously described residual method [19]. Inferential statistics were performed with the purpose of 1) comparing the groups with respect to oxidative stress and 2) constructing statistical models of the response variables of oxidative stress, considering as explanatory variables the intake of Se, plasma, erythrocyte and urinary levels of Se, intake of protein and carbohydrate, participant's sex and BMI, duration of treatment, and time posttreatment. The last two variables mentioned were, of course, applied only for the ALL group. By the large number of variables assessed, the explanatory variables were selected according to their importance to explain the oxidative stress. Variables with low importance were included only when they differed between groups. The least-squares method was employed in fitting the models [20]. Selection of the final statistical models of response variables was based on the lowest value of the Akaike Information Criterion (AIC) and the highest adjusted R^2. In the selection of the final model of the variable GPx, the Se variables were fixed because they are theoretically correlated with GPx activity and, therefore, important for the model. Diagnostic tests, sensitivity

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