Nutrition 32 (2016) 1238–1242

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Applied nutritional investigation

Influence of HIV infection and the use of antiretroviral therapy on selenium and selenomethionine concentrations and antioxidant protection

Lígia Moriguchi Watanabe M.Sc. [a,*], Fernando Barbosa Júnior Ph.D. [b], Alceu Afonso Jordão Ph.D. [c], Anderson Marliere Navarro Ph.D. [c]

[a] Department of Food and Nutrition, Faculty of Pharmaceutical Sciences, São Paulo State University - UNESP, São Paulo, Brazil
[b] Department of Internal Medicine, Faculty of Medicine of Ribeirão Preto, University of São Paulo - FMRP/USP, São Paulo, Brazil
[c] Department of Clinical and Toxicological Analyses and Bromatology, Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo - FCFRP/USP, São Paulo, Brazil

ARTICLE INFO

Article history:
Received 17 December 2015
Accepted 24 March 2016

Keywords:
HIV
Antiretroviral therapy
Selenium
Selenomethinine
Oxidative stress

ABSTRACT

Objective: The aim of the present study was to evaluate whether HIV infection and antiretroviral therapy (ART) use are associated with oxidative stress, concentrations of selenium and selenomethionine, and antioxidant protection.
Methods: Individuals were classified as HIV negatives: control group (CG; n = 40); HIV positives: group 1 (G1; taking ART for >5 y, n = 40) and group 2 (G2; taking ART for <5 y, n = 40). Plasma and erythrocyte selenium, selenomethionine, glutathione (GSH), glutathione peroxidase activity (GPX), and malondialdehyde (MDA) were evaluated.
Results: Selenium deficiency (plasma selenium 45 μg/L) was not observed in any of the participants, and plasma selenium in CG (69.4 μg/L) was lower than in G1 and G2 (88.4 and 72.5 μg/L, respectively). G1 and G2 showed higher concentrations of MDA and GPX and lower concentration of GSH than CG. Multiple linear regression analysis indicated an association of MDA, GPX, and GSH with HIV status. CG participants showed higher concentrations of selenomethionine than G1 and G2 individuals and we observed a significant negative correlation between the concentration of selenomethionine and the use of ART.
Conclusions: Prolonged ART use seems to increase the selenium in plasma, but influences the reduction of selenomethionine. HIV infection was associated with increased oxidative stress and appears to affect in protective activity of GPX. Finally, more studies are required to further address the importance of selenium and selenometabolites in the pathogenesis of infection and metabolism of HIV-positive individuals in prolonged use of ART.

Introduction

Oxidative stress has been widely documented in patients infected by HIV; once viral infections promote prolonged activation of the immune system contributed to the increase of the production of reactive oxygen species [1–3]. Several studies linked the use of antiretroviral therapy (ART) as a potential contributor to increase the reactive oxygen species (ROS) [4]. Furthermore, the use of ART has been associated with adverse effects that are related to important metabolic changes [5,6].

The level of oxidative damage in HIV-infected individuals can be influenced by both the extent of oxidative stress and the

The present research was supported by São Paulo Research Foundation - FAPESP, under grant: 2013/25228-4 and Coordination for the Improvement of Higher Education Personnel–CAPES. LMW and AMN were responsible for the conception, design, generation, collection, and assembly of the study, carried out the sample analyses; and the analysis and interpretation of data; drafted and revised the manuscript. AAJJ and FBJ carried out the sample analyses and revision of the manuscript. All authors read and approved the final manuscript. The authors have no conflicts of interest to declare.
* Corresponding author. Tel.: +55 169 810 10826; fax: +55 163 602 2563.
E-mail address: ligia.moriguchi@gmail.com (L. M. Watanabe).

http://dx.doi.org/10.1016/j.nut.2016.03.024
0899-9007/

activity of antioxidant defenses [7]. Increase in oxidative stress may accelerate disease progression, increasing HIV replication through signaling of nuclear factor (NF)-κB as well as the mutation rate of the viral RNA genome, leading to greater damage to the host [2,3]. On the other hand, balance in the activity of antioxidant defenses, originated or not from diet and antioxidant enzymes, protect against the oxidative stress and may delay progression of the HIV disease [2,7].

Selenium is an essential micronutrient that has been widely associated with an important role in HIV infection, mainly due to its involvement in regulating oxidative stress, intimately linked with the redox state of the cell and the redox regulation of genes, which are important for multiple immune functions [1,8]. Moreover, by incorporating selenoproteins, particularly glutathione peroxidase (GPX) and thioredoxin reductase, selenium has been shown to be a potent regulator in both the NF-κB activity and transcription of HIV [1,8].

The beneficial effects of selenium to human health are strongly dependent on their chemical form and concentration [9]. Therefore, it highlights the importance of chemical speciation in identifying and quantifying a number of metabolites that contains selenium [10].

The characterization of this metabolism and verification of the individual's health status can help in establishing new therapeutic strategies. However, currently studies that evaluate the selenium in HIV patients who have been using ART for a long time are limited. Additionally, no data is available related to the selenium metabolites and HIV infection. Thus, the present study aimed to evaluate whether HIV infection and use of ART are associated with oxidative damage, concentrations of selenium and selenomethionine, and antioxidant protection of glutathione (GSH) and GPX.

Methods

Study population

This was a prospective, observational, cross-sectional study conducted at the Clinics Hospital of Ribeirão Preto Medical School, University of São Paulo (HC/FMRP-USP). The study was approved by the Research Ethics Committee of the institution (process 551.355) and all participants signed the informed consent.

Recruitment of the study group patients was in the Special Treatment Unit in Infectious Diseases in HC/FMRP-USP. The control group was made up of staff and students in the different areas of the HC/FMRP-USP.

We evaluated 120 adults of both sexes. Of this total, 80 HIV-positive individuals using ART made up the study group and were divided into group 1 (G1), composed of 40 participants taking ART for >5 y and group 2 (G2), composed of 40 participants taking ART for <5 y. The arrangement of HIV-positive individuals in G1 and G2 according to the ART usage time was based on a prior analysis of the profile of patients attending in UETDI, in which the average of ART usage time was about 5 y. The control group (CG) was composed of 40 healthy HIV-negative individuals.

The exclusion criteria for participation in the project were drug users, the use of a supplement containing selenium in its composition, adjacent morbidities, thyroid disease, malabsorption syndrome, diabetes mellitus, renal failure, and chronic inflammatory diseases such as rheumatic and autoimmune diseases.

Anthropometric measurements

Body weight (kg) was measured on an electronic scale accurate to 0.1 kg and height in stadiometer accurate to 0.1 cm. Body mass index (BMI; in kg/m^2) was calculated for each participant.

Evaluation of selenium dietary intake

Selenium dietary intake was assessed by food record consecutively over 3 d. The information was processed in nutritional analysis program Dietpro®, 5 i version. For recommendation of selenium consumption, we considered Dietary Reference Intakes (DRIs) [11].

Quantification of viral load and CD4 + lymphocytes count

The quantification of viral load was determined by the Abbott Real Time method and was considered undetectable when the values were below 50 copies/mL [12].

The count of CD4+ lymphocytes was determined by the flow cytometry method using the kit Multitest® and Calibur® cytometer FACS (Becton Dickinson, San Jose, CA, USA) and the classification criteria for HIV/AIDS from the Centers for Disease Control and Prevention [13] were used.

Biochemical evaluation

The collection of blood samples was performed in the Clinical Research Unit (UPC) of HC/FMRP-USP after 8 h of fasting and the separation of whole blood to obtain serum, plasma, and erythrocytes occurred immediately after collection. The samples were stored at –80° until the time of analysis.

Evaluation of oxidative damage: Malondialdehyde

According to a previous methodology, to obtain the dosage of malondialdehyde (MDA), 100 μL plasma sample was used [14]. To this was added 300 μL of 10 mM solution of 1-methyl-phenylindole in acetonitrile and methanol (2:1, v/v) and 75 μL of HCl pure (37%). The Eppendorf tubes were then vortexed and incubated in a water bath at 45°C for 40 min. After the bath, the samples were cooled in ice and then the Eppendorf tubes were centrifuged at 4000*g* for 10 min. The supernatant was read for absorbance at a wavelength of 586 nm. The MDA concentration was calculated by comparing it to a curve 1,1,3,3-tetramethoxypropane (TMP) hydrolyzate.

Reduced glutathione

GSH activity was determined using a previously adapted method that proposed that 25 μL of plasma be used. One mL of Tris-EDTA, and 25 μL of DTNB concentration of sulfhydryl groups were calculated using a standard curve of GSH [15].

Glutathione peroxidase activity

GPX activity was measured using a previously adapted method in erythrocytes [16]. The method was based on the reaction in which GPX catalyzes the oxidation of GSH by a hydroperoxide. In the presence of GPX nicotinamide adenine dinucleotide phosphate (NADPH), oxidized GSH is converted to the reduced form with a concomitant oxidation of NADPH+. The decrease in absorbance at 340 nm was then measured.

Selenium

The determination of the total concentration of selenium in plasma and erythrocytes was performed according to a previously described method, by an inductively coupled plasma mass spectrometry (ICP-MS), fitted with a dynamic reaction cell (Perkin Elmer Sciex, Norwalk, CT, USA) [17]. Samples were diluted in the ratio 1:50 with a solution containing Triton X-100 0.01% (v/v), HNO_3 0.05% (v/v) and 10 mg/L-1 rhodium (Rh) as an internal standard. The concentration of the analytical calibration standards ranged from 0 to 50 μg/L.

Selenium speciation: Selenomethionine

The chemical speciation of selenium for identification of metabolite selenomethionine was performed in plasma of 15 individuals, 5 in each group, according to the previously adapted method [18]. The standards of seleno amino acids: seleno-L-methionine (S-3132), Se-(methyl)-selenocysteine (M-6680), and Protease XIV (P5147) were obtained from Sigma-Aldrich (St. Louis, MO, USA). For the preparation of plasma samples, 20 mg of protease XIV was added to a 900 μL sample and then vortexed. This mixture was incubated at room temperature for 24 h. After incubation, 100 μL of HNO_3 (1 N) was added to the mixture, vortexed, then centrifuged at 3500*g* for 10 min. The supernatant fraction was then filtered using a 0.25-μm filter. The sample was diluted five times and after dilution, 100 μL was injected into the high-performance liquid chromatography ICP-MS for selenomethionine speciation using metilselenocysteine as an internal standard.

For the chemical speciation of selenium, we used a pump LC Perkin Elmer Model G-200, a six-port injector (Rheodyne 9725) and a reverse-phase column (C8, 3 μM, 4.6 mm × 33 mm, Column Brownlee, PerkinElmer, Boston, MA, USA) constitute of the liquid chromatography system. The output from the chromatographic column was coupled to ICP-MS nebulizer. The instrumental conditions were Radio frequency power: 1400 W; plasma flow rate: 15 L/min; nebulizer flow rate: 1.25 L/min; standard resolution; scan mode: time resolved acquisition; residence time: 500 ms; and monitored isotope: mass 82. The mobile phase was composed of water-methanol (97: 3, v/v) and the flow rate was 1 mL/min.

Statistical analysis

Categorical variables

For categorical variables we applied the χ^2 exact test and the results were presented as frequencies and percent.

Comparison between groups

Comparison of plasma and erythrocyte selenium, GSH concentration, GPX, and MDA among CG, G1, and G2 groups was conducted by analysis of variance