Original article

Effect of Low Selenium Diet on Glutathione Peroxidase 3 Concentration in Male Sprague-Dawley Rats' Serum

Nikolina Kolobaric 1, Ana Stupin 1,2, Petar Susnjara 1, Lidija Baric 1, Anita Matic 1

1 Faculty of Medicine Osijek, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia
2 Faculty of Dental Medicine and Health in Osijek, Josip Juraj Strossmayer University of Osijek, Croatia

Corresponding author: Anita Matic, anita.cosic@mefos.hr

Abstract

Aim: Determination of antioxidative enzyme glutathione peroxidase 3 (GPx3) serum concentrations after consumption of food which contains different concentrations of selenium (Se).

Research subjects and methods: Four-week-old Sprague Dawley rats consumed food containing different concentrations of Se (food Divan) over a period of 10 weeks. The animals were divided into two groups: 1) normal Se (0.363 mg/kg Se) and 2) low Se (0.030 mg/kg Se). Each animal was weighed at the end of protocol, and serum samples were collected for determining GPx3 concentrations. All experimental procedures were in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes and approved by the Ethics Committee of the Faculty of Medicine in Osijek and the Ministry of Agriculture of the Republic of Croatia.

Results: Different concentrations of Se in food did not cause a change in body weight. Food containing the recommended intake of Se according to the guidelines of the World Health Organization significantly increased GPx3 enzyme concentration (13.96±0.42 mg/ml) when compared to low selective Se (12.04 ± 0.33 mg/ml, p = 0.002).

Conclusion: Serum concentration of the antioxidant enzyme GPx3 depends on the concentration of Se in food. It is shown that, in comparison with food with low Se levels, food containing a normal concentration of Se is enriched with the antioxidant GPx3 which, according to numerous studies, has a protective role in the human body.

Introduction

The most important antioxidant enzymes are catalase, glutathione peroxidase, glutathione reductase and superoxide dismutase, which have harmful oxidative metabolic intermediates. These enzymes require cofactors selenium (Se), zinc, copper, iron, and magnesium for their catalytic activity (1).

Glutathione peroxidase (GPx), which is the subject of our study, is a family of enzymes that metabolize hydrogen peroxide and lipid hydroperoxide into water. They have a binding site for Se, which oxidizes in reaction with hydrogen peroxide (2-4). Glutathione peroxidase is involved in protection against oxidative stress in that it participates in the transfer of amino acids through the plasma membrane, removes the hydroxyl radical and the singlet oxygen, thereby detoxifying the hydrogen peroxide and the lipid peroxide by the catalytic action of GPx. It can replenish the active forms of the most essential vitamins, i.e., vitamins C and E (5).

It is known that oxidative stress is one of the pathogenic mechanisms that cause disorders of the vascular system and contribute to development and progression of various cardiometabolic diseases (hypertension, diabetes, atherosclerosis, obesity). A diet enriched with trace elements, which increase the concentration of antioxidant enzymes, could provide better protection against oxidative stress (6).

As part of GPx, Se is important for the regulation of the oxidative system (7-9). It is an essential trace element which must be ingested in sufficient quantity through food. In many countries, there is malnutrition due to the lack of Se as a micronutrient. Inadequate Se intake causes many different disorders (necrotizing cardiomyopathy, peripheral myopathy, reduced muscle tone, concentration problems, hair loss and nail splitting) (10-12). In our previous animal study, we showed that low dietary Se content affects the function of aorta, reduces ACh-induced relaxation, which is dominantly mediated by NO, and also increases the level of local oxidative stress (13).

Human studies have shown that Se concentrations are inversely related to mortality and occurrence of cancer (14, 15). Se functions as an antimutagenic agent that prevents the transformation of healthy cells into malignant ones; it is assumed that these protective effects are primarily associated with the activity of GPx (16, 17).

Glutathione peroxidase 3 (GPx3) is the only enzyme in the GPx group that functions in extracellular space. The substrates for this enzyme are hydrogen peroxide and phospholipid hydroperoxides, which play a significant role in the antioxidative processes in the blood (18) in that they decrease oxidative stress by reducing H2O2 and organic hydroperoxides to their corresponding alcohols and oxygen (19). Since increased GPx3 activity is found in certain subtypes of tumors, like ovarian tumors (20), it can also be used as a biomarker.

This study aims to determine the level of the GPx3 antioxidative enzyme in serum samples with different levels of Se and to determine whether Se causes a change in body mass.

Materials and Methods

Ethical Approval

Experimental procedures complied with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Council of Europe No 123, Strasbourg 1986) and were approved by the Ethics Committee of the Faculty of Medicine, University of Osijek (Class: 602-04/14-08/06, No: 2158-61-07-14-05), and authorized by the Ministry of Agriculture of the Republic of Croatia (Class: UP/I-322-01/14-01/90, No: 525-10/0255-15-4). The tests were performed in the Laboratory for Vascular Physiology and Laboratory for Molecular and Clinical Immunology at the Department of Physiology and Immunology of the Faculty of Medicine Osijek, Croatia.
Experimental groups

Food was prepared at the Faculty of Agrobiotechnical Sciences Osijek, Croatia, based on the wheat briquettes recipe developed by Mucedola, Italy. Wheat was specially grown in soil with different concentrations of selenium and zinc, so that the concentration of said micronutrients was adjusted according to the requirements of the experiment. Four-week-old healthy male Sprague Dawley rats (N = 7 per group) consumed the above-mentioned food for ten weeks. The animals were randomly divided into groups:

1) normal Se group (0.363 mg/kg Se) and
2) low Se group (0.030 mg/kg Se).

Rats were housed doubly in shoebox-style cages with free access to food and tap water, housed in a temperature of -21°C-22°C, humidity- and light-controlled room, maintained on a 12:12 hour light : dark cycle.

Determination of Se (normal or low concentration) in food is consistent with previous studies (13, 21-24).

After 10 weeks of feeding, the animals were weighed and then anesthetized using a combination of ketanest S 75 mg/kg (Ketanest S 25 mg/ml, 2 ml ampoules, Pfizer) and midazolam 0.5 mg/kg (Midazolam Torrex 5 mg/ml, 3 ml, Torrex Chiesi Pharma). Blood samples were collected immediately after decapitation (arterial and venous blood) in empty tubes without anticoagulants to obtain serum and centrifuged at 3500 rpm for 10 minutes. The separated serums were stored in a refrigerator at -80 °C until analysis.

Glutathione peroxidase 3 (GPx3) determination in serum samples

GPx3 concentrations were determined using the commercially available enzyme immunoassay kit purchased from LifeSpan BioSciences, USA (LSBio Cat. No. LS-F6289). Each well of the supplied microtiter plate was precoated with an antibody. First, 100 μl of serum samples and standards (in duplicates) were put in their appropriate place on the ELISA plate and incubated for 1 hour at 37 °C. After the first incubation, the unbound standard or sample was washed away. 100 μl of biotin-conjugated detection antibody was added to each well and the same time and temperature incubation was continued. After that, it was washed 3 times and Avidin-Horseradish Peroxidase (HRP) conjugate was added, followed by a 30-minute incubation at 37 °C. A TMB substrate was then added, which reacted with the HRP enzyme, resulting in color development. At the end of the protocol, stop solution was added to terminate the color development reaction and optical density (OD) was measured at a wavelength of 450 nm on the PR 3100 TSC Microplate Reader in the Laboratory for Molecular and Clinical Immunology at the Department of Physiology and Immunology of the Faculty of Medicine, Josip Juraj Strossmayer University of Osijek.

Statistical analysis

Differences in normally distributed numerical variables between groups were tested with the Student t-test, and in case of deviations from normal distribution with the Mann-Whitney U test (SigmaPlot version 11.2, Systat Software, Inc., Chicago, USA). The level of significance was determined at p < 0.05. The sample size was determined using the Sigma Plot version 11.0 program. For the power of the test of 0.8, p value less than 0.05 and the minimum expected difference of 0.25, it was found that at least 4 animals per group were required. Results are presented below as the mean ± standard deviation (SD).

Results

Effect of diet on body weight

There was no difference in body weight between the normal Se and the low Se group (p < 0.05) (Figure 1).
Figure 1. Body weight in experimental rat groups

GPx3 serum concentration

The group of animals that consumed food with normal Se content had significantly increased GPx3 enzyme concentrations (13.96 ± 1.11 ng/ml) compared to the low selective Se content group (12.04 ± 0.89 ng/ml, p = 0.002) (Figure 2).

Figure 2. Concentration of the antioxidative enzyme GPx3 (ng/ml) in serum samples
Discussion

We recently showed that low dietary Se content increases oxidative stress, which is possibly caused by decreased gene expression of the antioxidant enzyme GPx1 in thoracic aortic tissue without changes of the same enzyme in serum samples (13). Gene expression of other antioxidant enzymes (Cu/Zn SOD and catalase) did not change significantly. GPx3 is a better isoform of GPx for determination of the antioxidant effect of Se in blood samples, so we decided to measure the more appropriate GPx isoform, which is readily detectable in the blood (25).

The main findings of this study are the following: a) serum concentration of the antioxidant enzyme GPx3 depends on the concentration of Se in food; b) our study once again confirmed that food with sufficient levels of Se (according to the World Health Organization (WHO) and the American Institute of Nutrition (AIN)) is richest with the antioxidant GPx3, which has a protective role in the body; c) different concentrations of Se have no effect on body weight.

Trace elements are essential for living organisms because they are necessary for normal function of the organism, its growth and performance of its metabolic functions. Amounts in the human body range from 1.5 mg to 4.5 g. It is characteristic for trace elements that very small amounts of a trace element affect the condition of the entire organism (26, 27). Levels of Se in food can vary in different parts of the world and countries (28, 29). Intakes of Se are high in America and Japan, and much lower in Europe, particularly Eastern Europe (30).

It was recently shown that Se has an important role in antioxidant selenoproteins used for protection against oxidative stress initiated by ROS and its compounds in different types of cancers, cardiovascular diseases, immune system diseases and aging (31, 32). The World Health Organization published findings that a diet which contains 0.1 mg Se per kilogram of food is sufficient for normal function of the organism (33), while the American Institute of Nutrition (AIN) and The TestDiet® AIN-93 Growth Purified Diet indicate that 0.24 mg Se/kg in food is optimal (21). According to these guidelines, we determined the values of low (0.030 mg/kg Se) and normal (0.363 mg/kg Se) levels of Se for our research. Measuring Se levels in blood samples seems to be a good indicator because plasma and serum contain about 75% of the Se. The level of Se found in these samples is directly related to recent dietary intakes (29).

Our research has shown that consuming food containing sufficient amounts of Se according to the guidelines provides a significantly higher level of GPx3 antioxidants in plasma compared to foods with low Se concentration (Figure 2). Such findings indicate that Se is directly responsible for increasing the antioxidative status of GPx and for lowering oxidative stress.

Se and its protective role against disease are mostly related to the removal of free radicals and the enzymatic breakdown of oxygen metabolites (34). For example, in tuberculosis patients, reduced oxidative stress was caused by ROS generation with Se supplementation (35). Of all types of tissue, the thyroid gland has the highest Se concentration, and in the form of GPx3, Se protects thyroid cells from free radicals and oxidative stress (36, 37). It was shown that in Hashimoto’s thyroiditis Se supplementation significantly lowered thyroid peroxidase autoantibody titer after 3 months (38). Furthermore, studies showed that Se has a beneficial effect on lowering the risk for different types of cancer (39- 43). It can reduce oxidative damage and prevent DNA damage (44). It can be used as alternative medicine by cancer patients undergoing radiotherapy (45 - 47). In addition, cellular and molecular processes that might be involved in the anti-cancer effects of Se are stabilization of the immune response, induction of programmed cell death and inhibition of angiogenesis (44).

Lack of the Se causes irreversible brain injury (48). Similar, lower serum Se was also found in children with epileptic seizures or febrile seizures and in adults with epileptic seizures (49, 50).
Se has also been proved to be important for maintaining the immune system. It has been shown that Se in a concentration of 400 μg per day significantly increased the number of T-cells (51), and supplementation with 100 μg per day for 6 months significantly increased the response to antigen challenge (52). Se deficiency may cause a slower immune response because of the negative influence it has on immune cells (53).

ROS is involved in regulating the synthesis of adhesion molecules on endothelial cells, and is essential for inflammatory responses during the early stages of a disease (54, 55). The expression of intercellular and vascular adhesion molecules increases significantly during inflammation and is involved in the firm adhesion of leukocytes and endothelial cells (54). Se nutritional status can directly influence vascular endothelial cell functions, e.g., under low Se conditions, aortic endothelial cells exhibit increased platelet activating factor biosynthesis, which causes vascular disorders that are affected by increased oxidative stress (56). It was also found that the production of prostaglandins was significantly decreased in Se-deficient endothelial cells, which are associated with the pathophysiology of several inflammatory diseases through significantly increased biosynthesis of thromboxane B2 (TXB2) and 15-hydroperoxyeicosatetraenoic acid (15-HPETE) (57, 58). Crucial role of Se in oxidative stress and endothelial influence has been demonstrated by the study Stupin et al. (2017), which showed that increased oxidative stress affects a NO-mediated response in low-Se aortas probably due to decreased NO bioavailability (13). Selenoproteins regulate vascular tone by establishing a balance between superoxide anion and nitrogen oxide (7), which was found to be the case in this study as well.

Numerous studies have shown the important role that Se plays in different health/disease states. Further research is necessary to make use of the benefits of this important element as much as possible. Observing the specific role of Se in the endothelium, glutathione peroxidase was found to have an indispensable role in the maintenance of normal vascular function. Specifically, low Se decreased tissue expression of GPx1 and blood concentration of GPx3, which caused an increase in oxidative stress and disrupted endothelial function (Figure 3).

Figure 3. Negative effect of low Se levels on changed vascular antioxidant status and consequent endothelial dysfunction
Sources of Funding

This study was supported by grants from the Ministry of Science, Education and Sports of the Republic of Croatia, #219-2160133-2034 and #079-0790462-0450; the Cro-Hu bilateral project “Common pathways for microcirculatory dysfunction in diabetes mellitus and hypertension” (2009–2011); and the Norwegian Ministry of Foreign Affairs Program in Higher Education, Research and Development HERD/Agriculture project “Cooperation in education and research on mineral improved crop production for human and animal health in Balkan Countries” (number 09/1548).

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