Effect of Selenium-Containing Products on Antioxidant Enzyme Activity in the Kidneys, Liver, and Blood of Guinea Pigs

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The antioxidant system of some tissues in guinea pigs with no selenium deficiency was studied after treatment with selenium-containing products. Activity of a selenium-containing enzyme glutathione peroxidase was significantly reduced in the kidneys and blood. The kidneys were characterized by low activity of glutathione reductase and high activity of SOD. These features illustrate the development of oxidative stress in the kidneys.

Key Words: *selenium; glutathione peroxidase; glutathione reductase; superoxide dismutase*

Selenium is an essential element in the body. This essential and biologically active microelement enters the composition of many hormones and enzymes. Therefore, selenium is functionally related to activity of all organs and tissues [1-4,9-13]. The demands in selenium are satisfied by its consumption with the plant and animal food. The bioavailability of dietary selenium is of considerable importance [3-4]. Selenium is involved in cell metabolism. Inorganic selenium (selenites and selenates) is transformed into hydrogen selenide. Selenium toxicity is related to the excess of hydrogen selenide. Organic selenium exists in the form of amino acids (selenomethionine and selenocysteine) that are present in proteins. In plant products, selenium is mainly presented by selenomethionine. Selenocysteine is found in humans and animals [3]. Selenium deficiency is associated with the dietary regimen and geographical location. The animals and humans receive selenium with food products (from the soil through the plants). Selenium deficiency is accompanied by immune dysfunction, changes in the antioxidant defense system, iodine deficiency, decrease in the genome stability [13], and preterm aging. These abnormalities contribute to the development of various diseases. Functions of selenium-containing proteins remain unknown [10-11]. Much attention was paid to enzymes of hormone metabolism (*e.g.*, selenoprotein P, 5'-iodothyronine deiodinase, selenoprotein W, and thioredoxin reductase [9,12]) and antioxidant enzyme glutathione peroxidase (GPx) [5].

Here we studied the antioxidant systems of the kidneys, liver, and blood in guinea pigs receiving selenium-containing products. The animals did not have selenium deficiency, but biological additives were used for prophylactic treatment.

MATERIALS AND METHODS

Functions of the antioxidant system were evaluated from changes in activities of GPx (EC 1.11.1.9; selenium containing-enzyme), glutathione reductase (EC 1.6.4.2, GR; enzyme for the regulation of intracellular reduced glutathione), and SOD (EC 1.15.1.1; enzyme for the neutralization of superoxide radicals). Enzyme activities were measured after dietary consumption of the following three products of selenium: organic selenium, Selen-Aktiv (Plant of Ecological Nutrition "Diod"); yeast preparation Bioselen (selenomethionine and selenocysteine); and inorganic sodium selenate ($Na_2SeO_4 \times 10H_2O$). Previous studies showed that addition of selenium preparations to food products is accompanied by a significant increase

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in selenium content in the kidneys and liver [4]. Activity of cytoplasmic antioxidant enzymes was measured in these tissues. Moreover, activity of these enzymes was measured in the blood.

Male guinea pigs (body weight 259±29 g) were divided into 3 groups to receive one of the selenium-containing products with feed.

Over the first 10 days, the dose of selenium was 12.5 μg/kg. In the follow-up period (17 days), the dose of dietary selenium was increased to 25 μg/kg. The animals received this additive for 27 days. Dry forms of organic selenium and yeast selenium were minced and added to the porridge. An aqueous solution of inorganic sodium selenate in the specified dose was also added to the porridge. The porridge was cooked from a mixture of pearl barley, oat flakes or ground wheat, or whole-grain oatmeal. Control animals fed the porridge with no additive of selenium. All animals also received the pelleted feed (in the nighttime), clover hay, vegetables, and water (*ad libitum*). The animals were maintained in a vivarium under standard conditions.

The animals were anesthetized with ether before the sampling procedure. They were decapitated with a guillotine. The left kidney and hepatic lobe were removed, put in a cold solution of 125 mM KCl and 10 mM HEPES buffer (pH 7.5), washed 2 times with the same buffer, weighted, squeezed, and homogenized in the buffer in the 1:2 ratio (1 g tissue per 2 ml buffer). The homogenate was centrifuged (6000 rpm) in ice for 15 min. Aliquot samples of the supernatant were stored at -20°C. Enzyme activity was measured within the next 2-3 days.

The blood (50 μl) and distilled water were put in Eppendorf tubes with physiological saline and heparin (10 U) to obtain the hemolysate. The preparation of samples and measurement of enzyme activity in blood hemolysate were performed as described elsewhere [7,15]. SOD activity in blood hemolysate was measured on the day of the experiment. Aliquot samples of the hemolysate were taken and stored at -20°C for 2-3 days to measure activities of GPx and GR.

Activities of antioxidant enzymes GPx, GR, and SOD were measured biochemically [7,15]. GPx activity was measured using 5,5'-dithiobis/2-nitrobenzoic acid (DTNB). GR activity was estimated from a decrease in the amount of NADPH required for the reduction of oxidized glutathione. SOD activity was evaluated in the superoxide-generating reaction of epinephrine autooxidation. Adrenochrome formation was recorded at 347 nm [6,7]. SOD activity in the supernatants of tissue homogenates was measured by a modified method. The study with tissue homogenates was performed using 0.1 M carbonate buffer (pH 10.6). The experiment with blood samples was performed with 0.2 M carbonate buffer. Before addition of epinephrine, the samples were preincubated for 40 sec to reach the linear region. Optical density was recorded for 2 min.

The measurements were performed on a Multiscan Plus device and Uvikon 923 spectrophotometer (time Driver regimen).

The correction for nonspecific oxidation of NADPH was introduced to evaluate the rate of NADPH oxidation with study enzyme. The sample was studied in the absence of oxidized glutathione (substrate). Protein concentration in supernatants was measured by the method of Lowry. Hemoglobin concentration was estimated as described previously [7]. Enzyme activity in blood hemolysate was calculated per 1 μl whole blood, 1 mg hemoglobin, and 0.1 optical density units (at 280 nm).

The results were analyzed by Student's *t* test (Microsoft Excel software).

RESULTS

Selenium-containing products had the most significant effect on enzyme activity in the kidney (Tables 1-3). The preparation of organic selenium produced a strong effect on activity of study enzymes in the kidneys. Activities of GPx and GR decreased by 40 and 18%, respectively. SOD activity was significantly elevated under these conditions (by 99%; Table 1). This preparation is an organic compound of selenium (Selexen, 9-phenyl-octahydroselenoxanthene). Selexen was synthesized at the Medical Radiological Research Center and Medbiofarm Company (V. V. Shakhtarin *et al.*). In the body, this product plays a metabolic role of GPx. These features probably contribute to a decrease in activity of endogenous GPx and GR. A relationship exists between GR and GPx, which maintains the pool of reduced glutathione. Feeding of the yeast preparation had a slight stimulatory effect on GR (32%), but did not modify activity of other antioxidant enzymes. Activation of GR can be associated with the presence of selenium and sulfur-containing amino acids (methionine and cysteine) in this product. Inorganic sodium selenate induced significant activation of SOD in the kidneys (89%). The increase in SOD activity under the influence of selenium-containing products reflects the generation of superoxide radicals. Hence, this treatment is accompanied by oxidative stress in the kidneys.

The yeast preparation also had a strong effect on antioxidant enzyme activity in the kidneys of guinea pigs. Activities of GPx and SOD decreased by 45 and 38%, respectively. GPx activity was elevated by 40% after treatment with inorganic sodium selenate (Table 2). The preparation of organic selenium did not modify activity of antioxidant enzymes in the liver (as differentiated from the kidneys; Table 2). The liver is an organ for detoxification and active metabolism.

TABLE 1. Effect of Consumption of Selenium-Containing Preparations on Antioxidant Enzyme Activity in the Kidney of Guinea Pigs (n=4, $M\pm m$)

Enzyme	Control	Organic selenium	Yeast product	Sodium selenate
GPx, μmol/mg protein/min	0.82±0.24	0.49±0.09*	0.63±0.05	0.78±0.08
GR, μmol NADPH/mg protein/min	206.9±26.2	169.3±11.1*	272.3±62.0*	204.7±14.0
SOD, arb. units/mg protein/min	5736±2607	11425±1339*	8490±3161	10837±1855*

Note. Here and in Tables 2 and 3: *p<0.05 compared to the control.

TABLE 2. Effect of Consumption of Selenium-Containing Preparations on Antioxidant Enzyme Activity in the Liver of Guinea Pigs (n=4, $M\pm m$)

Enzyme	Control	Organic selenium	Yeast product	Sodium selenate
GPx, μmol/mg protein/min	1.23±0.15	1.05±0.17	0.68±0.15*	1.70±0.03*
GR, μmol NADPH/mg protein/min	111.9±15.7	103.7±9.1	107.0±19.4	111.7±14.9
SOD, arb. units/mg protein/min	9386±1271	7905±941	6389±1582*	8966±1254

TABLE 3. Effect of Consumption of Selenium-Containing Preparations on Antioxidant Enzyme Activity in Whole Blood Hemolysate of Guinea Pigs (n=4, $M\pm m$)

Enzyme	Control	Organic selenium	Yeast product	Sodium selenate
GPx, μmol/mg protein/min	193.0±34.2	138.4±18.1*	174.3±34.0	101.8±9.7*
GR, μmol NADPH/mg protein/min	22.4±3.2	22.3±3.4	22.0±1.7	23.5±6.8
SOD, arb. units/mg protein/min	490±172	392±80	434±28	378±64

Functional activity of the studied enzymes in the liver is probably less dependent on exogenous factors.

Antioxidant enzyme activity was evaluated in the hemolysate of blood samples from animals after feeding of selenium-containing products (Table 3). Irrespective of the method of measuring enzyme activity, we showed that organic selenium and sodium selenate have little effect only on GPx (decrease in enzyme activity by 28 and 47%, respectively). Activities of GR and SOD in blood hemolysates did not change after feeding of biological additives (Table 3).

We conclude that feeding of selenium-containing products is followed by a decrease in activity of endogenous GPx in the kidneys and blood. These changes are accompanied by the development of oxidative stress in the kidneys. Antioxidant enzymes of the liver exhibited a weak reaction to biological additives. The consumption of selenium-containing preparations probably produces a good effect during selenium deficiency, since antioxidant enzymes "respond" to "exogenous" (dietary) selenium under these conditions. However, this treatment should be accompanied by the control over functional activity of the kidneys. The feeding of selenium-containing products in the absence of selenium deficiency can be followed by inactivation of endogenous GPx and development of oxidative stress in the kidneys. Selenium-rich products can be recommended for the prevention of selenium deficiency. Selenium content in these products decreases in the following order: coconut>pistachio>pork fat>garlic>salt-water fish>wheat bran>cep>eggs>soya>white rye bread>liver>crude rice>beef heart>chicken meat> beef>lentil>sunflower seeds [8]. The feeding of selenium-containing products should be under strict control during selenium deficiency and liver disorders.

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