Distribution of selenium and glutathione peroxidase in rabbits given selenite

Belma TURAN[1], Ertan DELİLBAŞI[2], Aşkın IŞTMER[3], Nejat DALAY[4]

[1]Department of Biophysics, Faculty of Medicine, Ankara University
department of Oral Surgery, Faculty of Dentistry, Gazi University
department of Pharmacology and Toxicology, Guillain-, Military Medical Academy, ANKARA
[4]Oncology Institute, Istanbul University, ISTANBUL

The effects of therapeutic selenium supplementation on an antioxidative enzyme glutathione peroxidase (GSH-Px) activities and selenium levels in plasma, erythrocyte, heart, liver and intestine tissues were investigated. Selenium as selenite was supplemented to rabbits as 50 \xg/kg body weight daily for 15 days. The selenium levels of the samples were measured by a new Zeeman graphite furnace atomic absorption spectrometry using a palladium-ascorbic acid chemical modifier instead of nickel modifier and more precision was obtained. An improved coupled test procedure was used to determine the GSH-Px activities in tissues and in blood samples.

Selenium levels, measured, in all experimental samples incerased significantly ($p<0.05$) with respect to controls. We observed that plasma selenium is more rapidly influenced by dietary supply modifications and is a more sensitive indicator of the short-term selenium status.

We also tried to demonstrate the possible effects of therapeutic selenium supplementation on GSH-Px activities. GSH-Px activities in tissues and in blood samples of supplemented group were measured as higher than the controls ($p<0.05$). It seems, therefore, that therapeutic selenium supplementation leads to an increase in selenium levels and GSH-Px activities of tissues and these increases are organ specific. [Turk J Med Res 1992, 10(2): 71-75]

KcyWords: Selenium, Nutrition, GlutaÜüone peroxidase, Tissue

Despite many similarities with sulfur, selenium occupies special position between metals and non-metals, and its strong tendency to change its oxidation levels has been utilized for some time in organic chemical synthesis in which redox-reactions are involved (1). While until 1957 only the toxic effects of selenium were known, different studies have revealed the importance of selenium in the glutathione-peroxidase (GSH-Px) activity thereafter (2,3). GSH-Px is an antioxidative enzyme which participates in the removal of toxic hydrogen peroxides from the cell, thus acting as an intracellular defence element in hydrogen peroxide catabolism (1,4,5). GSH-Px and similar antioxidative enzymes require essential elements as cofactors for their catalitic activities and one of them, GSH-Px utilizes selenium.

The activities of these antioxidative enzymes in the mammalian cell is a function of the complex mutual interactions between the mineral cofactor and the regulation of the enzyme synthesis rate. Deficiencies of Cu, Zn, Mn and Se lead to a decrease in the activity of the enzyme which requires one of these elements. However, the correlation of the deficiency of these elements and the decline in the antioxidative enzyme activity is still controversial (6). Several studies have shown that selenium deficiency may lead to certain diseases due to the incerased concentrations of peroxidation products and decreased GSH-Px activity (7-11). Dietary selenium deficiency induces changes and abnormalities in liver function, brain, heart, striate muscles, pancreas

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Yazışma Adresi: Belma TURAN
A.Ü.Tıp Fakültesi Biyofizik ABD
Sıhhiye 06100 - ANKARA

and genital tract (12) and has been shown to be associated with cardiomyopathy and other cardiovascular diseases (13). On the other hand, there has been evidence in supplementing human subjects with selenium for prevention of cancer, Keshan disease and other diseases (10).

With the growing appreciation of the role of dietary selenium in cardiac disease and cancer, a considerable number of reports have appeared where low selenium levels were correlated with a wide variety of diseases. Consequently, if a decline in the selenium concentration and the associated GSH-Px activity is likely to be an etiological factor in a particular disease, should be expected that selenium supplementation may reverse at least some of the disease symptoms. Studies conducted in Turkiye have revealed that the mean selenium levels are lower in Turkish population when compared to the developed countries (14,15). This study was undertaken to investigate the effects of selenium supplementation at a therapeutic dose level a) on the levels of selenium and b) on the activities of GSH-Px in blood and tissues.

MATERIALS AND METHODS

Initially, a total of 20 male new Zealand white rabbits with a mean weight of 2600 g (2500-2800g) were fed with a standard diet and water was available ad libitium for one week. The temperature and light/dark cycle were controlled. While all animals were fed with the same diet and housed under similar conditions, and were divided into two groups; 10 rabbits were intubated gastrically with a selenium solution (sodium selenite-Sigina) containing 50 ug Se/ml per kg bodyweight daily. Other 10 animals were kept as controls. After 15 days of selenium supplementation, blood samples from each animal were collected in heparinized plastic tubes and after centifucation they were frozen in liquid nitrogen and stored at -70°C until use. After that, all animals were killed by decapitation and exsanguination. The body cavities were opened by a mid-ventral incision and the hearts, livers and intestines were obtained. A11 samples were transfered to plastic containers and were frozen in liquid nitrogen and stored at -70°C until use.

Tissue samples were washed in deionized water, weighed before mincing and homogenized in 0.1 M phosphate buffer at pH 7.1. The improved test procedure as described by Giinzler et al. (16) was used to determine the GSH-Px activities of blood and tissue samples.

Selenium levels in blood and tissues were determined by a new Zeeman graphite furnace atomic absorption spectrometry (AA-30/40 Varian Spectrometer, GTA-96 graphite tube atomizer) procedure using a palladium-ascorbic acid chemical modifier instead of nickle modifier (17). The palladium modifier (Sigma) was injected into the graphite tube prior to addition of the samples which have been previously diluted (1:4) with a mixture of 0.5% Triton-x-100 (Merck) and 0.125 %L-ascorbic acid (Aldrich) as an antioxidizing agent. Calibration was made automatically by an autosampler using selenium stock standard solution (Sigma).

The significance levels of differences between the mean values of experimental and control groups were tested by using one way ANOVA test.

RESULTS AND DISCUSSION

Table 1 and 2 show the mean (± SD) GSH-Px activities and selenium levels of the plasma, erythrocyte, heart, liver and intestine selenium levels and GSH-Px activities in experimental and control groups as mean (± SD). GSH-Px activities and selenium levels in all samples of selenium supplemented group are higher than the control group. These high values measured in experimental group are statistically significant compared with the control group (Table 1 and 2).

In this study, a new Zeeman graphite furnace system was used as a method for the determination of selenium in all samples. Palladium-ascorbic acid chemical modifier gave eshanced thermal stability and sensitivity for selenium compared with the often used nickel modifier due to reduction of palladium to metallic state in the graphite tube. Using this chemical modifier we obtained more precise data for selenium levels of the samples and the level of our measurements is in agreement with the previous data (17).

Our results show that selenium supplemention for 15 days leads to an increase in the tissues selenium levels and the GSH-Px activities when compared to the levels of the control group. Several authors have reported an incerase in the selenium

Table 1. The mean (± SD) values of parameters measured in selenium supplemented and control groups

SAMPLES/ PARAMETERS	PLASMA		ERYTHROCYTE	
	Control	Experiment	Control	Experiment
Selenium (ng/ml)	65.12i5.70	200.45:5.71 p<0.05 X200	552.43:30.30	975.06:78.53 p<0.05 S77
GSH-Px (U/ml)	0.412:0.073	0.576-0.097 P<0.05 ¡540	19 08:5.64	35.34:5.62 p<0.05 885

p:The significance levels of differences between the values of supplemented and control groups.

??:The percentage rate of increasing of selenium and GSH-Px values measured in supplemented group with respect to control group.

content and the GSH-Px activity in different tissues in response to selenium supplementation (13,18,19,20). Previous studies on selenium supplementation, intake, metabolism and excretion have shown that acute 80-140 fig Se/kg body weight are subtoxic and toxic doses (29). Dietary selenium intake greately affects the GSH-Px activitity in several tissues in rats, chicken and sheep (12). Approximately 36% of the total selenium is estimated to be associated with Se-GSH-Px in rat liver. Hence the selenium status is highly correlated with the Se-GSH-Px activity (22). Tissue GSH-Px activities would appear to be a sensitive indicator of the blood selenium levels in animal models.

Baker and Cohen (23) have shown that a similar significant correlation has been found between blood selenium content and GSH-Px activity and between erythrocyte selenium levels and GSH-Px activity. Previous studies (23, 24) have demonstrated that there was a linear relationship between dietary selenium supply and blood selenium concentration in man. Plasma selenium is consistently lower than erythrocyte selenium and is more rapidly influenced by dietary supply modifications or by the selenium status than erythrocyte selenium (24). Thus these studies and our results (Table 1 and 2) show that plasma selenium (whicn was increased 200%) appears to be a more sensitive indicator of the short-term selenium status. On the other hand, the dependence of the GSH-Px activity upon the diet selenium content is also well known. Dietary selenium intake greatly affects the GSH-Px activity in several tissues of rats, chicken and sheep (1).

Oral or intraperitoneal supplementation of selenium leads to an incerase in the liver, kidney, lung and heart tissue selenium levels (20). We have measured the selenium levels in heart and liver tissues of supplemented group and increases of 36% and 51% respectively when compared to control groups while the GSH-Px activities increased 59% and 61% (Table 2). The highest level was observed in liver (Table 2). The previous results (22,23,24) and ours are in aggrement for selenium supplementation.

The selenium content and the GSH-Px activity were also measured in intestine of control and supplemented rabbits for the first time in present study. We observed that both of these values were increased with respect to controls in supplemented groups significantly ($p<0.05$). Recently, selenate uptake was shown to be faster than selenate uptake in rat intestine (25). Turner et al (26) using everted sacs of sheep ileum, demonstrated the selenate uptake to be more rapid than selenite uptake and a Na-K-AT-

Table2. The mean (± SD) values of parameters measured in selenium supplemented and control groups

SAMPLES/ PARAMETERS	HEART		LIVER		INTESTINE	
	Control	Experiment	Control	Experiment	Control	Experiment
Selenium (ug/g tissue)	0.86t0.03	1.17i0.08 P<0.05 %36	1.47±0. 1	2.22±0.19 P<0.05 %51	0.21 ±0.01	0.28i0.03 P<0.05 %33
GSH-Px (U7g tissue)	0.730±0.442	1.160±0.660 P<0.05 %59	1.28710. 04	2.069±0.946 P<0.05 %6\	0,212±0.042	0.392±0.137 P<0.05 %85

p:The significance levels of differences between the values of supplemented and control groups.

%:The percentage rate of increasing of selenium and 65H-Px values measured in supplemented group with respect to control group.

Pase was probably responsible for energizing the ileal brushborder transport of selenate.

Janghorbani et al. (27) indicated that selenium supplementation induces an increase in organ selenium levels and excretion of selenium by urine is less than the amount of intake by organs. A11 organs showed an increase in the selenium concentration during supplementation. The extent of this increase was found to be organ spesific. As an essential trace element selenium takes part not only in the direct protection of the endothelial cells againts the accumulation of aggressive oxygen species but also in the biosynthesis of arachidonic acid derivatives involved in platelet and leucocyte functions or in the regulation of cholesterol. Moreover, it prevents the toxic effects of cadmium and mercury and modulates the active transport of calcium (28).

There has been evidence that variations in the nutritional intake of selenium affect the function of the immune system, although the mechanisms involved are still unclear. It is evident, therefore, that this area merits more research not only in terms of maintance of status of general good health, but also in terms of the control of many disorders. Further studies should be devoted to the influence of the marginal deficiency in this trace element whose optimal requirement does not seem to be adequate by the usual dietary intake.

Selenit verilen tavşanlarda selenyum ve glutatyon peroksidaz dağılımı

Bu çalışmada terapötik selenyum uygulamasının plazma, eritrosit, kalp, karaciğer ve ince bağırsak dokularında selenyum seviyelerine ve bir antioksidan enzim glutatyon peroksidaz (GSH-Px) aktivitesine etkileri araştırıldı. Selenyum selenit olarak tavşanlara 15 gün boyunca günlük 50 \x g/kg vücut ağırlığı dozunda verildi. Bütün örneklerin selenyum seviyeleri yeni Zeeman grafit fırınlı atomik absorpsiyon spektrom etresi ile nikel m odifiyeryerine palladiunı-askorbik asit modifiyeri kullanılarak ölçüldü ve daha hassas sonuçlar elde edildi. Doku ve kan örneklerinin GSH-Px aktivitelerinin ölçümünde gelişmiş çiftli test işlemi kullanıldı.

Bütün deney örneklerinde selenyum seviyeleri kontrollere göre istatistiksel olarak anlamlı seviyede (p<0.05) yüksek ölçüldü. Plazma selenyumun beslenmeye ilişkin selenyum uygulaması ile çok daha hızlı etkilendiğini ve kısa süreli selenyum seviyeleri için çok daha duyarlı bir belirteç oludğunu gözledik.

Aynı zamanda bu çalışmada terapötik selenyum uygulamasının GSH-Px aktivitelerine olası etkilerini de sergilemeye çalıştık. Selenyum uygulanmış tüm hayvanların doku ve kan örneklerinde GSH-Px aktivitelerinin kontrollere göre istatistiksel olarak anlamlı seviyede (p<0.05) yüksek olduğu gözlendi. Böylece, selenyum suplementasyonunun doku selenyum seviyelerini ve GSH-Px aktivitelerini artırdığı ve bu artışın organa özel olduğu sonucuna ulaşıldı. [Türk Tıp Araştırma 1992, 10 (2): 71-75]

Anahtar Kelimeler: Selenyum, Beslenme, Glutatyon peroxidaz, Doku

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