Türk Biyokimya Dergisi
2002,,Cilt: 27, Sayı:2

EFFECT OF CADMIUM ION ON GLUTATHIONE REDUCTASE AND GLUTATHIONE PEROXIDASE ACTIVITY OF RAT LIVER AND THE RELATIONSHIP WITH DIETARY SELENIUM

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KADMİYUM İYONUNUN SIÇAN KARACİĞERİ GLUTATYON REDÜKTAZ VE GLUTATYON PEROKSİDAZ AKTİVİTELERİ ÜZERİNE ETKİSİ VE BU ETKİNİN DİYETDEKİ SELENYUM İLE İLİŞKİSİ

Özet: *Bu çalışmanın amacı, kadmiyum iyonunun sıçan karaciğeri glutatyon redüktaz ve glutatyon peroksidaz aktivitelerine etkisini ve bu etkinin diyetteki selenyum ile ilişkisini incelemektir. Bu amaçla bir grup Whistar sıçanı selenyumca fakir, diğer bir grup selenyumca zengin diyetle beslendi; kontrol grubuna ise normal diyet verildi. Bu gruplarda, Cd2+'nin karaciğer glutatyon redüktaz ve glutatyon peroksidaz aktivitelerine in vitro etkileri incelendi. Cd2+'nin (160 nmol/mg protein) glutatyon redüktaz aktivitesi üzerine inhibitör etkisi, kontrol grubu ile selenyumca fakir grup arasında farksızken, selenyumca zengin grupta anlamlı derecede düşük bulundu. Glutatyon peroksidazın Cd2+ ile inhibisyonu (10 mmol/mg protein) her üç grupta birbirinden farksızdı. Cd2+ konsantrasyonu iki katına çıkarıldığı zaman Cd2+'nin inhibitör etkisinin anlamlı derecede arttığı gözlendi.*

Anahtar Kelimeler:. *Glutatyon redüktaz, glutatyon peroksidaz, sıçan karaciğeri, kadmiyum, selenyum*

Summary: *This study is aimed to investigate the inhibitory effect of cadmium ion on glutathione reductase and peroxidase activities of rat liver and the relationship of this effect with dietary selenium. For this purpose one group of Whistar rats were fed with selenium deficient, another group with selenium excess diet, and the control group was fed with normal diet. In vitro inhibitory effects of Cd2+ on liver glutathione reductase and glutathione peroxidase activities of these groups were investigated. The inhibitory effect of Cd2+ (160 nmol/mg protein) on glutathione reductase activity was the same for selenium deficient and control groups, but significantly lower in the selenium excess group. There was no difference with respect to Cd2+ inhibition (10 mmol/mg protein) of glutathione peroxidase in all of the groups. When the concentration of Cd2+ was doubled, the inhibitory effect of Cd2+ became significantly higher in the selenium deficient group*

Key Words: *Glutathione reductase, glutathione peroxidase, rat liver, cadmium, selenium*

INTRODUCTION

Cadmium is one of the most toxic environmental and industrial pollutants. It induces severe alterations in various organs and tissues following acute or chronic exposure. One of the earliest effects induced by Cd2+ is the enhancement of lipid peroxidation, which is dependent on free oxygen radicals. Since under physiological conditions, Cd2+ is a poor electron acceptor and donor, the mechanism of free radical generation by Cd2+ is not well understood. Extensive studies have been carried out to identify mechanism of Cd2+ toxicity (1).

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Glutathione redox cycle is a system participating in the removal of free radicals from the cell. Glutathione reductase (GR) and glutathione peroxidase (GPx) is the components of this system (2). It was shown that when Cd2+ were given to rats, the activities of these enzymes were decreased. It was also shown that Cd2+ is the reversible inhibitor of yeast (3) and sheep brain GR (4).

On the other hand, selenium (Se) is an essential trace element for the mammalian nutrition. It is a structural component of GPx (5). An interesting finding published on Cd2+ and Se metabolism is that Se protects the cell from Cd2+ toxicity (6). The aim of this study is to investigate the inhibitory effect of Cd2+ on GR and GPx activities of rat liver at Se deficiency and excess.

MATERIALS AND METHODS

Animals and Housing:

Both sex weanling Whistar rats were divided into three groups and housed in stainless steel, wire bottomed cages. Each group consisted of five rats. They were maintained at an ambient air temperature of 22+1oC and a 12-h light/dark cycle. Selenium status of the animals was verified by determination of Se in blood and liver homogenates.

Diets and Feeding:

The deficient diet (Table I) was obtained commercially. Selenium was supplemented in aequate- and rich- diets as sodium selenite. Based on analysis of random batches of diet, the selenium concentration of the deficient diet was 9.8 mg/kg diet and the adequate and rich diets contained 225 mg Se/kg diet and 4.2 mg Se/kg diet respectively. Selenium content of the deionized water was negligible (<1 mg/l). The animals were fed with either an adequate diet (control group), a deficient diet (Se deficient group), or a rich diet (Se rich group). The animals were permitted free access to the diets and water for 14 weeks.

In order to obtain the liver tissue, rats were heparinized and anesthetized with sodium pentobarbital (30 mg/kg). Livers were homogenized with three volumes of 50 mM potassium phosphate buffer, pH 7.4. 14,000xg supernatants of the homogenates were used for the following measurements.

Table I- % composition of the diet

Torula yeast	30
Sucrose	58.7
Lard	3
Corn oil	2
Mineral mix (except Se)	5
Vitamin mix	1
DL-methionine	0.3

Glutathione reductase activity was measured according to modified Staal method (7). The incubation mixture contained 100 mM sodium phosphate buffer, pH 7.4, 1 mM GSSG, 100 mM NADPH, liver homogenate sample and Cd2+ ions when indicated. Decrease in the absorbance of NADPH at 340 nm was monitored spectro-photometrically, at 37oC. A unit of activity (U) was defined as the amount of enzyme that catalyses the oxidation of 1mmole of NADPH in one minute under these conditions.

Glutathione peroxidase activity was measured by modified Paglia and Valentine method (8). Tissue homogenate samples were incubated with 100 mM sodium phosphate buffer, pH 7.4, 1 mM GSH, 1 U/ml glutathione reductase, 4 mM sodium azide, 200 mM NADPH, (and Cd2+ ions when indicated) at 37oC, for 10 minutes. After the incubation period 1 mM H2O2 was added into the incubation medium and the decrease in the absorption of NADPH at 340 nm was monitored. A similar mixture excluding GSH was used as a blank. The definition of the unit was the same as that of GR.

Table II- GR and GPx activities as % of control when Cd2+is added into assay medium

	Se deficient diet	Normal diet	Se rich diet
GR (+160 nmol/mg Cd2+)	59.2 + 3.9	55.5 + 11.4	70.9 + 7.2*
GPx (+10mmol/mg Cd2+)	61.5 + 9.1	75.1 + 12.8	73.7 + 16.3
GPx (+20mmol/mg Cd2+)	37.4 +11.4*	54.2 + 8.8	51.7 + 12.4

($p < 0.05$)*

Proteins were measured by Bradford method (9). Bovine serum albumin was used as standart.

For the measurement of the enzyme activities LKB Ultraspec Plus, and for the measurement of proteins, Shimadzu UV-120-02 spectrophotometer was used.

All the chemicals were analytical grade.

Mann-Whitney U nonparametrical significance test was used for the statistical analyses (10).

RESULTS AND DISCUSSION

It was formerly observed that both GR and GPx activities were significantly lower in the groups that received either Se deficient or Se rich diet (11). The mean value of rat liver GR for the control, Se deficient and Se rich groups were 0.430+0.169, 0.162+0.026 and 0.188+0.042 respectively. The mean value of GPx obtained for these groups were also measured and found to be 26.170 + 4.593, 10.586 + 0.680 and 16.466 + 3.807 respectively.

There are some reports on the protective role of Se on Cd^2+ toxicity. The mechanism of this protection is not well known. It is thought that Cd^2+ might make a complex with Se.

In the former reports on the protective role of Se on Cd^2+ toxicity, the animals were fed with Se and/or Cd^2+ and individual and combined in vivo effects of these ions on GPx activity were studied (6). In the present study, in vitro effect of Cd^2+ on liver GR and GPx at different nutritional status with respect to Se was investigated. To our knowledge, this is the first report on the in vitro inhibition level of these enzymes by Cd^2+ and protective role of Se in the diet.

Inhibition of rat liver GR and GPx by Cd^2+ was investigated and the results are summarized in Table II. No difference was found between Se deficient and control groups with respect to inhibition pattern of GR by Cd^2+. On the other hand, inhibition effect of Cd2+ on GR as % of control is lower in animals which received Se rich diet as compared to normal or Se deficient group ($p<0.05$).

For GPx activity, there was no significant difference in the inhibition behaviour among all three groups when the concentration of Cd^2+ was 10 mmol/-mg. When the concentration of Cd^2+ was doubled however, Se deficient group became more susceptible to inhibition by Cd^2+ ($p<0.05$).

It is observed that, Cd^2+ is an inhibitor of GR at much lower concentrations as compared to GPx (160 nmol/mg vs 10 mmol/mg). This inhibition of GR might be responsible of the increase in the lipid peroxidation upon exposure to Cd2+.

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