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AN INVESTIGATION ON THE RELATIONSHIP BETWEEN VANADIUM AND ANTIOXIDATIVE ENZYME SYSTEM IN RATS

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Abstract

The aim of the study is to investigate the relationship between free oxygen radicals and vanadium in vanadium cytotoxicity. For this purpose erythrocyte copper zinc superoxide dismutase (CuZn-SOD) erythrocyte and plasma selenium dependent glutathione peroxidase (SeGSH-Px) enzyme activities, erythrocyte thiobarbituric acid reactive substances (TBARS) levels, erythrocyte and plasma vanadium levels were measured in Sprague Dawley rats fed with 0.15mgV/ml in tap water for the period of 15 days. And also water, food consumption and weight loss were followed during the test. Control rats were fed with normal tap water. Erythrocyte CuZn-SOD (175.4±61.40 U/ml) and plasma SeGSH-Px (0.50±0.05 U/ml) activities were significantly lower than that of control group (519.90±67.84 and 0.63±0.09 U/ml respectively) ($p<0.001$ and $p<0.05$ respectively). On the other hand erythrocyte SeGSH-Px activities (21.20±2.60 U/ml) were not different from control group (22.85±4.50 U/ml). There was significant difference between erythrocyte TBARS levels of test group and control group (7.91±3.00 nmol/ml, 6.03±3.00 nmol/ml respectively). Plasma vanadium levels (18±3.92 ng/ml) and erythrocyte vanadium levels (140.50±10.33 ng/ml) of test group were significantly higher than that of control group (3.22±0.83 ng/ml and 16.11±2.85 ng/ml respectively) ($p<0.001$). At the end of the test the body weight of test animals (133±6.25 g) were significantly lower than that of control animals (180.80±4.25 g) ($p<0.001$). As a result, it has been concluded that free radical production could be responsible for the vanadium cytotoxicty.

Key words: *Vanadium, Lipid peroxidation, Antioxidant enzymes*

Sıçanlarda Vanadyum ve Antioksidan Sistem Arasındaki İlişkinin Araştırılması

Bu çalışmanın amacı vanadyum (V) sitotoksisitesinde serbest oksijen radikalleri ve V arasındaki ilişkiyi incelemektir. Bu amaçla 15 gün süreyle mililitrede 0.15 mg V içeren içme sularıyla beslenen Sprague Dawley cinsi dişi sıçanlarda eritrosit bakır-çinko süperoksit dismutaz (CuZn-SOD), eritrosit tiobarbitürik asit reaktif ürünleri (TBARS), eritrosit ve plazma V seviyeleri ölçüldü. Aynı zamanda yiyecek ve su tüketimleriyle kilo kayıpları da deney boyunca takip edildi. Kontrol grubu sıçanlara normal çeşme suyu verildi. Eritrosit CuZn-SOD (175.4±61.40 U/ml) ve plazma SeGSH-Px (0.50±0.05 U/ml) aktiviteleri kontrol grubundaki değerlerden (sırasıyla 519.90±67.84 and 0.63±0.09 U/ml) anlamlı olarak daha düşüktü (sırasıyla $p<0.001$ and $p<0.05$). Eritrosit SeGSH-Px aktiviteleri (21.20±2.60 U/ml) kontrol grubundan (22.85±4.50 U/ml) farklı değildi. Deney ve kontrol gruplarının eritrosit TBARS düzeyleri (sırasıyla 7.91±3.00 nmol/ml, 6.03±3.00 nmol/ml) arasında anlamlı bir fark tespit edildi. Deney gruplarının plazma V düzeyleri (18±3.92 ng/ml) ve eritrosit V düzeyleri (140.50±10.33 ng/ml) kontrol grubundaki değerlerden (3.22±0.83 ng/ml and 16.11±2.85 ng/ml) daha yüksekti ($p<0.001$). Deney grubu sıçanların deney sonundaki vücut ağırlıkları (133±6.25 g) kontrol grubu sıçanların ağırlıklarından (180.80±4.25 g) anlamlı olarak düşük bulundu ($p<0.001$). Sonuç olarak serbest radikal üretiminin V sitotoksisitesinden sorumlu olabileceği kanısına varılmıştır.

Anahtar Kelimeler*: Vanadyum, Lipid peroksidasyonu, Antioksidan enzimler.*

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INTRODUCTION

Vanadium is widely recognized as a potentially toxic environmental pollutant (1,2). Due to fossil fuel burning, as much as 66 000 tons of vanadium is released into the atmosphere and further redistrubuted in the environment each year. Although vanadium is known to be essential for a number of species, its role as a micronutrient in humans has yet to be established (3). Vanadium enters the organism by inhalation, the gastrointestinel tract and the skin. Once absorbed, vanadate is reduced to vanadyl by the glutathio ne of erythrocytes or by ascorbic acid and other reducing substances in plasma and its transported by albumin and transferrin (4) . Vanadium is especially stored in certain organs, mainly in bone, kidney and liver (5).

There are now increasing evidence that vanadium can also be toxic to living system (6,7). Epidemiological studies have shown a correlation between vanadium exposure and the incidence of lung cancer in human (8-11). While the mechanisms of vanadium's toxicity and carcinogenecity remain to be investigated , it has been reported that this metal is able to regulate growth-factor-mediated signal transduction pathways, promote cell transformation, exert inhibitory effect on certain enzymatic systems, and decrease cell adhesion (12-14). Vanadium compunds were also reported to cause direct DNA damage (15).

There are many data that in vitro vanadium induces lipid peroxidation in purified and partially peroxidazed fatty acids, phospholipids and lipid tissue extracts (16-18), as well as in a great variety of biological systems: mitacondria (19), microsomes from liver, brain and human placenta (18-21), erthrocytes (22), isolated liver cells (23) and mouse and rat tissues (24). Some of the authors suggest that lipid peroxidation underlies vanadium toxicity (21-25).

It has long been known that vanadium can stimulate oxidation of fatty acid lipids (17). Recent report have described vanadium-dependent lipid peroxidation in human erythrocytes (22) and in animals treated acutely and chronically with vanadium (26). In all of these reports, the initial events are unclear, but strong evidence suggests possible free radical involvement. Lipid peroxidation, a process known to involve active oxygen species, can lead to pathological effects on biological membranes including permeability changes and possible cell lysis (27).

Little is also known about the in vivo effects of vanadate on antioxidant enzymes. Increased glutathione peroxidase and glutathione reductase activities in lungs (28) as well as unchanged CuZn-SOD and Mn-SOD activities in liver have been reported (29).

In this study we aimed to investigate the relationship between free oxygen radicals and vanadium in vanadium cytotoxicity. For this purpose erythrocyte CuZn-SOD activity, erythrocyte and plasma Se-GSH-Px enzyme activities, erythrocyte TBARS, erthrocyte and plasma vanadium levels were measured in Sprague Dawley Rats.

EXPERIMENTAL

Materials

Twenty Sprague Dawley rats were used in this study. Animals were divided equally into two groups of ten as control and exposed. Control group received water to drink. Animals of exposed group were given solely an aqueous solution of amonium meta vanadate (AMV) 0.15 mg/ml/V

for fifteen days. Food and fluid intakes were measured daily and body weight were monitored weekly during the test. Blood samples were taken. Whole blood was separated into plasma and erythrocyte fractions by centrifugation (4000g, 6min) at 4°C. Immediately after separation, the erythrocyte fractions were washed three times with an isotonic saline. Then, erythrocytes were lysed with cold distilled water (1:4); stored in a refrigerator at 4°C for 15 min and the erythrocyte membranes were removed. Plasma samples and erythrocyte lysates were stored at –70°C until assays.

All reagents used in this study were of analytical grade and obtained from Sigma Chemical Co. (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

Methods

CuZn-SOD activity measurement

CuZn-SOD activity in erythrocyte lysate was measured by the method described in our previous study (30). Briefly, each hemolysate was diluted 1:400 with 10mM phosphate buffer, pH 7.00. 25 µL of diluted hemolysate was mixed with 850 µL of substrate solution containing 0.05 mmol/L xanthine sodium and 0.025 mmol/L 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) in a buffer solution containing 50mmol/L CAPS and 0.94mmol/L EDTA pH 10.2. Then, 125 µL of xanthine oxidase (80U/L) was added to the mixture and absorbance increase was followed at 505 nm for 3 min against air. 25 µL of phosphate buffer or 25 µL of various standard concentrations in place of sample were used as blank or standard determinations. CuZn-SOD activity was expressed in U/ml.

SeGSH-Px activity measurement

SeGSH-Px activities in both erythrocyte lysate and plasma samples were measured by the method described in our previos study (30). The reaction mixture was 50mmol/L tris buffer, pH 7.6 containing 1mmol/L of Na_2EDTA, 2 mmol/L of reduced glutathione (GSH), 0.2 mmol/L of NADPH, 4 mmol/L of sodium azide and 1000U of glutathione reductase (GR) 50 µL of plasma and 950 µL of reaction mixture, or 20 µL of erythrocyte lysate and 980 µL of reaction mixture were mixed and incubated for 5 min. at 37°C. Then the reaction was initiated with 8 mmol/L H_2O_2 and the decrease in NADPH absorbance was fallowed at 340nm for 3 min. Enzyme activities were reported as U/ml in erythrocyte lysate and plasma.

TBARS level measurement

Erythrocyte TBARS levels were determined in erythrocyte lysates obtained after centrifugation and in accordance with the method described in our previous study (30). After the reaction of thiobarbituric acid with malondialdehyde, the reaction product was measured spectrophotometrically. Tetramethoxy propane solution was used as standard. TBARS level of erythrocyte were expressed as nmol/L .

Vanadium level measurement

Determination of vanadium was performed on a Varian 30/40 atomic absorption spectrophotometer, a varian GTA 96 graphite tube atomizer and a Varian DS-15 data station. The graphite furnace was purged with pre-purifed argon gas during operation. Gas 'stop condition' was used only during the atomization cycle. A varian autosampler was used to inject 10µl aliquots of sample solution. The standard solutions of Vanadium were prepared with nitric acid (Merck) at various concentrations such as 25, 50, 75, and 100 ng per ml. All standart solutions were prepared daily. Plasma and erythrocyte samples were diluted with triton X-100 (Sigma). Pyrolytically coated graphite tubes (i.d. 5.8 nm-o.d.80 mm and length 28.0 mm) were used. The mixtures were transfered to the autosampler of the graphite furnace and the results were taken from the printer. The instrument parameters were as follows:

RESULTS

The AMV exposed rats which received, as sole drinking liquid, the aqueous solution of AMV (0.15mg V/ml) for 15 days, we observed the typical symptoms of vanadium intoxication: diminished food and water consumption, supression of body weight increment (Table 1). The volume of liquid and the amount of food taken up by the vanadate-treated rats were decreased by 30% and 33% respectively low as compared to their controls. The present results in agreement with previous studies suggest that the supression of the body weight increment in animals intoxicated with AMV, is largely due to the decreased food and water uptake (31,32).

Table 1. The effect of amonium metavanadate (AMV) on food and fluid intake and body weight in tested rats.

Group of animals	Number of animals examined	Initial weight of animals (g)	Weight of animals after 15 days	Food intake g/rat/24h	Fluid intake ml/rat/24h
Control	10	144.55±5.65	180.80±4.25	18.30±0.75	29.80±1.20
AMV	10	149.70±5.34	133.00±6.25*	12.20±0.65*	21.00±1.80*

The results are given as mean values ± SEM
*$p<0.001$, significantly different from control group

Table 2 shows antioxidant enzyme activities of control and AMV treatment rats. Erythrocyte CuZn-SOD and plasma SeGSH-Px activities were significantly lower than that of control group ($p<0.001$ and $p<0.05$ respectively). On the other hand erythrocyte SeGSH-Px activities were not different from control group. There was no significant difference between TBARS levels of test and control group. Plasma and erythrocyte vanadium levels of test group were significantly higher than that of control group ($p<0.001$).

DISCUSSION AND CONCLUSION

In the investigations described here the supressed body weight increment appears to be due to a decrease in food consumption , rather than to a decrease in food efficiency. The smaller body weight increment recorded at present and previous studies (31,32).

Table 2. The comparison of antioxidant enzyme activities and malondialdehyde levels in control and AMV treatment rats.

	Control	AMV	p value
Erythrocyte CuZn-SOD (U/ml)	519.90±67.84	175.40±61.40	p<0.001
Plasma SeGSH-Px (U/ml)	0.63±0.09	0.50±0.05	p<0.05
Erythrocyte SeGSH-Px (U/ml)	22.85±4.50	21.20±2.60	p>0.05
Erythrocyte MDA (nmol/ml)	6.03±3.00	7.91±3.00	p<0.05
Plasma vanadium (ng/ml)	3.22±0.83	18.00±3.92	p<0.001
Erythrocyte vanadium (ng/ml)	16.11±2.85	140.50±10.33	p<0.001

The results are given as mean values ± SEM

Also we suggested that this anorexigenic effect of vanadium is due to a stimulation of glucose uptake by the central nervous system (33,34).

Free oxygen radicals are the molecules which contain unpaired electrons. They are also called as ‘oxidant molecules’ or ‘reactive oxygen species’. Reactive oxygen metabolites are constant products of normal aerobic cell metabolism. Healthy organisms are protected by various defense mechanisms against free oxygen radicals which are ceaselessly generated. These oxidants are degraded by enzyme systems such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-Px) and antioxidant such as ceruloplasmin, transferrin,reduced glutathione (GSH), methionine, vitamine E and C . MDA is the product of lipid peroxidation which occurs as a result of free radical injury and its formation is accerelated by oxidative stress. The amount of MDA produced, as measured by the thiobarbituric acid (TBA) assay, has been shown to be a true indicator of endogenous lipid peroxidation, Vanadium as sodium orthovanadate induces an increase in lipid peroxidation in the kidneys after a single subcutaneous or intraperitonal injection to rats or mice. The rate of MDA formation increased more than 100% 1 hour after 10 weeks in kidney homogenates of these animals but not in other tissues (26).

In this study, erythrocyte TBARS levels were found at higher concentration with vanadium. There was significant difference between TBARS levels of AMV and control group.The effect of vanadium treatment on TBARS levels is similar to previous reports. Donaldson and LaBella reported that rat and mouse tissues incubated with sodyum metavanadate ($NaNO_3$) exhibited high indices of lipid peroxidative damage, as measured by tissue MDA levels compared to control

tissues (24). Similarly, Elfant and Keen reported that pregnant and lactating rats fed with diets containing 75µg $NaVO_3$/g for 43 days showed high MDA levels both in whole liver homogenate and mitochondrial fragments compared to controls (29). These observations show that vanadium can directly or indirectly induce oxidative changes to erythrocytes and tissues.

Haider and El-Fakhri reported that, intraperitonal sodium metavanadate (2.5, 3.5 or 5.0mg/kg) administration to rats, for 7, 3 or 2 consecutive days enhanced lipid peroxidation in various rat brain fractions. However, an administration of alpha-tocopherol in combination with sodium metavanadate decreased the vanadium-stimulated lipid peroxidation (35) .

Lipid peroxidation was increased in liver and kidney rats received an aqueous solution of AMV (0.15mg/V/ml), but no increase in the erythrocytes was observed. However CuZn-SOD and SeGSH-Px enzyme activities in blood remained the same in contrast with our previous study (31).

It can be concluded with these results that free radical production can be responsible for vanadium toxicity. Heider and El-Fakhri demonstrated that antioxidant therapy prevented the cytotoxicity of vanadium (35). As our knowledge vanadium can reduce blood glucose levels in some previous studies (36,37). At that point if vanadium is used for antidiabetic activities, the antioxidant intake can be offer during the vanadium therapy.

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