Vitamin E Protects against Oxidative Damage Caused by Cadmium in the Blood of Rats

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ABSTRACT

Aim: *The protective effect of vitamin E (vit E) on cadmium (Cd) induced oxidative stress was studied in the blood of rats.*

Methods: *The rats were randomly divided in to three experimental groups: Control, Cd treated and Cd + vit E treated, each containing 10 animals. The Cd treated and Cd + vit E treated groups were injected subcutaneously daily with CdCl2 dissolved in isotonic NaCl in the amount of 2 mL/kg for 20 days, resulting in a dosage of 0.49 mg Cd/kg/d. In addition, Cd + vit E treated group received intramuscular injection of 150 mg/kg vit E until the end of the study.*

Results: *Cd treatment increased significantly malondialdehyde (MDA) levels and the antioxidant enzyme activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) in plasma and erythrocytes compared to the control group. Cd + vit E treatment, decreased significantly elevated MDA levels in plasma and erythrocyte and also reduced significantly the enhanced antioxidant levels. Cd treatment increased significantly the activity of iron levels in the plasma compared to the control group. Cd + vit E treatment, decreased the activity of iron levels in the plasma compared to the Cd treated group. In the control group, the histology of erythrocytes was normal. In Cd treated group, there was marked membrane destruction and there were hemolytic changes in erythrocytes. In Cd + vit E treated group, these changes were less than Cd treated group.*

Conclussion: *Our results show that vit E exerts a protective effect against cadmium toxicity.*

Key words: *Vitamin E, cadmium, hemolysis, oxidative stress, blood, rat.*

INTRODUCTION

Cadmium (Cd) is a wide-spread environmental pollutant, characterized by its toxicity to various organs, including kidney, liver, lung, testis, brain, bone, blood system (1-3). The molecular mechanisms of its toxicity are not yet well defined. Cd has been demonstrated to stimulate free radical production, resulting in oxidative deterioration of lipids, proteins and DNA, and initiating various pathological conditions in humans and animals (4,7). After the intake and resorption, Cd enters the blood where it binds to the red blood cell (RBC) membranes and plasma albumin (8). In the blood and tissues, Cd stimulates the formation of metallothioneins (9) and reactive oxygen species (ROS) thus causing oxidative damage in RBCs and in various tissues, which result in a loss of membrane functions (10). Cd also induces the onset of anemia, decreases the RBC count, hemoglobin concentration and hematocrit value as well as producing reduced blood iron levels (11). Moreover, a variety of accompanying changes in antioxidant defense enzymes were reported (12,13). Fariss (14) has shown that free radical scavengers and antioxidants are useful in protecting against Cd toxicity.

Vit E protects critical cellular structures against damage caused by oxygen free radicals and reactive products of lipid peroxidation. It has been reported that lipid peroxidation is prevented by vit E (15). Vit E inhibits peroxidation of membrane lipids by scavenging lipid peroxyl radicals, as a consequence of which it is converted into a tocopheroxyl radical. In fact, α-tocopherylquinone may act as a potent anticoagulant and as an antioxidant through its reduction to hydroquinone (16). Also, Boldyrev et al (17) reported that the protective role of vit E against the toxicity of oxidants may be due to the quenching of hydroxyl radicals.

The aim of this study was to investigate a possible protective effect of vit E treatment on the selected biochemical parameters and histological changes of RBCs in rat exposed to Cd.

MATERIAL and METHODS

Animals

Thirty healthy male Wistar albino rats, weighing 200-250 g and averaging 16 weeks old were supplied from The Center of Medical Investigations of Yuzuncu Yil University. The animals were given standard rat pellets (Murat animal food product co., Ankara, Turkey) and tap water ad libitum. The rats were house singly in temperature controlled (20-25°C) cages. They were housed in macrolon cages under standard laboratory conditions (light period 7.00 a.m. to 7.00 p.m., 21±2°C, relative humidity 55%).

Treatment of Rats

The rats were randomly divided in to three experimental groups: A (Control), B (Cd treated) and C (Cd + vit E treated), each containing 10 animals. The Cd treated and Cd + vit E treated groups injected subcutaneously daily with CdCl2 dissolved in isotonic NaCl in the amount of 2ml/kg for 20 days, resulting in a dosage of 0.49 mg Cd/kg/d (18). The control groups were injected with only isotonic NaCl (2 mL/kg/d) throughout the experiment. In addition, the Cd + vit E treated group received intramuscular injection of 150 mg/kg/d Vit E (Evigen, Turkey) until the end of the study. All animals received care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health.

At the end of the experiment, blood samples were obtained for biochemical and histopathological investigations. The rats in all groups were starved overnight for 12 h, and sacrificed under ketamine hydrocloride (Ketalar®, Eczacıbaşı Farma 10 mg/kg i.p.) anaesthesia. Blood from the heart was collected from the abdominal and thoracic cavities into potassium-EDTA tubes using 10 ml syringes (10.5 mg/7 mL). Blood samples were centrifuged at 3000 rpm for 5 minutes in a refrigerated centrifuge and the Plasma and buffy coat were discarded. Packed RBCs were washed twice with normal saline after 1/10 dilution to remove remaining leukocytes and plasma components. All reagents were of analytical grade. Trichloroacetic acid (TCA), 2-thiobarbituric acid (TBA) and hexane were purchased from Merck Chemical Company, Darmstadt, Germany. n-Heptane and α-α'dipyridyl were purchased from Sigma (St Louis, USA).

Biochemical Analysis

Malondialdehyde (MDA) was determined by the double heating method of Draper and Hadley (19). This method is based on the fact that lipid peroxides and TBA react to form a pink pigment with absorption maximum at 532 nm. For this purpose, 2.5 mL of 100 g/L TCA solution were added to 0.5 mL hemolysate/plasma in each centrifuge tube and placed in a boil-

ing water-bath for 15 min. After cooling in tap water, the mixture was centrifuged at 1000 g for 10 min, and 2 mL of the supernatant was added to 1 mL of 0.67% (w/v) TBA solution in a test tube and placed in a boiling water-bath for 15 min. The solution was then cooled in tap water and its absorbance was measured using a Shimadzu UV-1601 (Japan) spectrophotometer at 532 nm. The concentration of MDA was calculated by the absorbance coefficient of MDA-TBA complex, 1.56 × 105 cm-1 M-1, and is expressed in nmol/g hemoglobin (Hb) and in plasma nmol/mL. All samples were assayed in duplicate.

Blood for the determination of antioxidant status was centrifuged to separate plasma and RBCs. Isolated RBCs were washed three times with 3 volumes of ice-cold 155 mmol/L NaCl and hemolysates containing about 50g Hb/L prepared according to McCord and Fridovich (20) were used for the determination of catalase (CAT) and glutathione peroxidase (GSH-Px) activities. CAT activity was determined according to Beutler, (21) while the activity of GSH-Px was assayed by the subsequent oxidation of NADPH at 340 nm with t-buthyl-hydroperoxide as a substrate (22). For determination of superoxide dismutase (SOD) by the method of Misra and Fridovich, (23) lysates were diluted with distilled water (1:7 v/v) and treated by chloroform-ethanol (0.6:1 v/v) in order to remove hemoglobin. The clinical chemical analysis of plasma was performed on a Cobas integra 800 automatic analyzer of Roche Diagnostic (USA). Analysis of blood was carried out at 37°C with potassium-EDTA plasma. Analysis of iron was done according to the instructions of the manufacturer of the automatic analyzer. Hb measurement was by mixing blood with Drabkin solution, which contains potassium ferricyanide and potassium cyanide.

Morphological Evaluation

Blood samples using sterile potassium-EDTA tubes. Immediately after mixing with blood by inverting the tubes, blood-smears were made by spreading one drop on a slide and then stained with the Giemsa solution. RBCs morphology was determined using a Nikon Optiphot-2 microscope (Tokyo, Japan) with an oil immersion 100/1.25 objective.

Image Analysis

The system we used consisted of a PC with hardware and software (Image-Pro Plus 5.0-Media Cybernetics, USA) for image acquisition and analysis, Spot Insight QE (Diagnostic Instruments, USA) camera and optical microscope. The method requires preliminary software procedures of spatial calibration (micron scale) and setting of color segmentation for quantitative color analysis. The image analysis program was used for morphologic evaluation of the samples. Twenty areas from each group were chosen randomly. The number of intact and hemolysed RBCs were measured. The investigators who performed these measurements were unaware of the experiment.

Statistical Analysis

The data were expressed as mean ± standard deviation (SD) and analysed using repeated measures of variance. Tukey test was used to test for differences among means when ANOVA indicated a significant ($p<0.05$) F ratio.

RESULTS

Plasma MDA, and RBC MDA, SOD, GSH-Px and CAT were presented in (Table 1). As shown in table 1, Cd treatment increased significantly MDA levels in plasma and RBC ($p<0.01$) and also increased significantly the antioxidant levels (SOD, GSH-Px and CAT)

Table 1. *Comparison of Plasma MDA, and RBC MDA, SOD, GSH-Px and CAT of A (Control), B (Cd treated) and C (Cd + vit E treated) groups. Statistical analysis was by a one-way ANOVA with Tukey's test. Values are expressed as mean ± SD, and n: 10 for all groups.*

Parameters	*A*	*B*	*C*
RBC MDA (nmol/g Hb)	*14.8±1.6*	*19.7±2.3[a]*	*16.8±1.9[b]*
RBC SOD (U/g Hb)x10³	*4.98±0.42*	*11.94±1.22[a]*	*7.96±1.05[b]*
RBC GSH-Px (nmol NADPH/min/g Hb) x10³	*58.08±1.73*	*79.04±2.13[a]*	*68.16±1.94[b]*
RBC CAT (µmol H_2O_2/min/g Hb) x10³	*69.14±1.13*	*88.92±3.02[a]*	*78.37±2.02[b]*
Plasma MDA (nmol/ml)	*2.26±0.31*	*5.81±0.56[a]*	*3.89±0.36[b]*

[a] *p<0.01 when compared with group A*

[b] *p<0.05 when compared with group B*

(p<0.01) compared to the control group. Vit E treatment decreased significantly elevated MDA levels in plasma and RBC (p< 0.05) and also reduced significantly the enhanced antioxidant levels (p<0.05).

As shown in Table 2, Cd treatment increased significantly the activity of iron levels (p<0.05) in the plasma compared to the control group. Vit E treatment decreased the activity of iron levels (p<0.05) in the plasma compared to the Cd treated group. It was found that the RBC counts decreased (p<0.05) in Cd treated rats. Vit E treatment increased (p<0.05) the lowered RBC counts. It was also found that the hemolysed RBC counts increased (p<0.001) in Cd treated rats. Vit E treatment reduced (p<0.01) hemolysed RBC counts number (Table 3).

In control group, histology of RBCs was normal (Figure 1). In Cd treated group, there were remarkable membrane destruction and hemolytic changes in RBCs (Figure 2). In Cd + vit E treated group, these changes were less than Cd group (Figure 3).

DISCUSSION

Cd is a toxic metal that is widely used in different industries. It promotes an early oxidative stres and afterward contributes to the development of serious pathological conditions because of its long retention in some tissues (24). The present results have clearly demonstrated the ability of Cd to induce oxidative stress in rat plasma and RBC as evidenced by increased lipid peroxidation after 20 days of Cd treatment. This finding is in agreement with several reports demonstrating that Cd induces oxidative stress in tissues by increasing lipid peroxidation and by altering the antioxidant status in several tissues (4-6,25).

RBC membrane is rich in polyunsaturated fatty acids which are very susceptible to free radical mediated peroxidation. Membrane to induce lipid peroxidation and eventually cause hemolysis (26).

***Table 3**. Image analysis of A (Control), B (Cd treated) and C (Cd + vit E treated) groups. Statistical analysis was by a one-way ANOVA with Tukey's test. Values are expressed as mean ± SD, and n = 10 for all groups.*

Groups	*Intact RBC (%)*	*Hemolysed RBC (%)*
A	*98±0.79*	*2±0.13*
B	*59±2.33[a]*	*31±1.01[c]*
C	*84±3.19[b]*	*16±0.67[d]*

[a]p<0.05 when compared with group A
[b]p<0.05 when compared with group B
[c]p<0.001 when compared with group A
[d]p<0.01 when compared with group B

Lipid peroxidation is associated with a wide variety of toxicological effects, including decreased membrane fluidity and function, impaired mitocondrial and Golgi apparatus functions, and inhibition of enzymes. MDA is an end product of lipid peroxidation and is a frequently measured index of these processes. MDA can croos-link with membrane constituents of RBC (27,28).

The deformability of destructed RBC and increased RBC hemolysis may be due to increased production of free radicals. It has been shown that parenteral application of α-tocopherol caused a marked decrease in lipid peroxidation in the RBC membrane on chronic hemodialysis patients (29). In our study, we found that the administration of vit E decreased RBC MDA levels significantly as compared to Cd treated group.

Previous investigations showed that chronic treatment with Cd induced oxidative damage in RBCs of rats and goldfish, causing destruction of cell membranes and increased lipid peroxidation, as well as alteration of the antioxidant defence system, energy metabolism and the appearance of anemia (11,25,30-34). The data of other authors showed that Cd caused the damage of the RBC membrane resulting in hemolysis. Some antioxidants can exert a protective role against Cd-induced destruction of RBCs (7).

***Table 2**. The values of RBCs and iron concentration of plasma in the blood samples of A (Control), B (Cd treated) and C (Cd + vit E treated) groups. Statistical analysis was by a one-way ANOVA with Tukey's test. Values are expressed as mean ± SD, and n = 10 for all groups.*

Parameters	*A*	*B*	*C*
RBC (x 106/µl)	*7.5±0.76*	*5.1±0.41[a]*	*6.3±0.55[b]*
Iron (µg/dl)	*63.5±5.3*	*85.2±7.4[a]*	*72.1±6.4[b]*

[a] p<0.05 when compared with group A
[b] p<0.05 when compared with group B

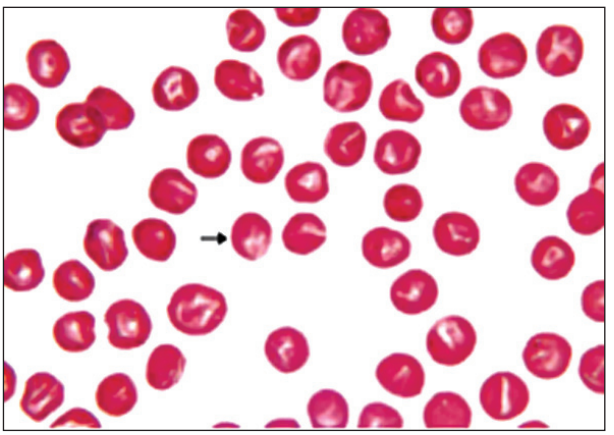

***Figure 1**. The morphology of normal RBCs in control rats (arrow: normal RBCs, Giemsa; 1150x).*

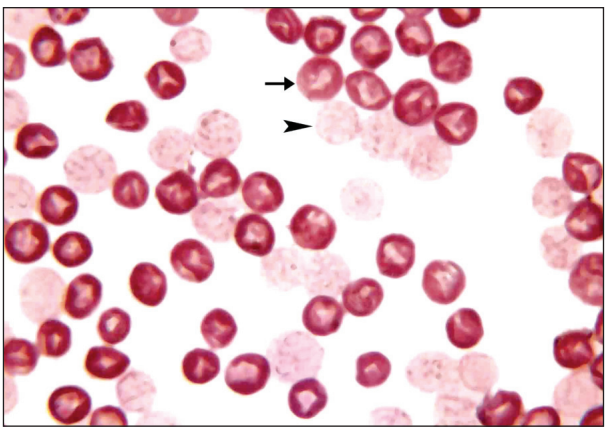

***Figure 2**. In Cd treated rats, remarkable membrane destruction and hemolytic changes are seen in the RBCs (arrow: normal RBCs, arrowhead: hemolytic RBCs, Giemsa; 1150x).*

Treatment with Cd increased lipid peroxide concentration in the blood of rats which was accompanied by increased formation of ROS (6,35). As a consequence, enhanced lipid sulfhydryl homeostasis as well as marked disturbances of antioxidant defence system occurred (36,38). Pretreatment with vitamin E exhibited a protective role on the toxic effects of Cd on the hematological values, lipid peroxide concentration as well as on enzymatic and non-enzymatic components of antioxidant defence system (34). In our study, Cd treatment increased significantly MDA levels in plasma and RBC compared to the control group. Cd + vit E treatment decreased significantly elevated MDA levels in plasma and RBC. These findings supported by El-Demerdash et al (39) who reported that treatment of vitamin E with CdCl2 produces a significant reduction in CdCl2-induced increase in TBARS in different rat tissues. This finding is paralleled by modulation of GST in plasma and liver. As a consequence of ameliorated oxidative stress and ROS, the activities of plasma, liver, testes and brain enzymes were partially restored. Thus, the present results indicate that adequate antioxidant status may attenuate Cd-induced oxidative stress and cellular damage.

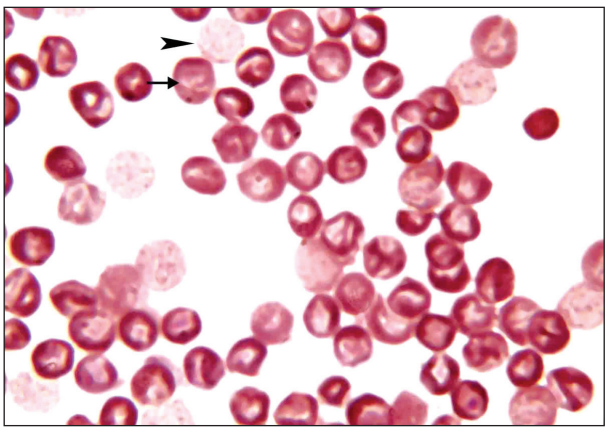

***Figure 3**. Reduced hemolysis of RBCs in Cd + vit E treated rats are seen (arrow: normal RBCs, arrowhead: hemolytic RBCs, Giemsa; 1150x).*

Activities of SOD, CAT and GSH-Px were significantly increased in RBC of Cd- treated rats. It is known that Cd induces the formation of superoxide anion radicals in RBCs and it is reasonable to expect an increased activity of SOD (11,25,33,34,37). Cd induced an increase in CAT and GSH-Px activities which may be explained by their influence on hydrogen peroxide as substrate which is formed in the process of dismutation of superoxide anion radicals (7). The pretreatment with vit E prior to Cd administration decreased RBC SOD, CAT and GSH-Px activities indicating that Vit E eliminates the toxic effects of Cd on the activity of these enzymes (34). Our result is in accordance with a recent report of Kanter et al (25) who showed that Cd treatment increased significantly the antioxidant levels (SOD, GSH-Px and CAT) in plasma and RBC compared to the control group. Cd + Nigella sativa treatment decreased significantly elevated antioxidant levels in plasma and RBC. In contrast, Sarkar et al (6) indicated that the RBC SOD and CAT activities decreased significantly with Cd and the pretreatment with vit E and/or selenium prior to Cd administration partially reversed these changes. In addition, Beytut et al (40) observed that the Cd treatment decreased the GSH-Px activity of RBC.

Armutcu et al. (41) demonstrated that plasma iron levels were significantly increased in the acetone-treated rats. It has been suggested that this increasing might be associated with hemolysis in the RBCs. Increasing of plasma iron levels reflect the rise in hemolysis of RBCs. Iron concentration of plasma is elevated in hemolysis due to iron of haemoglobin (42). Our previous investigation showed that Cd treatment increased significantly the activity of iron levels in the plasma compared to the control group. Cd + Nigella sativa treatment decreased the activity of iron levels in the plasma compared to the Cd treated group (25). Similar to these results our present data show that Cd causes significant increase of iron levels. But cotreatment of rats with Cd and vit E combination caused a significant decrease the levels of iron. However, Kostić et al (11) indicated that the chronic exposure of Cd decreases the level of iron in the blood and causes the decrease of the RBC count, hemoglobin concentration and hematocrit value. The decrease of hematocrit value in hemolyzed plasma of rats exposed to Cd indicates the increased destruction of RBCs (43,44). It was found in this work that the RBC counts decreased in Cd treated rats. Vit E treatment increased the lowered RBC counts. It was also found that the hemolysed RBC counts increased in Cd treated rats. Vit E treatment reduced hemolysed RBC counts number.

It can be concluded from presented results that Cd induced oxidative damage in RBCs leads to anemia, loss of membrane function by enhancing of lipid peroxide concentration as well as alteration of the activity of antioxidant defense system enzymes. Our results show that vit E expressed protective role against toxic influence of Cd on all examined parameters in rat blood.

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