Kragujevac J. Sci. 23 (2001) 115-126.

PROTECTIVE ROLE OF VITAMIN E ON ANTIOXIDANT DEFENSE SYSTEM AND LIPID PEROXIDE CONCENTRATION IN THE BLOOD OF RATS ACUTELY EXPOSED TO CADMIUM

Branka I. Ognjanović[a], Slađan Z. Pavlović[b], Snežana D. Maletić[a], Radoslav V. Žikić[a], Andraš Š. Štajn[a], Zorica S. Saičić[b] and Vojislav M. Petrović[b]

[a]Institute of Biology, Faculty of Sciences, University of Kragujevac, Radoja Domanovića 12, 34000 Kragujevac, Serbia, Yugoslavia
[b]Institute for Biological Research "Siniša Stanković", Department of Physiology, 29. Novembra 142, 11060 Belgrade, Serbia, Yugoslavia

(Received March 16, 2001)

ABSTRACT. Male *Wistar albino* rats, 3 months old, were acutely treated with cadmium (0,4 mg Cd/kg b.m., i.p., 24^h before the sacrificing) and with vitamin E + Cd (20 IU Vit E/kg b.m., i.m., 48^h + 0,4 mg Cd/kg b.m., i.p., 24^h before the sacrificing). The haematological values: red blood cells (RBC) count, haematocrite (Htc) value, haemoglobin (Hb) and lipid peroxide (LP) concentrations were determined. The status of antioxidant defense system (AOS): copper zinc containing superoxide dismutase (CuZn SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GR), glutathione-S-transferase (GST) activities and reduced glutathione (GSH), ascorbic acid (AsA) and vitamin E (Vit E) concentrations were also determined in the blood of rats. RBC count, Htc value and Hb concentration were significantly decreased in the blood of rats acute treated by Cd. Vit E prevents anaemia caused by Cd. Acute administration of Cd significantly increased LP concentration, while Vit E reversed this changes. All examined components of AOS in the blood were significantly increased in Cd-treated rats. Pretreatment of rats with Vit E abolished the toxic effects of Cd on AOS.

INTRODUCTION

Cadmium (Cd) is a very toxic heavy metal, an important pollutant of environment (present in soil, water, air, food and in cigarette smoke), which causes poisoning in various tissues of humans and different species of laboratory animals [1-3]. After the intake and resorption, Cd enters the blood where it binds to the erythrocyte membranes and proteins of low molecular mass forming metalothioneins [4, 5]. Binding of Cd to red blood cells (RBC) causes their destruction and increased haemolysis and haematological values alters. It also induced decreased absorption of intestinal iron and anaemia appears [6, 7].

Recently, it has been suggested that Cd may induce oxidative damage in various tissues by enhantion peroxidation of membrane lipids and by inhibition of enzymes involved in the removal of

certain reactive oxygen species (ROS) [8, 9]. Cd induced the increases of ROS production, including superoxide anion radical [10], hydrogen peroxide [11], hydroxyl radical [12] and lipid peroxidation [13-15]. A variety of accompanied changes in antioxidant defense system (AOS) were reported [16-22]. Studies of Fariss [23] have shown, that free radical scavengers and antioxidants are useful in protecting against cadmium toxicity.

Vitamin E (Vit E) is the primary liposoluble antioxidant, which may have an important role in scavenging of free oxygen radicals and stabilizes the cell membranes maintaining its permeability [22-25]. Vit E is present in all cellular membranes where it is bound to the protein complexes in the inner mitochondrial membranes and may affect oxidative changes which occur in organelles [26]. Moreover, it is known that antioxidants, such as vitamin E, coenzyme Q, ascorbic acid, glutathione and selenium may act synergically, preventing lipid peroxidation and cell destruction [12, 17, 26-32].

The aim of this study was to investigate a possible protective role of Vit E pretreatment on the haematological values, lipid peroxide concentration and on AOS in the blood of rats acutely treated with cadmium.

MATERIALS AND METHODS

In our experiments male *Wistar albino* rats, 3 months old (weighing 280 ± 30 g) were used. The animals were kept at 21 ± 1°C and exposed to 12 h light - 12 h dark cycle. The animals were injected with $CdCl_2$ (0,4 mg Cd/kg b.m., i.p., 24^h before the sacrificing) and with vitamin E + Cd (20 IU Vit E/kg b.m., i.m., 48^h + 0,4 mg Cd/kg b.m., i.p., 24^h before the sacrificing). Control rats were drunk by tap water *ad libitum*. The exposed rats were housed in individual cages and given a standard diet and water *ad libitum*. Each experimental group consisted of six animals.

The animals were sacrificed by decapitation always between 8 and 10 A.M. and fresh blood was immediately collected into heparinized test tubes. Red blood cells (RBC) count and haematocrite (Htc) value were determined by standard haematological techniques [33]. The haemoglobin (Hb) concentration was determined by the cyanmethaemoglobin method [34].

The concentration of lipid peroxides (LP) in the blood was determined as thiobarbituric acid-reactive substances (TBARS) according to Ohkawa et al. [35]. Concentration of reduced glutathione (GSH) in whole blood was measured by standard method of Beutler [36].

Blood for the determination of antioxidant defense system was centrifuged to separate plasma and red blood cells. Plasma specimens were used for determination of ascorbic acid (AsA) with 2,4,6-Tripyridyl-S-triazine (TPTZ) by the method of Day et al. [37], while vitamin E (Vit E) was determined by the method suggested by Desai [38]. Glutathione-S-transferase (GST) activity toward 1-chloro-2,4-dinitrobenzene (CDNB) as substrate was determined according to Habig et al. [39].

For the estimation of the RBC enzyme activities, isolated red blood cells were washed three times with 5 vol. of cold 155 mM/l NaCl. Haemolysates containing about 50 g Hb/L were prepared according to McCord and Fridovich [40]. Copper zinc containing superoxide dismutase (CuZn SOD) activity was measured by the epinephrine method of Misra and Fridovich [41].

Catalase (CAT) was determined according to Beutler [42], while the activity of seleno-enzyme glutathione peroxidase (GSH-Px) was assayed by following the oxidation of NADPH with t-buthyl-hydroperoxide [43]. Glutathione reductase (GR) was determined by measuring NADPH oxidation in the presence of oxidized glutathione [44]. All enzyme assays were performed at 26°C.

Data are given as mean ± SE and differences between experimental and control groups were evaluated. Statistical analysis of the result was based on the Student′s paired t-test, considering the significance at a level of $p < 0.05$ [45].

RESULTS AND DISCUSSION

Results presented in Table 1 clearly show that administration of Cd results in a significant decrease of RBC count ($p<0.01$), Htc value and Hb concentration ($p<0.02$). Pretreatment with Vit E partially improved these changes.

Table 1: The haematological values: red blood cells (RBC) count, haematocrite (Htc) value and haemoglobin (Hb) concentration in the blood of control rats (C), rats exposed to cadmium (Cd) and vitamin E + cadmium (Vit E + Cd). The values are means ± SE from 6 animals in each experimental group.

Significantly different from C: * $p<0.05$; ** $p<0.02$, ***$p<0.01$ and **** $p<0.005$. Significantly different from Cd: [a] $p<0.05$; [b] $p<0.02$, [c] $p<0.01$.

	RBC (10^{12}/L)	Hct (L/L)	Hb (mmol/L)
C	7.91 ± 0.21	0.45 ± 0.02	8.35 ± 0.12
Cd	5.11 ± 0.09 ****	0.42 ± 0.02 ***	7.57 ± 0.10 ***
Vit E + Cd	6.51 ± 0.25 ** b	0.45 ± 0.03 c	8.06 ± 0.14 * a

Literature data showed that Cd induced oxidative damage in erythrocytes, causing destruction of cell membrane by increasing lipid peroxidation. It also induced an alteration of AOS and energy metabolism, as well as appearance of anaemia [6, 7, 9, 46-48]. Many authors have documented that free radical scavengers and antioxidants are useful in protecting against Cd toxycity [9, 12, 17-19, 22, 23].

Our results show that acute treatment with Cd induces anaemia, e.g. decrease of RBC count, Htc value and Hb concentration (Table 1). The results have confirmed our previous investigations [6, 48] and the results of other authors [7, 9, 46, 49-51]. It is well known, that the presence of Cd in the organism decreases the level of iron in the blood [6] and causes the decrease of Hb concentration. The

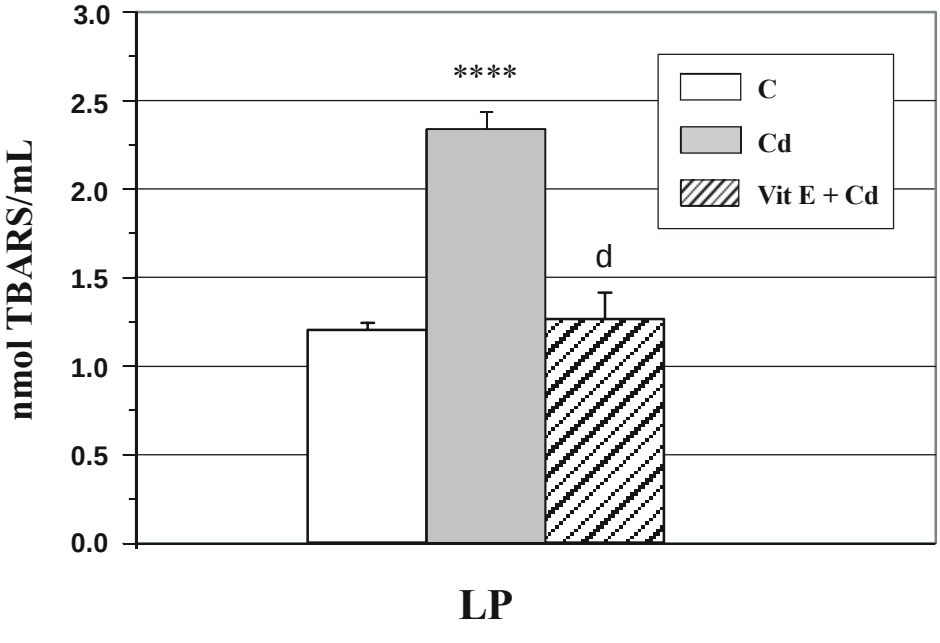
decrease of Htc value in haemolysed plasma of rats exposed to Cd indicates an increased destruction of erythrocytes [6, 7, 9, 46-51]. Cd induced anaemia is characterized by pronounced reticulocytosis and hypochromia [6]. Cd also caused the damages of the erythrocyte membrane resulting in haemolysis in the same way like other metals such as Pb, Cu and Zn [7, 51, 52].

In rats pretreated with Vit E before Cd-treatement, RBC count, Htc value and Hb concentration were significantly increased in comparison to the rats received Cd only. Our results suggest a very important role of pretreatment with Vit E before intoxication with Cd. Previous data showed that Vit E decreased the toxic effects of Cd on the haematological value and has the protective role in anaemia [21, 22, 53, 54].

The LP concentration was significantly increased in the blood of rats after acute administration of Cd ($p<0.01$), while Vit E pretreatment reversed this changes to control values (Figure 1). Recent studies on mammals have shown that Cd stimulates formation of ROS, including superoxide anion radical [10], hydrogen peroxide [11] and probably hydroxyl radical [12]. As a consequence enhanced LP, DNA damage, altered calcium and sulfhydryl homeostasis, as well as marked disturbances of AOS occurs [2, 9, 20-22, 52, 53]. In addition increased intake of Cd results in its retention and in peroxidative damage in erythrocytes and in soft tissues [6, 9, 13-16, 54]. Manca et al. [13] examined the susceptibility of liver, kidneys, brains, lungs, and testes of rats given intraperitoneal doses of $CdCl_2$. These results indicate that LP is an early and sensitive indicator of Cd toxicity.

Figure 1: The concentration of lipide peroxides (LP) in the blood of rats exposed to cadmium (Cd) and vitamin E + cadmium (Vit E + Cd). The values are means ± SE from 6 animals in each experimental group.

Significantly different from C: ****$p<0.005$.
Significantly different from Cd: ^{d} $p<0.005$.



Pretreatment with Vit E was very effective in the prevention of oxidative damage induced by Cd. The concentration of LP was significantly lower in the Vit E cotreated animals than in the group received Cd only (Figure 1). Vit E is capable to scavenge free oxygen radicals, as well as lipide and lipide peroxyl radicals [54]. By inhibiting LP in the blood of rats, Vit E can exhibit protective role against acute Cd-induced toxicity. Coadministration of some antioxidants, such as AsA, Vit E, GSH, coenzyme Q and Se, resultet in the uptake and distribution of Cd in liver and kidney of rats, and no rise in LP concentration was recorded [12, 17, 25-32, 54-59].

The data presented in Figures 2 and 3 show that Cd induced significant changes in activities of AOS enzymes. In animals exposed to Cd the activities of CuZn SOD ($p<0.02$) and CAT ($p<0.01$) (Figure 2), as well as activities of GSH-Px ($p<0.02$), GR ($p<0.02$) and GST ($p<0.02$) (Figure 3) were significantly increased. The pretreatment with Vit E prior to Cd intoxication, partially reversed these changes. The activities of CuZn SOD ($p<0.01$), CAT ($p<0.02$), GR ($p<0.02$) and GST ($p<0.01$) were significantly decreased as compared with animals given Cd alone, while the GSH-Px activity was significantly increased ($p<0.05$). Similarly, the activities of GSH-Px, GR and GST ($p<0.02$), (Figure 3), as well as concentration of GSH ($p<0.02$), (Figure 4) was significantly increased by Cd intoxication.

Figure 2: The activities of copper zinc containing superoxide dismutase (CuZn SOD) and catalase (CAT) in erythrocytes of rats exposed to cadmium (Cd) and vitamin E + cadmium (Vit E + Cd). The values are means ± SE from 6 animals in each experimental group.
Significantly different from C: ** $p<0.02$, **** $p<0.005$.
Significantly different from Cd: ^{b} $p<0.02$, ^{c} $p<0.01$.

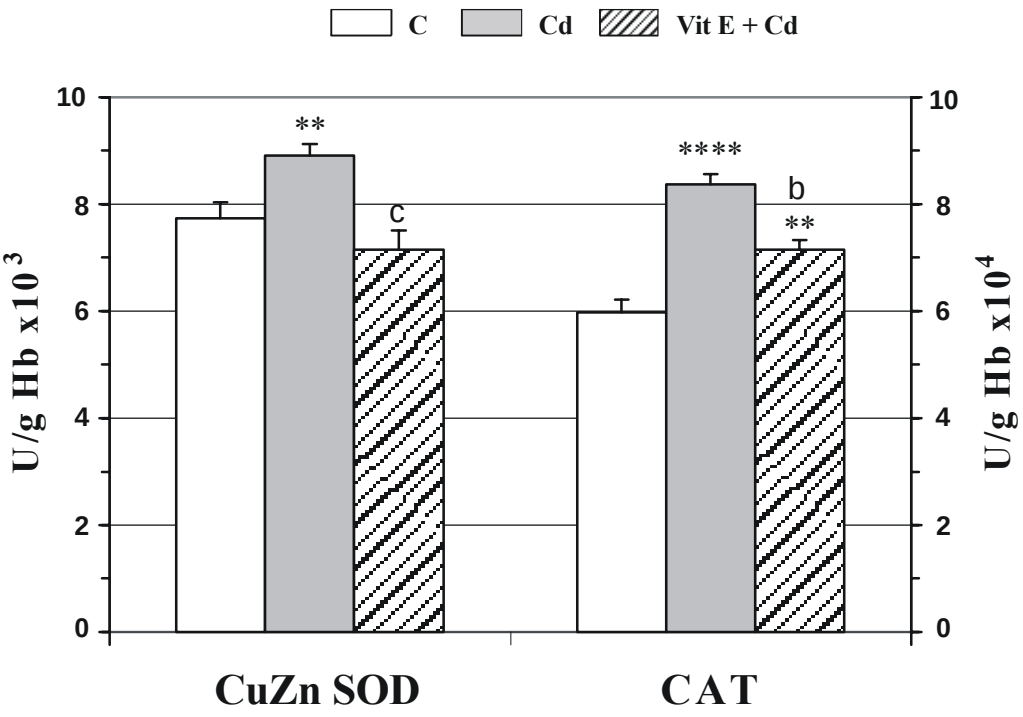
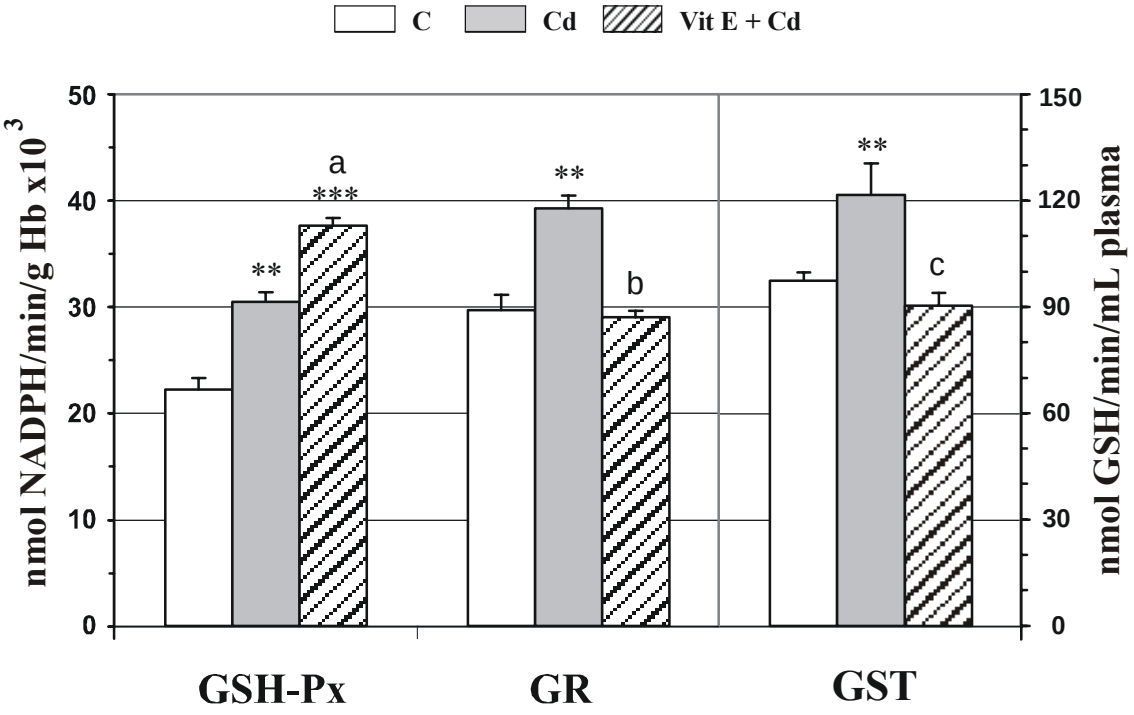


Figure 3: The activities of glutathione peroxidase (GSH-Px), glutathione reductase (GR) in erythrocytes and glutathione-S-transferase (GST) in the plasma of rats exposed to cadmium (Cd) and vitamin E + cadmium (Vit E + Cd). The values are means ± SE from 6 animals in each experimental group.

Significantly different from C: ** p<0.02, ***p<0.01.
Significantly different from Cd: [a] p<0.05; [b] p<0.02, [c] p<0.01.



It is known that Cd induces formation of superoxide anion radicals in the erythrocytes and it is reasonable to expect as a biological response an increased activity of CuZn SOD in RBC of Cd-treated animals [6, 8, 9]. Cd-induced increase of CAT and GSH-Px activities can be explained by their action on hydrogen peroxide as substrate, which is formed in the process of superoxide anion radicals dismutation [9, 22, 60]. This action is followed by increased reduction of oxidized glutathione (GSSG) by GR to form GSH [6, 18, 54, 55, 61, 62]. GST has an important role in detoxification of lipid hydroperoxides contributing thus to the protection of the cell integrity [63, 64].

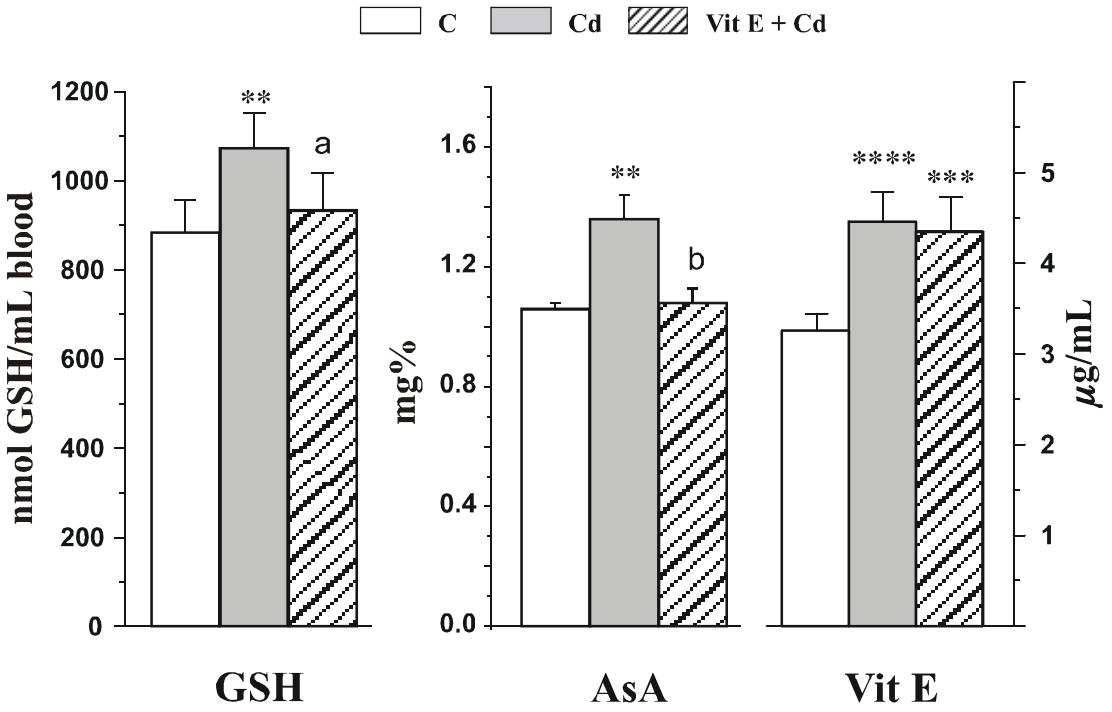
Pretreatment with Vit E prior to Cd administration decreased the activities of SOD, CAT and GR activities in RBC, as well as GST activity in the plasma, remaining at a level similar to the control values and thus Vit E eliminates the toxic effects of Cd on the activities of these enzymes. Vit E is an important factor in the system for protecting tissue against oxidative damage and the toxic action of Cd. These data are similar with the findings of other investigators [9, 15, 22, 60, 65].

The erythrocyte GSH-Px activity was markedly increased after the concomitant treatment with Vit E+Cd in respect to the rats exposed to Cd only. The Vit E treatments before Cd administration helped to maintain the erythrocyte GSH concentration [9, 53, 54, 66] and stimulate GSH-Px activity [67]. Singhal et al. [55] have shown that intracellular GSH functions in protection against Cd toxicity, and that this tripeptide provides a first line of defense against Cd before induction of metallothionein

synthesis. Our results confirmed the results of other investigators [6, 12, 18, 61] that Cd produced dose- and time- dependent increases in intracellular GSH concentration and the activity of GSH-Px (GSH-Px utilize GSH as a substrate to inactivate hydrogen peroxide and free oxygen radicals).

In our experiments concentrations of AsA ($p<0.05$) and Vit E ($p<0.01$) in the plasma were significantly increased in Cd-treated rats (Figure 4). Pretreatment with Vit E prior Cd administration reversed concentrations of GSH and AsA to control values, while Vit E level is the same as values given for the Cd treatment.

Figure 4: The concentrations of glutathione (GSH) in whole blood and ascorbic acid (AsA) and vitamin E (Vit E) in the plasma of rats exposed to cadmium (Cd) and vitamin E + cadmium (Vit E + Cd). The values are means ± SE from 6 animals in each experimental group.
Significantly different from C: ** $p<0.02$, ***$p<0.01$, **** $p<0.005$.
Significantly different from Cd: [a] $p<0.05$, [b] $p<0.02$.



Our previous investigations showed that chronic treatment with Cd induced decrease of AsA concentration in the liver and kidneys of young and adult rats [17, 18, 65]. However, Cd increases the concentration of Vit E in the rat liver [17, 65], kidneys [18] and in the plasma [6, 65]. It is known that increased accumulation of Cd in the liver induces LP and increases the production of malondialdehyde, which consequently inhibits the enzyme L-gulonolactone oxidase [14, 65] necessary for the synthesis of AsA. It has been shown that AsA deficiency results in intracellular oxidative damage in the guinea-pig [14, 68, 69]. In comparison to the studies of chronic treatment, our acute treatment with Cd show that increase of AsA and Vit E concentrations may be due to a defense response of the organism to oxidant injuries caused by Cd. AsA decreases endogenous lipid

peroxidation, decreases protein oxidative damage, protects the liver from oxidative damage, reduced erythrocyte membrane nitroxide radicals [29] and have protective effect on anaemia and have an important role in process of regeneration of reduced form of Vit E [31, 57, 70]. At the same time, Vit E is an important liposoluble antioxidant [22, 24, 25] that stabilizes the cell membranes maintaining its permeability and integrity [24-32].

Pretreatment with Vit E prior to Cd intoxication decreased the concentration of AsA in comparison to the results obtained in Cd-treated animals (Figure 4). On the other hand, plasma Vit E concentration did not significantly alter. Similar results were obtained in our previous investigations [65]. Vit E is a lipide soluble antioxidant that functions as an intramembraneous scavenger of oxygen radicals, thereby preventing the lipid peroxidation of polyunsaturated fatty acids [22-32]. In previous investigations it was shown that plasma Vit E was in dynamic equilibrium with Vit E in the erythrocyte membranes, maintaining thus the stability and permeability of these membranes [28-54]. In addition, AsA may have an important role in the process of regeneration of reduced form of Vit E [31, 57, 70, 71]. The increased concentration of Vit E in the plasma could be explained by its protective role against the toxic effects of Cd on the erythrocyte membranes.

CONCLUSIONS

The results of this study show that Cd induced oxidative damage in erythrocytes causes appearance of anaemia, loss of membrane function by enhantion of lipid peroxide concentration, as well as alteration of AOS (CuZnSOD, CAT, GSH-Px, GR, GST, GSH, AsA and Vit E).

The pretreatment of rats with Vit E prior to Cd intoxication showed any protective or preventive effects against influence of Cd on antioxidant defense system and lipid peroxide concentration.

ACKNOWLEDGEMENTS

This research was supported by the Ministry of Science and Technology of Serbia, Yugoslavia, Grant Nos. 03E18 and 03E23.

References

[1.] Shukla G.S. and Singhal R.L., *The present status of biological effects of toxic metals in the environment: lead, cadmium and manganese,* Can. J. Physiol. Pharmacol. 62 (1984), 1015-1031.

[2.] Stohs S.J. and Bagchi D., *Oxidative mechanisms in the toxicity of metal ions,* Free Rad. Biol. Med. 18 (1995), 321-336.

[3.] Swiergosz R., Zakozenska M., Sawicka-Kapusta K., Bacia K. and Jankovska L., *Accumulation of cadmium and its effect on bank whole tissues after chronic exposure,* Ecotoxicol. Environ. Saf. 41 (1998), 130-136.

[4.] Bauman J.W., Liu J. and Klaassen C.D., *Production of metallothionein and heat-shock proteins in response to metals,* Fund. Appl. Toxicol. 21 (1993), 15-22.

[5.] Wormser U. and Ben Zakine S.*, Cadmium-induced metallothionein synthesis in the rat liver slice system,* Toxic. In Vitro 4 (1990), 791-794.

[6.] Kostić M.M., Ognjanović B., Dimitrijević S., Žikić R.V., Štajn A.Š., Rosić G.L. and Živković R.V., *Cadmium-induced changes of antioxidant and metabolic status in red blood cells of rats: in vivo effects,* Eur. J. Haematol. 51 (1993), 86-92.

[7.] Hamada T., Tanimoto A., Arima N., Ide Y., Sasaguri T., Shimayiri S. and Sasaguri Y., *Pathological Study of Splenomegaly Associated with Cadmium-Induced Anemia in Rats,* Sangyo Ika Daigaku Zasshi 20 (1998), 11-19.

[8.] Shukla G.S. and Chandra S.V., *Cadmium toxicity and bioantioxidants: status of vitamin E and ascorbic acid of selected organs in rat,* J. Appl. Toxicol. 9 (1989), 119-122.

[9.] Sarkar S., Yadav P. and Bhatnagar D., *Cadmium-induced lipid peroxidation and the antioxidant system in rat erythrocytes: the role of antioxidants,* J. Trace Elem. Med. Biol. 11 (1997), 8-13.

[10.] Amoruso M.A., Witz G. and Goldstein B.D., *Enhancement of rat and human phagocyte superoxide anion radical production by cadmium in vitro,* Toxicol. Lett. 10 (1982), 133-138.

[11.] Zhong Z., Troll W., Koenig K.L. and Frenkel K., *Carcinogenic sulfide salts of nickel and cadmium induce H_2O_2 formation by human polymorphonuclear leukocytes*, Cancer Res. 20 (1990), 7564-7570.

[12.] Ochi T., Otsuka F., Takahashi K. and Oshawa M., *Glutathione and metallothioneins as cellular defense against cadmium toxicity in culture chinese hamster cells,* Chem. Biol. Interact. 65 (1988), 1-14.

[13.] Manca D., Ricard A.C., Trottier B. and Chevalier G., *Studies on lipid peroxidation in rat tissues following administration of low and moderate dose of cadmium chloride,* Toxicology 67 (1991), 303-323.

[14.] Hudecova A. and Ginter E., *The influence of ascorbic acid on lipid peroxidation in guinea pigs intoxicated with cadmiumm,* Food Chem. Toxicol. 30 (1992), 1011-1013.

[15.] Casalino E., Sblano C. and Landriscina C., *Enzyme activity alteration by cadmium administration to rats: the possibility of iron involvement in lipid peroxidation,* Arch. Biochem. Biophys. 346 (1997), 171-179.

[16.] Kostić M.M., Ognjanović B., Žikić R.V., Štajn A.Š. and Rosić G.L., *Effects of cadmium on antioxidant enzymes, glutathione and lipid peroxidation in brown adipose tissue,* Iugoslav. Physiol. Pharmacol. Acta 29 (1993), 137-145.

[17.] Ognjanović B., Žikić R.V., Štajn A., Saičić Z.S., Kostić M.M. and Petrović V.M., *The effects of selenium on the antioxidant defense system in the liver of rats exposed to cadmium,* Physiol. Res. 44 (1995), 293-300.

[18.] Štajn A., Žikić R.V., Ognjanović B., Pavlović Z.S., Kostić} M.M. and Petrović V.M., *Effect of cadmium and selenium on the antioxidant defense system in rat kidneys,* Comp. Biochem. Physiol. 117C (1997), 167-172.

[19.] Žikić R.V., Štajn A.Š., Ognjanović B.I., Saičić Z.S., Kostić M.M., Pavlović Z.S. and Petrović V.M., *The effect of cadmium and selenium on the antioxidant enzyme activities in rat heart,* J. Environm. Pathol. Toxicol. Oncol. 17 (1998), 259-264.

[20.] Pavlović Z.S., Ognjanović B.I., Štajn A.Š., Žikić R.V., Saičić Z.S. and Petrović V.M., *The effects of cadmium and coenzyme Q_{10} on antioxidant enzyme activities in the blood of rats,* Current Topics of Biophysics, 24 (2000), 159-165.

[21.] Sarkar S., Yadav P. and Bhatnager D., *Lipid peroxidative damage on cadmium exposure and alterations in antioxidant system in rat erythrocytes: a study with relation to time,* Biometals 11 (1998), 153-157.

[22.] Shaikh Z.A., Vu T.T. and Zaman K., *Oxidative Stress as a Mechanism of Chronic Cadmium-Induced Hepatotoxicity and Renal Toxicity and Protection by Antioxidants,* Toxicol. Appl. Pharmacol. 154 (1999), 256-263.

[23.] Fariss M.W., *Cadmium toxicity: Unique cytoprotective properties of alpha tocopheryl succinate in hepatocytes,* Toxicology 69 (1991), 63-77.

[24.] Chow C.K., *Vitamin E in plasma, erythrocytes and erythrocyte membranes,* Biol. Synth. Memb. (1989), 445-451.

[25.] Björneboe A., Björneboe G.E.A. and Drevon C.A., *Absorption, Transport and Distribution of Vitamin E,* J. Nutr. 120 (1990), 233-242.

[26.] Ernster L., Fosmark P. and Nordenbrand K., *The mode of action of lipid-soluble antioxidants in biological membranes. Relationship between the effects of ubiquinol and vitamin E as inhibitors of lipid peroxidation in submitochondrial particles,* J. Nutr. Sci. Vitaminol. Spec. No. (1992), 548-551.

[27.] Zamora R., Hidalgo F.J. and Tappel A.L., *Comparative antioxidant effectiveness of dietary beta-carotene, vitamin E, selenium and coenzyme Q_{10} in rat erythrocytes and plasma,* J. Nutr. 121 (1991), 50-56.

[28.] Kumar C.T., Reddy V.K., Prased M. Thyagaraju K. and Reddanna P., *Dietary supplementation of vitamin E protects heart tissue from exercise-induced oxidant stress,* Mol. Cell Biochem. 111 (1992), 109-115.

[29.] Beyer R.E., *The role of ascorbate in antioxidant protection of biomolecules: Interaction with vitamin E and coenzyme Q,* J. Bioenerg. Biomemb. 26 (1994), 349-358.

[30.] Galleano M. and Puntarulo S., *Role of antioxidants on the erythrocytes resistance to lipid peroxidation after acute iron overload in rats,* Biochim. Biophys. Acta 1271 (1995), 321-326.

[31.] Niki E., Noguchi N., Tsuchihashi H. and Gotoh N., *Interaction among vitamin C, vitamin E, and beta-carotene,* Am. J. Clin. Nutr. 62 (1995), 1322S-1326S.

[32.] Shi H., Noguchi N. and Niki E., *Comparative study on dynamics of antioxidative action of α-tocopheryl hydroquinone, ubiquinol, and α-tocopherol against lipid peroxidation,* Free Rad. Biol. Med. 27 (1999), 334-346.

[33.] Chanarin I., *Laboratory haematology. An Account of Laboratory Techniques,* Hong Kong, Churchill Livingstone (1989).

[34.] Drabkin D. and Austin H., *Spectrophotometric studies preparations from washed blood cells,* J. Biol. Chem. 112 (1935), 51-55.

[35.] Ohkawa H., Okishi N. and Yogi K., *Assay for lipid peroxides in Animal Tissues by Thiobarbituric Acid Reaction,* Anal. Biochem. 95 (1979), 351-358.

[36.] Beutler E. *Glutathione. In: "Red Cell Metabolism, a Mannual of Biochemical Methods",* ed. Beutler E., Grune and Stratton, New York, (1975) pp. 112-114.

[37.] Day B.R., Williams D.R. and Marsh C.A., *A rapid manual method for routine assay of ascorbic acid in serum and plasma,* Biochem. 12 (1979), 22-26.

[38.] Desai I.D., *Vitamin E Analysis Methods for Animal Tussues. Methods in Enzymology* 105 (1984), 138-147.

[39.] Habig W.H., Pubst M.J. and Jakoby W.B., *Glutathione S-transferase,* J. Biol. Chem. 249 (1974), 7130-7139.

[40.] McCord J.M. and Fridovich I., *Superoxide dismutase and enzymic function for erythrocupreine (hemocupreine),* J. Biol. Chem. 244 (1969), 6049-6056.

[41.] Misra H.P. and Fridovich I., *The role of superoxide anion in the autooxidation of epinephrine and simple assay for superoxide dismutase,* J. Biol. Chem. 247 (1972), 3170-3175.

[42.] Beutler E., *Catalase. In: "Red Cell Metabolism, a Mannual of Biochemical Methods",* ed. Beutler E., Grune and Stratton, New York, (1982), pp.105-106.

[43.] Gunzler W.A., Vergin H., Muller I. and Flohe L., *Glutathione peroxidase. VI, die reaction der glutathione-peroxidase mit verschiedenen hydroperoxiden. Hoppe-Seylers,* Z. Physiol. Chem. 353 (1972), 1001-1004.

[44.] Glatzle D., Vulliemuier J.P., Weber F. and Decker K., *Glutathione reductase test with whole blood, a convenient procedure for the assesment of the riboflavin status in humans,* Experientia (1974), 665-668.

[45.] Hoel P.G., *Introduction to mathematical statistics,* John Wiley, New York (1966), p.p. 402-403.

[46.] Kunimoto M., Miysaka K. and Miura T., *Changes in membrane properties of rat red blood cells induced by cadmium accumulating in the membrane fraction,* J. Biochem. Tokyo 99 (1986), 397-406.

[47.] Petronijević M.R., Maletić S.D., Žikić R.V. and Kostić M.M., *Glycolysis and oxidative pentose phosphate pathway in red blood cells of rats chronically intoxicated with cadmium,* Coll. Sci. Pap. Fac. Sci. Krag. 17 (1995), 191-201.

[48.] Žikić R.V., Štajn A.Š., Ognjanović B.I., Pavlović S.Z. and Saičić Z.S., *Activities of superoxide dismutase and catalase in Erythocytes and Transaminases in the Plasma of Carps (Cyprinus carpio L.) exposed to Cadmium,* Physiol. Res. 46 (1997), 391-396.

[49.] Prigge E.P., Baumert H.P. and Muhle H., *Effects of dietary and inhalative cadmium on hemoglobin and hematocrite in rats,* Bull. Environ. Contam. Toxicol. 17 (1977), 585-590.

[50.] Kunimoto M. and Miura T., *Density increment and decreased survival of rat red blood cells induced by cadmium,* Environ. Res. 39 (1986), 86-95.

[51.] Hamada T., Tanimoto A., Arima N., Ide Y., Sasaguri T., Shimayiri S. and Sasaguri Y., *Altered membrane skeletion of red blood cells participates in cadmium-induced anemia,* Biochem. Mol. Biol. Int. 45 (1988), 841-847.

[52.] Skoczynsska A. and Smolik R., *The effect of combined exposure to lead and cadmium on serum lipids and lipid peroxides levels in rats,* Int. J. Occup. Med. Environ. Health 7 (1994), 263-271.

[53.] Shukla A., Shukla G.S. and Srimal R.C., *Cadmium-induced alterations in blood-brain barrier permeability and its possible correlation with decreased microvessel antioxidant potential in rat,* Hum. Exp. Toxicol. 15 (1996), 400-405.

[54.] Rana S.V.S. and Verma S., *Protective effects of GSH, vitamin E, and selenium on lipid peroxidation in cadmium-fed rats,* Biol. Trace. Elem. Res. 51 (1996), 161-168.

[55.] Singhal R.L., Anderson M.E. and Meister A., *Glutathione, a first line of defense against cadmium toxicity,* FASEB J. 1 (1987), 220-223.

[56.] Scaccini C., Nurdini M., D'Aquino M., Gentili V., Di Felice M. and Tomassi G., *Effect of dietary oils on lipid peroxidation and on antioxidant parameters of rat plasma and lipoprotein fractions,* J. Lipid. Res. 33 (1992), 627-633.

[57.] Buettner G.R., *The pecking order of free radicals and antioxidants: lipid peroxidation, alpha-tocopherol and ascorbate,* Arch. Biochem. Biophys. 300 (1993), 535-543.

[58.] Chudinova V.V., Alekseev S.M., Zakharova E.I. and Evstigneeva R.P., *Lipid peroxidation and mechanism of antioxidant effect of vitamin E,* Bioorg. Khim. 20 (1994), 1029-1046.

[59.] Tsuchihashi H., Kigoshi M., Iwatsuki M. and Niki E., *Action of beta-carotene as an antioxidant against lipid peroxidation,* Arch. Biochem. Biophys. 323 (1995), 137-147.

[60.] Krajcovicova-Kudlackova M. and Ozdin L., *Effect of fatty acid composition, cadmium and vitamin E intake on prooxidative-antioxidative state of rat liver,* Vet. Med. 40 (1995), 293-298.

[61.] Munday R. and Winterbourn C.C., *Reduced glutathione in combination with superoxide dismutase as an important biological antioxidant defence mechanism,* Biochem. Pharmacol. 38 (1989), 4349-4352.

[62.] Mullineaux P.M. and Creissen G.P., *Glutathione Reductase: Regulation and Role in Oxidative Stress. Oxidative Stress and the Molecular Biology of Antioxidant Defenses* (1997), 667-713.

[63.] Grose E.C., Richards J.H., Jaskot R.H., Menache M.G., Graham J.A. and Dauterman W.C., *Glutathione peroxidase and glutathione transferase activity in rat lung and liver following cadmium,* Toxicology 44 (1987), 171-179.

[64.] Pickett C.B. and Lu A.Y.H., *Glutathione S-transferases: gene structure, regulation, and biological function,* Annu. Rev. Biochem. 58 (1989), 743-764.

[65.] Žikić R.V., Štajn A., Ognjanović B., Pavlović S.Z. and Kostić M.M., *The effects of cadmium and selenium on the ascorbic acid and Vitamin E contents in the plasma and liver of young and adult rats,* Coll. Sci. Pap. Fac. Sci. Krag. 17 (1995), 203-213.

[66.] Chan H.M. and Cherian M.G., *Protective role of metallothionein and glutathione in hepatotoxicity of cadmium,* Toxicology 72 (1992), 281-290.

[67.] Gaetani G.F., Galiano S., Canepa L., Ferraris A.M. and Kirkman H.N., *Catalase and glutathione peroxidase are equally active in detoxication of hydrogen peroxide in human erythrocytes,* Blood 73 (1989), 334-339.

[68.] Nagyova A., Galbavy S. and Ginter E., *Histopathological evidence of Vitamin C protection against Cd-nephrotoxicity in guinea pigs,* Exp. Toxicol. Pathol. 46 (1994), 11-14.

[69.] Tatara M. and Ginter E., *Erythrocyte membrane fluidity and tissue lipid peroxides in female guinea-pigs on graded vitamin C intake,* Physiol. Res. 43 (1994), 101-105.

[70.] Chen H. and Tappel A.L., *Protection by Vitamin E, selenium, trolox c, ascorbic acid, palmitate, acetylcysteine, coenzyme Q, β-carotene, canthaxantine, and (+)-catechin against oxidative damage to liver slices measured by oxidized heme proteins,* Free Rad. Biol. Med. 16 (1994), 437-444.

[71.] Tanaka K., Hashimoto T., Tokumara S., Iguchi H. and Kojo S., *Interactions between Vitamin C and Vitamin E are observed in tissues of inherently scorbutic rats,* J. Nutr. 127 (1997), 2060-2064.