Protective Influence of Vitamin E on Antioxidant Defense System in the Blood of Rats Treated with Cadmium


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Received July 11, 2002
Accepted November 11, 2002

Summary
The effects of acute exposure to cadmium (Cd) on the blood antioxidant defense system, lipid peroxide concentration and hematological parameters, as well as the possible protective role of vitamin E were studied. Male Wistar albino rats (3 months old) were treated with cadmium (0.4 mg Cd/kg b.m., i.p., 24 h before the experiment) or 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 experiment). The hematological parameters were assessed: red blood cell counts, hematocrit value and hemoglobin concentration were significantly decreased in the blood of Cd-treated rats. Intoxication with cadmium was also followed by significantly increased lipid peroxide concentrations. We also observed increased activity of antioxidant defense enzymes: copper zinc containing superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase as well as concentrations of non-enzymatic components of antioxidant defense system: reduced glutathione, vitamin C and vitamin E. Pretreatment with vitamin E exhibited a protective role on the toxic effects of cadmium on the hematological values, lipid peroxide concentration as well as on enzymatic and non-enzymatic components of antioxidant defense system.

Key words
Vitamin E • Cadmium • Blood • Lipid peroxides • Antioxidant defense system

Introduction
Cadmium (Cd) is a very toxic heavy metal and an important environmental pollutant which is present in the soil, water, air, food and in cigarette smoke. Cadmium causes poisoning in various tissues of humans and animals (Swiergosz et al. 1998, Stohs et al. 2000). After the intake and resorption Cd enters the blood where it binds to the erythrocyte membranes and plasma albumin (Bauman et al. 1993). In the blood and tissues, Cd stimulates the formation of metallothioneins (Simpkins et al. 1998) and reactive oxygen species (ROS) thus causing oxidative damage in erythrocytes and in various tissues, which results in a loss of membrane functions (Sarkar et al. 1995). Cadmium also induces the onset of anemia, decreases the red blood cell count, hemoglobin concentration and hematocrit value as well as reduced blood iron levels (Kostić et al. 1993a). Moreover, a
variety of accompanying changes in antioxidant defense enzymes were reported (Shukla and Chandra 1989, Kostić et al. 1993b, Ognjanović et al. 1995, Štajn et al. 1997, Žikić et al. 1998). Fariss (1991) has shown that free radical scavengers and antioxidants are useful in protecting against Cd toxicity.

Vitamin E (Vit E) is the primary liposoluble antioxidant, which may have an important role in scavenging free oxygen radicals and in stabilizing the cell membranes, thus maintaining its permeability (Bjørneboe et al. 1990, Navarro et al. 1999). Vitamin E may also affect oxidative changes which occur in other cell organelles (Ibrahim et al. 2000). Moreover, it is known that antioxidants, such as Vit E, coenzyme Q, vitamin C (Vit C), glutathione (GSH) and selenium may act synergically, preventing lipid peroxidation and cell destruction (Beyer 1994, Chen and Tappel 1995, Navarro et al. 1999, Lass and Sohal 2000).

The aim of our study was to investigate a possible protective influence of Vit E pretreatment on some hematological parameters, antioxidant defense system (AOS) and lipid peroxide (LP) concentrations in the blood of rats acutely treated with cadmium. The following parameters were determined: red blood cells count (RBCs), hematocrit value (Ht), hemoglobin (Hb) and LP concentrations, RBCs copper zinc containing organelles (CuZn SOD) and selenium may act against Cd toxicity.

Blood for the determination of antioxidant status was centrifuged to separate plasma and RBCs. Plasma specimens were used for determination of Vit C by the method of Day et al. (1979), while Vit E was determined by the method suggested by Desai (1984). GST activity towards 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate was measured according to Habig et al. (1974).

Isolated RBCs were washed three times with 3 volumes of ice-cold 155 mmol/l NaCl and hemolysates containing about 50 g Hb/l (prepared according to McCord and Fridovich 1969) were used for the determination of CAT, GSH-Px and GR activities. For the determination of CuZn SOD activity by the epinephrine method of Misra and Fridovich (1972), 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 (Tsuchihashi 1923).

CAT activity was determined according to Beutler (1982), while the activity of GSH-Px was assayed by the subsequent oxidation of NADPH at 340 nm with t-buthyl-hydroperoxide as a substrate (Maral et al. 1977). The activity of GR was determined by measuring NADPH oxidation at 340 nm in the presence of oxidized glutathione (Glatzle et al. 1974).

Data are given as means ± S.E.M. All obtained results were compared with respect to the control animals (C), as well as to the animals treated with cadmium (Cd) in order to elucidate a possible protective role of Vit E pretreatment on cadmium toxicity. Data were analyzed using the non-parametric Mann-Whitney two-tailed test and differences at p<0.05 were considered as significant.

### Methods

In our experiments, male 3-month-old Wistar albino rats weighing 280 ± 30 g were used. The animals were kept at 21±1 °C and exposed to a 12 h light - 12 h dark cycle. The animals received an injection of Cd (0.4 mg Cd/kg b.m., i.p., 24 h before the experiment) or of 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 experiment). All rats were housed in individual cages and given a standard diet and water ad libitum. Each experimental group consisted of 7 animals.

After the treatment, the animals were sacrificed by decapitation always between 8:00 and 10:00 and fresh blood was immediately collected into heparinized test tubes. RBCs count and Ht value were determined by standard hematological techniques (Chanarin 1989). The Hb concentration was determined by the cyanmethemoglobin method (Drabkin and Austin 1935).

The concentration of lipid peroxide (LP) was assayed as thiobarbituric acid reactive substances (TBARS) in the blood according to Ohkawa et al. (1979). Concentration of GSH in whole blood was measured by a standard method according to Beutler (1975).

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### Results

Table 1 clearly show that intraperitoneal cadmium administration resulted in significant decreases of RBCs count, Ht value and Hb concentration (p<0.05) when compared to control animals. Pretreatment with Vit E diminished the negative effects of cadmium indicating that Vit E prevents anemia caused by cadmium.

Lipid peroxide concentration was significantly increased in the blood of rats after acute administration of Cd (p<0.05), while Vit E pretreatment reversed this change to control values (Fig. 1).
Table 1. Red blood cells (RBC) count, hematocrit (Ht) value and hemoglobin (Hb) concentration in the blood of control rats (C), treated with cadmium (Cd) and treated with vitamin E and cadmium (Vit E + Cd).

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>Cd</th>
<th>Vit E + Cd</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (10^12/l)</td>
<td>7.91 ± 0.21</td>
<td>5.11 ± 0.09 *</td>
<td>6.51 ± 0.25 *#</td>
</tr>
<tr>
<td>Ht (l/l)</td>
<td>0.45 ± 0.02</td>
<td>0.42 ± 0.02 *</td>
<td>0.45 ± 0.03 #</td>
</tr>
<tr>
<td>Hb (mmol/l)</td>
<td>8.35 ± 0.12</td>
<td>7.57 ± 0.10 *</td>
<td>8.06 ± 0.14 *#</td>
</tr>
</tbody>
</table>

Means ± S.E.M. from 7 animals in each group. Significantly different from controls: * p<0.05. Significantly different from Cd: # p<0.05.

The data presented in Figures 2 and 3 shows significant changes in the activities of AOS enzymes during the treatment of rats with Cd and Vit E. In animals exposed to Cd the activities of CuZn SOD and CAT (p<0.05) (Fig. 2), as well as the activities of GSH-Px, GR and GST (p<0.05) (Fig. 3) were significantly increased in comparison to controls. The pretreatment with Vit E prior to Cd intoxication partially reversed these changes. The activities of CuZn SOD, CAT, GR and GST (p<0.05) were significantly decreased as compared with animals given Cd alone.

The results of our experiments show that the concentrations of GSH in the whole blood as well as Vit C and Vit E levels in the plasma were significantly increased (p<0.05) in Cd-treated rats in comparison to the control animals (Fig. 4). Pretreatment with Vit E reversed the concentrations of GSH and Vit C to the control levels.

Discussion

Our previous investigations showed that chronic treatment with cadmium induced oxidative damage in erythrocytes of rats and goldfish, causing destruction of cell membranes and increased lipid peroxidation, as well as alteration of the AOS, energy metabolism and the appearance of anemia (Kostić et al. 1993a, Žikić et al. 1997, Ognjanović et al. 2000, Pavlović et al. 2001, Žikić et al. 2001).

The results obtained in our present study show that treatment with Cd induces anemia (decrease of RBCs...
count, Ht value and Hb concentration) in rats (Table 1). It is well known that the presence of Cd in the organism decreases the level of iron in the blood (Kostić et al. 1993a) and causes the decrease of Hb concentration. The decrease of Ht value in hemolyzed plasma of rats exposed to Cd indicates the increased destruction of erythrocytes (Shukla et al. 1996, Hamada et al. 1998).

Vitamin E pretreatment decreased the toxic effects of Cd on the hematological values and has a protective role in anemia (Table 1). The data of other authors showed that Cd caused the damage of the erythrocyte membrane resulting in hemolysis. Some antioxidants can exert a protective role against Cd-induced destruction of RBCs (Shaikh et al. 1999).

Treatment with cadmium (Fig. 1) increased LP concentration in the blood of rats which was accompanied by increased formation of ROS (Ochi et al. 1988, Shi et al. 1999). As a consequence, enhanced lipid peroxidation, DNA damage, altered calcium and sulfhydryl homeostasis as well as marked disturbances of antioxidant defense system occurred (Skoczynska and Smolik 1994, Sarkar et al. 1998, Stohs et al. 2000). Pretreatment with Vit E was very effective in the prevention of oxidative damage induced by Cd which resulted in significantly lower LP concentration (Fig. 1).

Fig. 3. Glutathione peroxidase (GSH-Px) and glutathione reductase (GR) activities in red blood cells and glutathione-S-transferase (GST) in the plasma of control rats (C), treated with cadmium (Cd) and treated with vitamin E and Cd (Vit E + Cd). Means ± S.E.M. from 7 animals in each group. Significantly different from controls: *p<0.05. Significantly different from Cd: #p<0.05.

Fig. 4. Concentrations of reduced glutathione (GSH) in whole blood and vitamin C (Vit C) and vitamin E (Vit E) in the plasma of control rats (C), treated with cadmium (Cd) and treated with vitamin E and Cd (Vit E + Cd). Means ± S.E.M. from 7 animals in each group. Significantly different from controls: *p<0.05. Significantly different from Cd: #p<0.05.
In animals exposed to Cd, the activities of CuZn SOD, CAT, GSH-Px, GR and GST as well as concentration of GSH were significantly increased (Figs. 2, 3 and 4). It is known that Cd induces the formation of superoxide anion radicals in erythrocytes and it is reasonable to expect an increased activity of SOD (Kostić et al. 1993a, Sarkar et al. 1998). 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 (Shaikh et al. 1999). This action is followed by increased reduction of oxidized glutathione by glutathione reductase to form GSH (Mates 2000, Štajn et al. 1997). The GST enzyme has an important role in detoxification of the lipid hydroperoxides thus contributing to the protection of the cell integrity (Grose et al. 1987, Ognjanović et al. 1995).

The pretreatment with Vit E prior to Cd administration decreased erythrocyte CuZn SOD, CAT and GR activities indicating that Vit E eliminates the toxic effects of Cd on the activity of these enzymes. At the same time, the activity of plasma GST and blood GSH concentration remain at the level of control values which confirm the protective role of Vit E. The erythrocyte GSH-Px activity was markedly increased in Vit E+Cd treated animals. The antioxidant treatment before Cd administration helped to maintain the erythrocyte GSH content (Singhal et al. 1987, Rana and Verma 1996).

As is shown in Figure 4, a significantly increased Vit C and Vit E concentrations in the plasma of rats after acute Cd treatment were observed. Our previous investigations showed that chronic treatment with Cd induces a decrease of Vit C concentration in the liver (Ognjanović et al. 1995, Žikić et al. 1995) and kidneys (Štajn et al. 1997) of young and adult rats, while Cd increases the concentration of Vit E in the rat liver (Ognjanović et al. 1995, Žikić et al. 1995), kidneys (Štajn et al. 1997) and plasma (Kostić et al. 1993a, Žikić et al. 1995, Pavlović et al. 2001).

Vitamin C is a potent scavenger of free oxygen radicals and it has been shown that marginal Vit C deficiency results in intracellular oxidative damage in the guinea-pig (Hudécová and Ginter 1992, Nagyová et al. 1994, Tatara and Ginter 1994). In comparison to the chronic exposure, the acute treatment demonstrated that increased concentrations of Vit C and Vit E may be due to a defense response of the organism to oxidant injuries caused by Cd.

Our results showed that pretreatment with vitamin E prior to Cd intoxication decreased the concentration of Vit C as compared to Cd-treated animals (Fig. 4). Increased concentration of Vit E in the plasma of Vit E+Cd treated animals could explain the protective role of Vit E on Cd-induced oxidative stress. In addition, Vit C may have an important role in the regeneration of reduced form of Vit E (Beyer 1994, Chen and Tappel 1995, Tanaka et al. 1997).

It can be concluded from presented results that cadmium induced oxidative damage in erythrocytes leads to anemia, loss of membrane function by enhancing of LP concentration as well as alteration of the activity of AOS enzymes: CuZn SOD, CAT, GSH-Px, GR and GST and concentrations of GSH, Vit C and Vit E. Our results show that vitamin E expressed protective role against toxic influence of cadmium on all examined parameters in rat blood.

Acknowledgements
This research was supported by the Ministry of Science, Technologies and Development of Serbia, Serbia, Yugoslavia, Grant No 1669.

References


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Reprint requests
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