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ANTIOXIDANT EFFECT OF COENZYME Q_{10} IN BLOOD FROM CADMIUM-EXPOSED RATS

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The effects of acute exposure to cadmium (Cd) on the blood antioxidant defense system (AOS), lipid peroxide (LP) concentration and hematological parameters, and the possible protective role of coenzyme Q_{10} (CoQ_{10}) was studied. Male *Wistar albino* rats 3 months old were treated with cadmium as $CdCl_2$ (0,4mg Cd/kg b.m., i.p., 24^h before the sacrificing) or with coezyme Q_{10} + Cd (20mg CoQ_{10}/kg b.m., i.m., 48^h + 0,4 mg Cd/kg b.m., i.p., 24^h before the sacrificing). The hematological parameters: red blood cells count (RBCs), hemoglobin (Hb) concentration and hematocrite (Ht) value were significantly decreased in the blood of Cd treated rats. Intoxication with Cd was also followed by significantly increased of LP concentration. We also observed increased concentrations of non-enzymatic components of antioxidant defense

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system (AOS): reduced glutathione (GSH), vitamin C (Vit C) and vitamin E (Vit E). Pretreatment with CoQ_{10} exhibited a protective role on the toxic effects of Cd on the hematological values, LP concentration as well as on endogeneous antioxidant components.

Key words: Coenzyme Q_{10} - Cadmium - Blood - Rat

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 different organisms (STOHS and BAGCHI, 1995). After the intake and resorption, cadmium enters the blood were it binds to erythrocytes and proteins of low molecular mass forming metalothioneins (MT). Cadmium is then transported into most of tissues and organs in which it also induces the forming of metalothioneins (WORMSER and BEN ZAKINE, 1990). From totally accumulated cadmium in organism, about 75% is deposited in liver and kidneys (OGNJANOVIĆ *et al.*, 1995; ŠTAJN *et al.*, 1997). However, cadmium is accumulated in most of other tissues and organs, such as pancreas, salivary glands, testes, heart, brain or brown adipose tissue (KOSTIĆ *et al.*, 1993a; ŽIKIĆ *et al.*, 1998).

Binding of cadmium to erythrocytes causes their destruction and increased hemolysis, haematological values alterates (decrease of haematocrite values, haemoglobin concentration and total red blood cells count), absorption of intestinal iron is decreased and anemia appears (KOSTIĆ *et al.*, 1993b; OGNJANOVIĆ *et al.*, 2003). Above mentioned parameters can be taken as the sensitive indicators of cadmium toxicity. Moreover, a variety of accompanying changes in antioxidant defense enzymes were reported (SHUKLA and CHANDRA, 1989; KOSTIĆ *et al.*, 1993a; OGNJANOVIĆ *et al.*, 1995; ŠTAJN *et al.*, 1997; ŽIKIĆ *et al.*, 1998; OGNJANOVIĆ *et al.*, 2003). Studies by FARISS (1991) have shown that free radical scavengers and antioxidants are useful in protection against Cd toxicity.

Coenzyme Q_{10} (CoQ_{10}), which is also known as ubiquinone, is a lipid-soluble molecule and an integral part of most biomembranes. It is also a mobile constituent of mitochondrial respiratory chain (BEYER, 1994). CoQ_{10} also functions in its reduced form (ubiquinol) as an antioxidant, preventing the initiation and/or propagation of lipid peroxidation in biological membranes and in serum low-density lipoprotein (IBRAHIM *et al.*, 2000). CoQ_{10} can regenerate the active form of Vit E from Vit E radical and stabillize extracellular ascorbate into the organism (BEYER, 1994). However, CoQ_{10} also protects biological membranes, liposomes, LDL, proteins and DNA from oxidation caused by lipid peroxidation and protects organism from oxidative stress induced by various toxic agents (IBRAHIM *et al.*, 2000). Degenerative diseases and aging may be manifestations of a decreased capacity to maintain adequate ubiquinol levels.

The results of this study indicate that, (1) cadmium is capable of causing increases in parameters that are indicative of oxidative stress in blood following Cd exposure, (2) causing changes in concentration of endogeneous antioxidant

components (GSH, Vit C and Vit E), and (3) pretreatment with CoQ_{10} is capable of reversing these parameters.

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 Cd as a solution of $CdCl_2$ x $2H_2O$ in deionized water (0,4 mg Cd/kg b.m., i.p., 24^h before the sacrificing) or with coenzyme Q_{10} (CoQ_{10}) + Cd (20 mg CoQ_{10}/kg b.m., i.m., 48^h + 0,4 mg Cd/kg b.m., i.p., 24^h before the sacrificing). 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 and 10 A. M. 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 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 standard method of BEUTLER (1975).

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 described by DESAI (1984).

Data are given as mean ± SEM. All obtained results were compared in 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 CoQ_{10} pretreatment on Cd toxicity. Data were analyzed using the non-parametric Mann-Whitney two-tailed test and differences at $p<0.05$ were considered as significant.

RESULTS

Results presented in Table 1. clearly show that intraperitoneal administration of Cd results in significant decreases of RBCs count, Hb concentration and Ht value ($p<0.05$) when compared to control animals. Pretreatment with CoQ_{10} diminished the negative effects of Cd indicating that CoQ_{10} prevents anemia caused by Cd.

Lipid peroxide concentration was significantly increased in the blood of rats after acute administration of Cd ($p<0.05$), while CoQ_{10} pretreatment reversed this change to control values (Table 2).

The results of our experiments show that the concentrations GSH in the whole blood as well as Vit C and Vit E in the plasma were significantly increased ($p<0.05$) in Cd treated rats in respect to the controls animals (Table 3).

Pretreatment with CoQ_{10} reversed concentrations of GSH and Vit C to the control levels. CoQ_{10} pretreatment significantly increased only of Vit E concentrations ($p<0.05$) in comparison to both control and Cd-treated rats.

Table 1. - Hematological parameters (red blood cells count - RBC, hemoglobin - Hb and hematocrit - Htc) from whole blood in control and experimental groups

Groups	RBC (10^{12}/l)	Hb (mmol/l)	Htc (l/l)
Control	7.91 ± 0.21	8.35 ± 0.12	0.45 ± 0.03
Cd	5.11 ± 0.09 *	7.57 ± 0.10 *	0.42 ± 0.02 *
CoQ_{10} + Cd	7.02 ± 0.42 #	8.17 ± 0.07 #	0.44 ± 0.02 #

All values represent means ± S.D.
Groups have 6 to 8 animals.
* $p<0.05$, compared to the corresponding value of control group.
# $p<0.05$, compared to the corresponding value of Cd group.

Table 2. - Concentration of lipid peroxides (LP) from whole blood in control and experimental groups

Groups	LP (nmol/ml)
Control	1.21 ± 0.04
Cd	2.35 ± 0.09 *
CoQ_{10} + Cd	1.68 ± 0.09 * #

All values represent means ± S.D.
Groups have 6 to 8 animals.
* $p<0.05$, compared to the corresponding value of control group.
# $p<0.05$, compared to the corresponding value of Cd group.

Table 3. - Nonenzymic antioxidants in the blood of control and experimental groups

Groups	GSH (nmol/g Hb)	Vit C (mg%)	Vit E (mg/ml)
Control	65.82 ± 6.59	1.06 ± 0.09	3.25 ± 0.21
Cd	88.09 ± 7.52 *	1.36 ± 0.02 *	4.48 ± 0.27 *
CoQ_{10} + Cd	66.56 ± 4.38 #	1.15 ± 0.03 #	5.18 ± 0.46 * #

All values represent means ± S.D.
Groups have 6 to 8 animals.
* $p<0.05$, compared to the corresponding value of control group.
# $p<0.05$, compared to the corresponding value of Cd group.

DISCUSSION

Our previous investigations showed that chronic treatment with Cd induced oxidative damage in erythrocytes of rats and goldfish, causing destruction of cell membrane and increase lipid peroxidation, as well as alteration of the AOS, energy metabolism and the appearance of anemia (KOSTIĆ *et al.*, 1993b; ŽIKIĆ *et al.*, 1997; PAVLOVIĆ *et al.*, 2001; OGNJANOVIĆ *et al.*, 2003).

The results obtained in our 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 organism decreases the level of iron in blood (KOSTIĆ *et al.*, 1993b) and causes the decrease of Hb concentration. The decrease of Ht value in hemolysed plasma of rats exposed to Cd indicates the increased destruction of erythrocytes (HAMADA *et al.*, 1998; OGNJANOVIĆ *et al.*, 2003).

CoQ_{10} pretreatment decreased the toxic effects of Cd on the hematological values and has the protective role in anemia (Table 1). The data of other authors showed that Cd caused the damages of the erythrocyte membranes resulting in hemolysis. Some antioxidants can exert protective role against Cd induced destruction of RBCs (SHAIKH *et al.*, 1999; OGNJANOVIĆ *et al.*, 2003).

Treatment with Cd (Table 2.) increased LP concentration in the blood of rats which is acompanied with increased formation of ROS (SHI *et al.*, 1999; OGNJANOVIĆ *et al.*, 2003). As a consequence, enhanced lipid peroxidation, DNA damage, altered calcium and sulfhydryl homeostasis as well as marked disturbances of antioxidant defense system were occur (SARKAR *et al.*, 1997).

Pretreatment with CoQ_{10} was very effective in the prevention of oxidative damage induced by Cd which resulted in significantly lower LP concentration (Table 2).

The results of our experiments show that the concentrations GSH in the whole blood as well as Vit C and Vit E in the plasma were significantly increased ($p<0.05$) in Cd treated rats in respect to the controls animals (Table 3). Acute Cd-induced toxicity may be due to the exhaustion of GSH stores and the increase in oxidative stress (RANA and VERMA, 1996). Oxidative stress induced by acutic Cd administration was reduced by CoQ_{10} pretreatment. A variety of experiments have suggested that Cd causes oxidative damage to cells. GSH also has a high affinity for heavy metals and, as a result, constitutes the first line of defense against Cd toxicity (SINGHAL *et al.*, 1987). GSH is an antioxidant and can also form complexes with Cd to altre Cd distribution and excretion (RANA and VERMA, 1996). Several protective agents, including glutathione and metallothionein, as well as vitamin E, play an important role in detoxification of endogenous and exogenous compounds (CHEN and TAPPEL, 1995; OGNJANOVIĆ *et al.*, 2003).

Our previous investigations showed that chronic treatment with Cd induces decrease of Vit C concentration in the liver (OGNJANOVIĆ *et al.*, 1995) and kidneys (ŠTAJN *et al.*, 1997) of young and adult rats, while Cd increases the concentration of Vit E in rat liver (OGNJANOVIĆ *et al.*, 1995), kidneys (ŠTAJN *et al.*, 1997) and plasma (KOSTIĆ *et al.*, 1993b; PAVLOVIĆ *et al.*, 2001; OGNJANOVIĆ *et al.*, 2003).

Vit 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 (HUDECOVA and GINTER, 1992). In comparison to the chronic exposure our acute treatment shows that increased concentration 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 CoQ_{10} prior to Cd intoxication decreased concentration of GSH and Vit C, vs Cd treated animals (Table 3).

The increased concentration of Vit E in plasma of Cd intoxicated rats (Table 3) could be explained by its protective role against the toxic effects of Cd on the erythrocyte membrane. Vit E is a liposoluble antioxidant that functions as an intramembraneous scavenger of oxygen radicals, thereby preventing the lipid peroxidation of polyunsaturated fatty acids (HUDECOVA and GINTER, 1992; SHAIKH *et al.*, 1999; SHI *et al.*, 1999). Similarly, increased concentration of Vit E in the plasma of CoQ_{10} + Cd treated animals could explain the protective role of CoQ_{10} on Cd induced oxidative stress. In addition, CoQ_{10} and Vit C may have an important role in the regeneration of reduced form of Vit E (BEYER, 1994; CHEN and TAPPEL, 1995).

CONCLUSION

In 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 endogeneous antioxidant components (GSH, ascorbic acid and Vit E). Our results show that CoQ_{10} expossed protective role against toxic influence of Cd on all examined parameters in rat blood.

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Izvod

Cilj ovog rada je bio da se ispita uticaj kadmijuma (Cd) na antioksidacioni zaštitni sistem (AOS), koncentraciju lipidnih peroksida (LP) i hematološke parametre u krvi, kao i zaštitna uloga koenzima Q_{10} (CoQ_{10}). Mužjaci pacova *Wistar albino*, stari tri meseca, akutno su tretirani kadmijumom (0,4mg Cd/kg t.m., i.p., 24^h pre žrtvovanja) i koenzimom Q_{10} + Cd (20mg CoQ_{10}/kg t.m., i.m., 48^h + 0,4mg Cd/kg t.m., 24^h pre žrtvovanja). Hematološki parametri: broj eritrocita (RBCs), koncentracija hemoglobina (Hb) i hematološka vrednost (Ht) su značajno smanjeni u krvi pacova posle tretmana kadmijumom. Cd značajno povećava i koncentraciju LP, kao i koncentracije neenzimskih komponenti AOS-a: redukovani glutation (GSH), vitamin C (Vit C) i vitamin E (Vit E). Eksperimenti sa pacovima koji su izazvanu anemiju i oksidaciona oštećenja (smanjuje koncentraciju LP), kao i značajno umanjuje toksične efekte Cd na komponente AOS-a.

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