UC 577,1; 61 ISSN 0354-3447

Jugoslov Med Biohem 24: 121–127, 2005 *Originalni naučni rad*
Original paper

A POSSIBLE PROTECTIVE ROLE OF COENZYME Q_{10} ON ANTIOXIDANT DEFENSE SYSTEM IN THE HEART OF RATS TREATED WITH CADMIUM

Slađan Z. Pavlović[1], Branka I. Ognjanović[2], Andraš Š. Štajn[2], Radoslav V. Žikić[2], Ratko M. Radojičić[3], Mihajlo B. Spasić[1], Zorica S. Saičić[1]

[1]*Institute for Biological Research »Siniša Stanković«, Department of Physiology, 11060 Belgrade*
[2]*Institute of Biology, Faculty of Sciences, University of Kragujevac, 34000 Kragujevac*
[3]*Institute of Biochemistry and Physiology, Faculty of Biology, University of Belgrade, 11000 Belgrade, Serbia, Serbia and Montenegro*

Summary: The effect of cadmium (Cd), coenzyme Q_{10} (CoQ_{10}) and Cd+CoQ_{10} on the activities of superoxide dismutases (total SOD), manganese containing superoxide dismutase (Mn SOD) and copper-zinc containing superoxide dismutase (Cu,Zn SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione-S-transferase (GST), glutathione reductase (GR) and concentrations of ascorbic acid (AsA) and vitamin E (Vit E) in the heart of male *Wistar albino* rats were studied in comparison to the controls and cadmium treated animals. Cd induces a significant increase of total SOD, Cu, Zn SOD and GSH-Px activities, as well as AsA and Vit E concentrations, but leads to a significant decrease of CAT and GR activities. CoQ_{10} induces a significant increase of total SOD, Mn SOD, Cu, Zn SOD and GSH-Px activities, as well as AsA and Vit E concentrations. In the same group of animals the activities of CAT, GST and GR were significantly decreased. By concomitant treatment of rats with Cd+CoQ_{10} the activities of total SOD, Mn SOD and GSH-Px, as well as concentrations of AsA and Vit E were markedly increased. In the same group of animals the activities of Cu, Zn SOD, CAT and GR were significantly decreased. In respect to the Cd treated rats in Cd+CoQ_{10} partialy are reversed changes (Cu,Zn SOD) of antioxidant defense system in the heart.

Key words: cadmium, coenzyme Q_{10}, rats, heart, antioxidant defense system

Introduction

Cadmium (Cd) is an environmental pollutant which in human and animals affects many cellular functions, but little is known about the mechanisms of its toxicity and cellular defense against it (1). A variety of mechanisms have been proposed to Cd-induced toxicity. Cadmium interferes with intracellular signaling network and gene regulation at multiple levels, induces lipid peroxidation and alterations in activity of antioxidant enzymes (2). Cadmium may induce oxidative damage in different tissues by enhancing peroxidation of membrane lipids and altering the antioxidant defense system (AOS) of the cells (3). An important role in the process of Cd detoxification plays metallothioneins, which are small, cysteine-rich, metal-binding proteins (4). In the heart, prolonged Cd intoxication disturbed intracellular metabolism by damaging ultra structural elements and suppressing the incorporation of precursors of RNA and protein synthesis (5). It is reported that Cd produce cardiotoxicity at doses and exposure conditions that cause no effects in other tissues, such as liver and kidneys. It appears that heart mitochondria are the site of the primary biochemical lesions by Cd involving changes of AOS and increased lipid peroxidation (6). The problem of prevention and therapeutic intervention in Cd intoxication may be approached in two ways: 1. chelation of Cd that has been localized intracellularly bound to metallothionein mainly in liver and kidney after the exposure and 2. free radical scavenging by antioxidants and enzymatic defense system (7).

Address for correspondence:
Slađan Z. Pavlović, Ph.D.
Institute for Biological Research »Siniša Stanković«
Department of Physiology
Bulevar despota Stefana 142
11060 Belgrade, Serbia, Serbia and Montenegro
Phone: (+ 381) 11 2078 341
Fax: (+ 381) 11 2761 433
e-mail: sladjan@ibiss.bg.ac.yu

Coenzyme Q_{10} (CoQ_{10}) has an important function in mitochondrial bioenergetics and is also a powerful antioxidant in its reduced form ($CoQ_{10}H_2$) (8). It has a large number of clinical applications, especially in the treatment of congestive heart failure (9). Recent studies show novel functions of CoQ_{10}: 1. as a essential cofactor of uncoupling proteins which acts to down regulate mitochondrial membrane potential; 2. as a H^+/e^- donor through which sulphydryl/disulfide intra-protein crosslinks are altered and 3. also the protein conformations determined as a regulator of gene expression by way of redox modulation, superoxide formation and its conversion to hydrogen peroxide (H_2O_2) production, utilizing H_2O_2 as a second messenger (10). Coenzyme Q_{10} has the potential to incorporate into the heart mitochondrial membranes and to improve energy production in mitochondria by bypassing defective components in the respiratory chain, as well as by reducing the effects of oxidative stress (11).

The recent results indicate that Cd influences heart AOS and that CoQ_{10} has a benefit effect on heart functions. From this reason, the aim of the present study was to evaluate the effects of Cd, CoQ_{10} and Cd+CoQ_{10} on AOS in the heart of rats. After 30 days of treatment the activities of total superoxide dismutase (total SOD), manganese containing superoxide dismutase (Mn SOD) and copper zinc containing superoxide dismutase (Cu, Zn SOD), (EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), glutathione peroxidase (GSH-Px, EC 1.11.1.9), glutathione-S-transferase (GST, EC 2.5.1.18) and glutathione reductase (GR, EC 1.6.4.2), as well as concentrations of ascorbic acid (AsA) and vitamin E (Vit E) were estimated.

Material and Methods

In the experiments 60 days old *Wistar albino* rats, weighing 190 ± 20 g at the beginning of experiments were used. The animals were housed in individual cages at 21 ± 1 °C and exposed to 12 h light – 12 h dark cycles. The rats were fed chow pellets (Veterinarski Zavod, Zemun, Serbia and Montenegro) and drank tap water *ad libitum*. The animals were divided in four experimental groups and treated during the course of 30 days. The first group of animals was control group (C, drinking tap water). The second group was treated with cadmium (Cd, 200 mg $CdCl_2 \times 5\ H_2O$ in drinking water during 30 days + 100 μL of olive oil, i.m., every fifth day). The third group was treated with coenzyme Q_{10} (CoQ_{10}, 20 mg CoQ_{10} dissolved in olive oil, i.m., every fifth day, drinking tap water). The fourth group was treated concomitantly with cadmium and coenzyme Q_{10} (Cd+CoQ_{10}, 200 mg $CdCl_2 \times 5H_2O$ in drinking water during 30 days + 20 mg CoQ_{10}, i.m. every fifth day). The average intake of 17 mg Cd/day/kg body mass was calculated from the water consumed during the 30 days of treatment. The average intake of CoQ_{10} was 16 mg/kg body mass every fifth day. Each group consisted of 7 animals. After the treatment the animals were sacrificed by decapitation between 8 and 10 A.M. in order to avoid any possible cyclic changes in metabolic and antioxidant levels. All animal experiments were carried in such a manner that all unnecessary animal discomfort and pain were avoided.

The hearts of rats were isolated and dissected out within 3 minutes, placed in ice-cold 155 mmol NaCl and washed with the same solution. The heart tissue was then minced and homogenized in 10 volumes of 25 mmol/L sucrose containing 10 mmol/L Tris-HCl, pH 7.5 at 1500 rpm using Thomas Sci Co. glass homogenizer (Teflon pestle). Homogenates were then centrifuged at 4 °C at 100 000 × g for 90 minutes. All chemicals were SIGMA (St. Louis, MO, USA) products.

Total SOD activity was assayed in the supernatant by the epinephrine method (12) based on the capacity of SOD to inhibit autooxidation of adrenaline to adrenochrome. For the determination of Mn SOD activity the assay was performed after the preincubation with 8 mmol/L KCN. Cu,Zn SOD activity calculated as a difference between total SOD and Mn SOD activities. SOD activities were expressed as U/g wet mass. CAT activity was measured as suggested by Beutler (13) and expressed as mmol H_2O_2/min/g wet mass. The activity of GSH-Px was determined following the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) as a substrate at 340 nm with t-butylhydroperoxide (14) and expressed in nmol NADPH/min/g wet mass. GST activity toward 1-chloro-2,4-dinitro benzene (CDNB) as a substrate was assayed according to Habig et al. (15) and expressed in nmol GSH/min/g wet mass. The activity of GR was evaluated as suggested by Glatzle et al. (16) and expressed in nmol NADPH/min/g wet mass. The AsA concentration was measured by the dinitrophenyl-hydrazine method (17) and expressed in mg/100 g tissue, while Vit E concentration was determined by the method of Desai (18) and expressed in mg/g tissue.

Data are given as means ± SE. All obtained results were compared with respect to control animals (C), as well as with respect to the Cd treated animals (Cd). Statistical analysis of results was based on Student–s paired t-test considering the significance at the level of $p<0.05$ (19).

Results

The results presented in this paper revealed that Cd and CoQ_{10} influenced enzymatic and non-enzymatic components of AOS in the heart of rats. The activities of total SOD and CuZn SOD (*Figure 1*) were significantly increased ($p<0.005$ and $p<0.01$, respectively) in the heart of rats treated with Cd in comparison to the control animals.

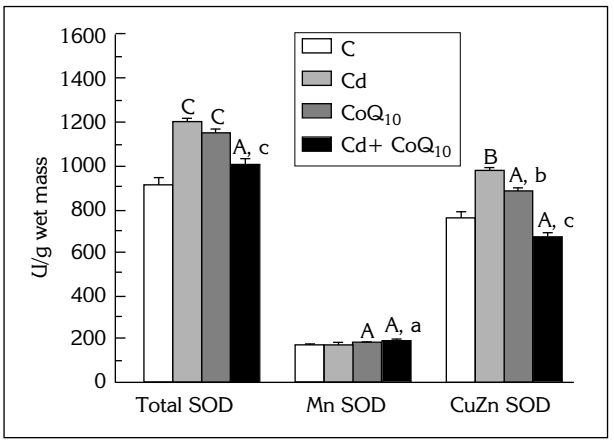

Figure 1. Activities of superoxide dismutases: total (total SOD), manganese containing (Mn SOD) and copper zinc containing (Cu,Zn SOD) expressed in U/g wet mass in the heart of: control rats (C), rats treated with cadmium (Cd), rats treated with coenzyme Q_{10} (CoQ_{10}) and concomitantly treated rats with cadmium and coenzyme Q_{10} (Cd+ CoQ_{10}) during 30 days. The values are means ± SE from seven animals.

Significantly different from controls (C):
[A] $p<0.05$ [B] $p<0.01$ [C] $p<0.005$
Significantly different from Cd treated rats (Cd):
[a] $p<0.05$ [b] $p<0.01$ [c] $p<0.005$

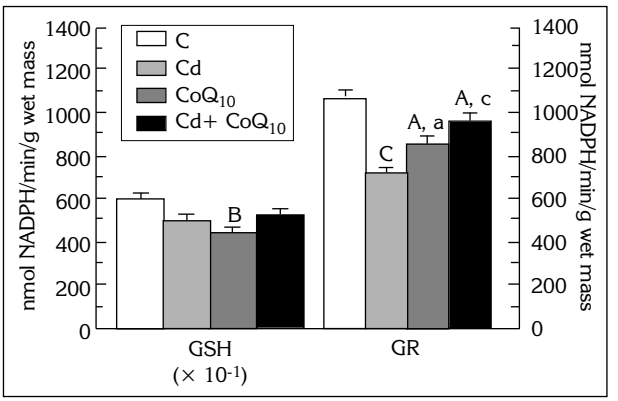

Figure 3. Activities of glutathione-S-transferase (GST) expressed in nmol GSH/min/g wet mass mass (real activity was 10 times higher than presented) and glutathione reductase (GR) expressed in nmol NADPH/min/g wet mass (real activity was 10 times higher than presented) in the heart of rats. The same experimental groups were examined as in *Figures 1* and *2*. The values are means ± SE from seven animals.

Significantly different from controls (C):
[A] $p<0.05$ [B] $p<0.01$ [C] $p<0.005$
Significantly different from Cd treated rats (Cd):
[a] $p<0.05$ [c] $p<0.005$

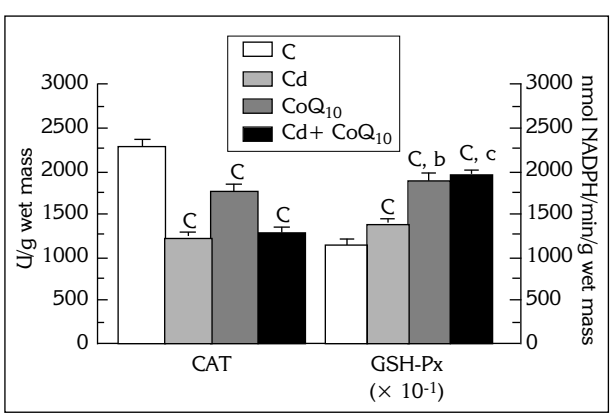

Figure 2. Activities of catalase (CAT) expressed in mmol H_2O_2/min/g wet and glutathione peroxidase (GSH-Px) expressed in nmol NADPH/min/g wet mass mass (real activity was 10 times higher than presented) in the heart of rats. The same experimental groups were examined as in *Figure 1*. The values are means ± SE from seven animals.

Significantly different from controls (C):
[C] $p<0.005$
Significantly different from Cd treated rats (Cd):
[b] $p<0.01$ [c] $p<0.005$

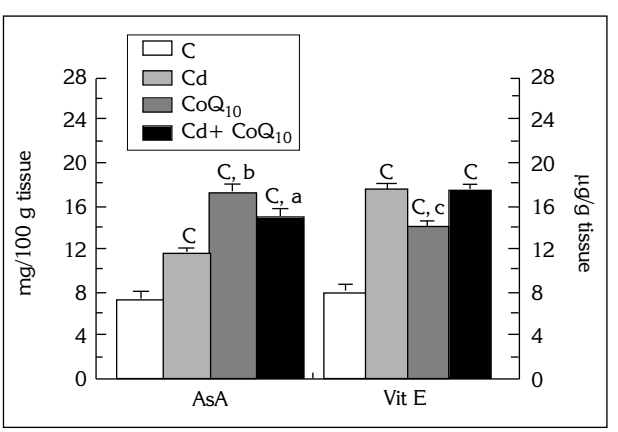

Figure 4. Concentrations of ascorbic acid (AsA) expressed in mg/100 g tissue and vitamin E (Vit E) expressed in mg/g tissue in the heart of rats. The same experimental groups were examined as in *Figures 1, 2* and *3*. The values are means ± SE from seven animals in each group.

Significantly different from controls (C):
[C] $p<0.005$
Significantly different from Cd treated rats (Cd):
[a] $p<0.05$ [b] $p<0.01$ [c] $p<0.005$

Cadmium also induces a significant increase of GSH-Px activity ($p<0.005$) (*Figure 2*), as well as AsA ($p<0.005$) and Vit E ($p<0.005$) concentrations (*Figure 4*). At the same time Cd induces a significant decrease ($p<0.005$) of CAT (*Figure 2*) and GR ($p<0.005$) activities (*Figure 3*).

In rats treated with CoQ_{10}, we observed a significant increase of heart total SOD ($p<0.005$), Mn SOD ($p<0.05$) and Cu,Zn SOD ($p<0.05$) activities (*Figure 1*). Coenzyme Q_{10} also influences a significant increase of GSH-Px ($p<0.005$) activity (*Figure 2*), as well as AsA ($p<0.005$) and Vit E ($p<0.005$) concentrations (*Figure 4*). Contrary to that, CoQ_{10}

significantly decreased CAT ($p<0.005$), (*Figure 2*), GST ($p<0.01$) and GR ($p<0.05$) activities (*Figure 3*). By concomitant treatment of animals with Cd+CoQ_{10}, the activities of total SOD ($p<0.05$), Mn SOD ($p<0.05$), (*Figure 1*) and GSH-Px ($p<0.005$), (*Figure 2*), as well as concentrations of AsA ($p<0.005$) and Vit E ($p<0.005$) were significantly increased in respect to the control rats (*Figure 4*).

On the other hand, co-exposure of animals with Cd+CoQ_{10} induces a significant decrease of heart CuZn SOD ($p<0.05$) (*Figure 1*), CAT ($p<0.005$) (*Figure 2*) and GR ($p<0.05$), (*Figure 3*) activities.

In relation to the rats treated with Cd, in animals administered with CoQ_{10} the activities of CAT ($p<0.005$), GSH-Px ($p<0.01$), (*Figure 2*), GR ($p<0.05$), (*Figure 3*), as well as AsA concentration ($p<0.01$), (*Figure 4*) were significantly increased, while CuZn SOD activity ($p<0.01$) (*Figure 1*) and Vit E concentration ($p<0.005$), (*Figure 4*) were significantly decreased.

In Cd+CoQ_{10} treated rats, the activities of Mn SOD ($p<0.05$), (*Figure 1*), GSH-Px ($p<0.005$), (*Figure 2*) and GR ($p<0.005$), (*Figure 3*), as well as AsA concentration ($p<0.05$), (*Figure 4*) were markedly increased in comparison to the Cd treated animals. At the same time, Cd+CoQ_{10} treatment induced a significant decrease of total SOD activity ($p<0.005$), (*Figure 1*).

Discussion

Conflicting results have been reported on the activities of antioxidant enzymes in the conditions of oxidative stress induced by Cd in various organs of laboratory animals (20). Cadmium is known to deplete glutathione and protein-bound sulphydryl groups, resulting in enhanced production of reactive oxygen species (ROS), such as superoxide anion radicals, hydroxyl radicals and hydrogen peroxide (21). As a consequence of increased superoxide anion radicals production, we obtained an increased activities of total SOD and Cu,Zn SOD in the heart of Cd treated rats (*Figure 1*), which is in accordance with our earlier experiments on rats (22). Contrary to that, some other authors (23) using young rats and higher concentrations of Cd (six months of exposure) were obtained a decreased activity of Cu,Zn SOD in the heart. The activities of total SOD, Mn SOD and Cu, Zn SOD were significantly increased in the heart of rats treated with CoQ_{10} in respect to the control animals (*Figure 1*). It is demonstrated that SOD exhibits a novel function as a superoxide semiquinone oxidoreductase (24). In this reaction, SOD reacts with hidroquinones and together with enzyme DT-diaphorase (NAD(P)H: quinone acceptor oxidoreductase (EC 1.6.99.2) inhibits autooxidation of hydroquinones. This reaction is important for maintenance of semiquinones on its reduced, biologically active form. Increased activity of Mn SOD is probably result of increased incorporation of CoQ_{10} in heart mitochondrial respiratory chain and increased production of superoxide anion radicals in mitochondria (25). Cotreatment of rats with Cd+CoQ_{10} diminished only the toxic effect of Cd on Cu,Zn SOD activity, while the activities of total SOD and Mn SOD where significantly increased.

The CAT activity was significantly decreased (*Figure 2*) in all experimental groups, i.e. Cd, CoQ_{10} and Cd+CoQ_{10} treated animals. An increased activity of SOD leads to an increased production of H_2O_2, and both CAT and GSH-Px participates in its detoxification. Increased activity of GSH-Px during the treatment of animals indicate that this system have a main role in the H_2O_2 removal. At the same time many authors (26) demonstrates that Cd can inhibit the activity of most antioxidant enzymes, which can explain decreased activity of CAT. Our earlier experiments (27) also show that CoQ_{10}, by elevating GSH-Px activity can protect the cells against Cd peroxidative damage. The present results confirm our earlier findings on rats that Cd did not alter the activity of GST in the heart (22). Treatment of rats with CoQ_{10} decreased activity of GST in the heart (*Figure 3*). CoQ_{10} by quenching ROS can be indirectly involved in the regulation of gene expression and in modulation of activities of most enzymes. At the same time, CoQ_{10} has an important role in the prevention of lipid peroxidation and oxidative damage of tissues, and thus induce a decreased activity of GST (10). The role of GR is reduction of oxidized glutathione (GSSG) to its reduced, biologically active form (GSH) utilizing NADPH as a cofactor (16). In earlier experiments we shown a significantly decreased activity of GR in all examined group of animals and these results may be explained by Cd inhibitory effect (27). Compared to the rats treated with Cd, concomitant treatment of rats with Cd and CoQ_{10} partially reversed the activity of GR to the control level. On the other hand CoQ_{10} induced an increased activity of NADPH-CoQ reductase and increased production of reducing equivalents necessary for antioxidant defense of cellular membranes. Reducing equivalents produced in cytosol may be transferred for regeneration of Vit E in biological membranes and for GSH redox cycling. Other authors also reported that CoQ_{10} can improve tissue aminothiol redox status (28).

Increased concentrations of AsA and Vit E in the heart of rats treated with Cd were also obtained (*Figure 4*). Similar results we were obtained in skeletal muscle of rats treated with Cd (27). AsA is a potent scavenger of superoxide anion radicals and singlet oxygen and increased concentration of AsA in the heart of rats can contribute to the better protection against Cd toxicity. Increased concentration of Vit E in the heart of rats treated with Cd could be explained by its protective role in toxic influence of Cd which is the physiological adaptation to Cd toxicity. Vit E is an

important lipid-soluble antioxidant acting as a scavenger of free oxygen radicals, inhibiting lypoxygenases and reducing peroxides in association with lypoxygenases. The results presented in *Figure 4* reveal that in the heart of rats treated with CoQ_{10}, as well as concomitantly treated with Cd+CoQ_{10}, AsA and Vit E concentrations were significantly increased in comparison to the controls. Increased concentration of AsA in the heart of rats treated with CoQ_{10} is in accordance with increased concentration of Vit E because they may act sinergically as antioxidants and each can exert sparing effect in the absence of the other (29). It is well known that CoQ_{10} and its NADPH-dependent reductase stabilized the extracellular ascorbate in the organism (30). On the other hand, Vit E radicals (Vit E$^{\bullet}$, α-tocopheroxyl radical) would be regenerated by reduced form of CoQ_{10} ($CoQ_{10}H_2$) and this could be the explanation for increased concentration of Vit E in the heart of rats treated with CoQ_{10} (31). The potential of CoQ_{10} to regenerate Vit E *via* electron transport from Vit E radicals serves to preserve other cellular reductants, such as AsA and GSH, which otherwise could provide only limited maintenance of reduced Vit E during oxidative stress. CoQ_{10} is directly reduced by cytochrome b_5 reductase, ferredoxin reductase and glutathione reductase, it maintains both AsA and Vit E in their reduced state. These facts suggest that CoQ_{10} and system involved in its oxidation/reduction might behave as regulators of cellular redox status and antioxidant capacity and should be considered as a part of the protective cellular response to oxidative injuries (32).

From the presented results, *it can be concluded* that CoQ_{10} exerts beneficial effects on AOS in the heart of rats treated with Cd, especially on non enzymatic components, such as AsA and Vit E and thus induced an increase in antioxidant defense potential in the heart of rats. Considering the influence of CoQ_{10} on the activity of antioxidant enzymes, we suppose that CoQ_{10} is involved in more complex mechanisms of their regulation including its role as a modulator of gene expression.

Acknowledgments. This work was supported by the Ministry of Science, Technology and Development, Republic of Serbia, Grant No 1669.

MOGUĆA ZAŠTITNA ULOGA KOENZIMA Q_{10} NA SISTEM ZAŠTITE OD OKSIDACIONIH OŠTEĆENJA U SRCU PACOVA TRETIRANIH KADMIJUMOM

Slađan Z. Pavlović[1], Branka I. Ognjanović[2], Andraš Š. Štajn[2], Radoslav V. Žikić[2], Ratko M. Radojičić[3], Mihajlo B. Spasić[1], Zorica S. Saičić[1]

[1]*Institut za biološka istraživanja »Siniša Sanković«, Odeljenje za fiziologiju, 11060 Beograd*
[2]*Institut za biologiju, Prirodno-matematički fakultet, Univerzitet u Kragujevcu, 34000 Kragujevac*
[3]*Institut za biohemiju i fiziologiju, Biološki fakultet, Univerzitet u Beogradu, 11000 Beograd, Srbija, Srbija i Crna Gora*

Kratak sadržaj: Ispitivan je uticaj kadmijuma (Cd), koenzima Q_{10} (CoQ_{10}) i Cd+ CoQ_{10} na aktivnost ukupne superoksid dismutaze (Uk SOD), mangan sadržavajuće superoksid dismutaze (Mn SOD), bakar cink sadržavajuće superoksid dismutaze (Cu,Zn SOD), katalaze (CAT), glutation peroksidaze (GSH-Px), glutation-S-transferaze (GST) i glutation reduktaze (GR), kao i na koncentracije askorbinske kiseline (AsA) i vitamina E (Vit E) u srcu mužjaka *Wistar albino* pacova u poređenju sa kontrolnom grupom i grupom tretiranom sa kadmijumom. Cd u srcu indukuje značajno povećanje aktivnosti Uk SOD, Cu,Zn SOD i GSH-Px, kao i koncentracija AsA Vit E uz značajno smanjenje aktivnosti CAT i GR. CoQ_{10} izaziva značajno povećanje aktivnosti Uk SOD, Mn SOD, Cu,Zn SOD i GSH-Px, kao i koncentracija AsA i Vit E. U istoj grupi životinja aktivnosti CAT, GST i GR su značajno smanjene. Istovremeni tretman sa Cd+CoQ_{10} dovodi do značajnog povećanja aktivnosti Uk SOD, Mn SOD i GSH-Px, kao i koncentracija AsA i Vit E. U istoj grupi životinja aktivnosti Cu,Zn SOD, CAT i GR su značajno smanjene. U poređenju sa pacovima tretiranim sa kadmijumom kod Cd+CoQ_{10} grupe dobijen je delimični zaštitni efekat (Cu,Zn SOD) na antioksidacioni zaštitni sistem u srcu pacova.

Ključne reči: kadmijum, koenzim Q_{10}, srce, antioksidacioni zaštitni sistem

References

1. Yamada H, Koizumi S. DNA microarray analysis of human gene expression induced by a non-lethal dose of cadmium. Ind Health 2002; 40: 159–66.
2. Wang Y, Fang J, Leonard SS, Krishna Rao KK. Cadmium inhibits the electron transfer chain and induces reactive oxygen species. Free Radic Biol Med 2004; 36: 1434–43.
3. Sarkar S, Yadav P, Trivedi R, Bansal AK, Bhatnagar P. Cadmium-induced lipid peroxidation and the status of the antioxidant system in the rat. J Trace Elem Med Biol 1995; 9: 144–9.
5. Saydam N, Adams TK, Steiner F, Schaffner W, Freedmand JH. Regulation of metallothionein transcription by the metal-responsive transcription factor MTF-1. Identification of signal transduction cascades that control metal-inducible transcription. J Biol Chem 2002; 277: 20438–45.
6. Skowerski M, Jasik K, Kmecki J. Effects of interaction between cadmium and selenium on heart metabolism in mice: the study of RNA, protein, ANP synthesis activities and ultrastructure in mouse heart. Med Sci Monit 2000; 6: 258–65.
7. Lamaye DA, Shaikh ZA. Cytotoxicity of cadmium and characteristics of its transport in cardiomyocytes. Toxicol Appl Pharmacol 1999; 154: 59–66.
8. Tandon SK, Singh S, Prasad S, Khandekar K, Dwivedi VK, Chatterje M, Mathur N. Reversal of cadmium induced oxidative stress by chelating agent, antioxidant or their combination in rat. Toxicol Lett 2003; 145: 211–7.
9. Kucharska J, Braunova Z, Ulicna O, Zlatos L, Gvozdjakova A. Defect of coenzyme Q in heart and liver mitochondria of rats with streptozocin-indued diabetes. Physiol Res 2000; 49: 411–8.
10. Langsjoen H, Langsjoen P, Langsjoen P, Willis R, Folkers K. Usefulness of coenzyme Q_{10} in clinical cardiology: a long-term study. Mol Asp Med 1994; 15: S165–75.
11. Linnane AW, Kopsidas G, Zhauch C, Yarovaya N, Kovalenko S, Papakostopoulos P, Eastwood H, Graves S, Richardson M. Cellular redox activity of coenzyme Q_{10}: Effect of coenzyme Q_{10} supplementation on human skeletal muscle. Free Radic Res 2002; 36: 445–53.
12. Rosenfeldt FL, Pepe S, Linnane A, Nagley P, Rowland M, Ou R, Marasco S, Lyon W, Esmore D. Coenzyme Q_{10} protects the ageing heart against stress: studies in rats, human tissues and patients. Ann NY Acad Sci 2002; 959: 355–9.
13. Misra HP, Fridovich I. The role of the superoxide anion in the autooxidation of epinephrine and simple assay for superoxide dismutase. J Biol Chem 1972; 247: 3170–5.
14. Beutler E. Catalase. In: Beutler E ed. Red Cell Metabolism, a Manual of Biochemical Methods. New York: Grune and Stratton, 1982: 105–6.
15. Tamura M, Oschino N, Chance B. Some characteristics of hydrogen and alkylhydroperoxides metabolizing systems in cardiac tissue. J Biochem 1982; 92: 1019–31.
16. Habig WH, Pabst MJ, Jakoby WB. Glutathione-S-transferase. J Biol Chem 1974; 249: 7130–9.
17. Glatzle D, Vulliemuier JP, Weber F, Decker K. Glutathione reductase test with whole blood, a convenient procedure for the assesment of the riboflavin status in humans. Experientia 1974; 30: 665–8.
18. Roe HJ. Chemical determination of ascorbic, dehydroascorbic and diketogulonic acid. In: Glick D ed. Methods of Biochemical Analysis. New York: Intersci. Publ., 1957: 115–39.
19. Desai ID. Vitamin E analysis methods for animal tisues. Meth Enzymol 1984; 105: 138–47.
20. Hoel PG. Introduction to Mathematical Statistics. New York: John Wiley, 1966: 402–3.
21. Casalino E, Calzaretti G, Sblano C, Landriscina C. Molecular inhibitory mechanisms of antioxidant enzymes in rat liver and kidney by cadmium. Toxicology 2002; 179: 37–50.
22. Stohs SJ, Bagchi D, Hassann E, Bagchi M. Oxidative mechanisms on the toxicity of chromium and cadmium ions. J Environm Pathol Toxicol Oncol 2000; 19: 201–13.
23. Žikić RV, Štajn AŠ, Ognjanović BI, Saičić ZS, Kostić MM, Pavlović SZ, Petrović VM. The effect of cadmium and selenium on the antioxidant enzyme activities in rat heart. J Environm Pathol Toxicol Oncol 1998; 17: 259–64.
24. Jamall IS, Sprowls J. Effects of cadmium and dietary selenium on cytoplasmic and mitochondrial antioxidant defense system in the heart of rats fed high dietary copper. Toxicol Appl Pharmacol 1987; 87: 102–10.
25. Cadenas E, Mira D, Brunmark A, Lind C, Segura-Anguilar J, Ernster L. Effect of superoxide dismutase on the autooxidation of various hydroquinones-a possible role of superoxide dismutase as a superoxide: semiquinone oxidoreductase. Free Radic Biol Med 1988; 5: 71–9.
26. Lenaz G, Bovina C, Formiggini G, Parenti-Castelli G. Mitochondria, oxidative stress, and antioxidant defences. Acta Biochem Pol 1999; 46: 1–21.
27. Shukla GS, Srivastava RS, Chandra SV. Glutathione metabolism in liver, kidney and testis of rats exposed to cadmium. Ind Health 1987; 25: 139–46.
28. Pavlović SZ, Ognjanović BI, Štajn AŠ, Žikić RV, Saičić ZS, Petrović VM. Antioxidant defense system in skeletal

muscle of rats treated with cadmium. A possible protective role of coenzyme Q_{10}. Jugoslov Med Biohem 2001; 20: 229–35.

29. Kishi T, Takahashi T, Usui A, Hashizume N, Okamoto T. Cytosolic NADPH-UQ reductase, the enzyme responsible for cellular ubiquinone redox cycle as an endogenous antioxidant in the rat liver. BioFactors 1999; 9: 189–97.

30. Tanaka K, Hashimoto T, Tokumara S, Iguchi H, Kojo S. Interactions between Vitamin C and Vitamin E are observed in tissues of inherently scorbutic rats. J Nutr 1997; 127: 2060–4.

31. Kozlov AV, Gille L, Staniek K, Nohl H. Dihydrolipoic acid maintains ubiquinone in the antioxidant active form by two-electron reduction of ubiquinone and one-electron reduction of ubisemiquinone. Arch Biochem Biophys 1999; 363: 148–54.

32. Ernster L. P/O rations-the first fifth years. FASEB J 1993; 7: 1520–4.

33. Kwong LK, Kamzalov S, Rebrin I, Bayne ACV, Jana CK, Morris P, Forster MJ, Sohal RS. Effects of coenzyme Q_{10} administration on its tissue concentrations, mitochondrial oxidant generation, and oxidative stress in the rat. Free Radic Biol Med 2002; 33: 627–38.

Received: September 1, 2004

Accepted: September 14, 2004