Acta Veterinaria (Beograd), Vol. 59, No. 2-3, 167-175, 2009.

DOI: UDK 619:615.916

ULTRASTRUCTURAL AND BIOCHEMICAL CHANGES IN THE TURKEYS LIVER AFTER THE CHRONIC CADMIUM EXPOSURE

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(Received 13. November 2008)

The aim of our study was to observe the chronic effects of cadmium and cadmium with zinc on the structure and histological changes in turkey liver by means of transmission electron microscopy and to examine whether concentrations of metals influence the activity of superoxide dismutase and lipid peroxidation in liver tissue. The ultrastructural changes were investigated in hepatocytes after 71 days exposure to cadmium (Cd group) and cadmium + zinc (Cd/Zn group). In Cd group, ultrastructural changes included swollen and round mitochondria with injured cristae, dilated rough endoplasmatic reticulum and depletion of glycogen granules. In the Cd/Zn group hepatocytes showed morphological features similar to those from the control group except the mitochondria. They had an oval or elliptical shape similar as in the control group, but the cristae were obviously loose and dissolved.

The activity of superoxide dismutase (SOD) and the content of thiobarbituric acid reactive substances (TBARS) were determined. The specific activity of SOD was significantly increased in Cd group. In Cd/Zn group, zinc co-administration shows a protective effect. Alterations in the activitiy of superoxide dismutase showed that the toxic effect of cadmium is not only due to its high affinity for thiol groups, but also in the production of reactive oxygen species. Cadmium induced ultrastructural changes in the hepatocytes were not accompanied by a significant increase in tissue TBARS concentrations.

Key words: morphology, hepatocytes, cadmium, zinc, superoxide dismutase

INTRODUCTION

Cadmium is a toxic heavy metal and a well-known environmental pollutant. It is present in the soil, water, air and consequently in the food. Quantifying the transfer of cadmium from food to target organs is a key in the estimation of health

risk (Chan *et al.*, 2004). Cadmium causes poisoning in various tissues of humans and animals. The mechanism of cadmium toxicity is not fully understood. Various possible mechanisms have been suggested to explain the damage induced by cadmium. It is known that cadmium reacts with thiol groups present in proteins and this event can affect various metabolic processes. The metal can block the functional sites of the catalytic domains of enzymes or modify protein conformation. These changes can lead to loss of catalytical activity. Another possible mechanism could be the displacement of the metal, which is essential for biological activity of a molecule by another one. Eybl *et al*. (2006) and Ikediobi *et al.* (2004) suggest that cadmium toxicity can cause oxidative stress and that lipid peroxidation is an early and sensitive consequence of acute cadmium exposure.

Cadmium is highly cumulative, especially in the liver and kidney. The bioaccumulation and toxicity of cadmium is modified by many dietary components. Therefore, the content of cadmium was studied in the liver - with high metabolic activity, cumulative capability, and detoxifying capacity. Cadmium derives its toxicological properties from its chemical similarity to zinc an essential micronutrient for plants, animals and humans.

The aim of our study was to observe the chronic effects of single cadmium and cadmium with zinc co-administration on the ultrastructure of hepatocytes in turkey liver by means of transmission electron microscopy and to examine whether metals concentrations influence the activity of superoxide dismutase and lipid peroxidation in this tissue.

MATERIAL AND METHODS

Animals and diets

The experiment was carried out on 18 female turkeys of BIG-6 breed at the age of 35 days. The animals were divided into 3 groups of 6 animals each after 30 days of acclimatisation. Turkeys were firstly fed with the HYD 14 feed mixture. HYD 15 was given from week 9. Two weeks before finishing the experiment the animals received feed mixture HYD 16. Food and water were offered *ad libitum*. The first group was the control (group C) without any treatment. The second group (Cd) received cadmium as $CdCl_2$ (aqueous solution) in the amount of 0.5 mg/kg (food) from the 65^{th} day of age. The third group (group Cd/Zn) received the same dose of cadmium as in Cd group plus zinc as $ZnSO_4$ (aqueous solution) in the amount, which represents the double of the recommended dose (90 mg/kg food). The application time of the substances was 71 days. The animals were killed at 136 days of age. The experiment was performed following ethical requirements for animal handling.

Transmission electron microscopy (TEM)

The samples intended for ultrastructural examinations were fixed in 3% glutaraldehyde, postfixed in 1% OsO_4 (both in a phosphate buffer pH 7.2-7.4), dehydrated in acetone en embedded in Durcupan ACM. The ultrathin sections were cut on the ultramicrotome Tesla BS 490, stained with uranyl acetate and lead citrate and evaluated using a transmission electron microscope Tesla BS 500.

Preparation of tissue extracts

The pieces of liver were homogenized in 5 mmol/dm^3 Tris-HCl buffer pH 7.8 containing 1 mmol/dm^3 Na_2EDTA, 0.15 mol/dm^3 KCl and 2 mmol/dm^3 GSH using Ultra-Turrax homogenizer. The homogenates (25% w/v) were centrifuged at 105 000 g at 4°C, for 60 min and stored at – 60°C until used for enzyme assays.

Analysis

Superoxide dismutase activity (SOD, EC 1.15.1.1) was determined by measuring the inhibition of cytochrome c reduction using xanthine/xanthine oxidase $O_2^{\bullet -}$ generating system at 550 nm (25°C) (Flohé and Otting, 1984). One unit of SOD activity was defined as the amount of enzyme that causes 50% inhibition of cytochrome c reduction under assay conditions.

Protein concentration was measured by the method of Bradford (1976), using bovine serum albumin as a standard.

Lipid peroxidation products measured at 535 nm as thiobarbituric acid reactive substances (TBARS) were determined according to Gutteridge (1984). The content of TBARS was expressed in absorbance/mg^1 of protein.

Metals (Cd, Zn) were determined by means of atomic absorption spectroscopy using the method of Kocourek (1992).

All reagents were of the highest purity from Sigma, Merck and Boehringer.

The results are given as means ± SD of at least three independent determinations in 6 different batches. Statistical analysis was done by one-way analysis of variance (ANOVA) with the *post hoc* Turkey 's multiple comparison test.

RESULTS

The data in Table 1 demonstrate the accumulation of cadmium in the liver of turkeys during cadmium intoxication. The content of cadmium was 11 times higher in the Cd group compared with the control. Cadmium is highly interactive with high concentrations of dietary zinc, which reduce the rate of cadmium absorption from various food sources. The accumulation of cadmium in our experiment was lower in Cd/Zn group.

Table 1. Metals content in the liver of turkeys

Group (n = 6)	Metal content (mg.kg^{-1} of tissue)	
	Cd	Zn
Control	0.07 ± 0.03	26 ± 8
Cd	0.78 ± 0.08	21 ± 1
Cd/Zn	0.41 ± 0.05	20 ± 3

The liver is a parenchymatose organ. The morphologic characteristics of hepatocytes in the control group were clearly observed. Polygonal hepatocytes

were radially arranged a trabecular fashion around a central vein. The nuclei were large, round, with predominant euchromatin and a small amount of heterochromatin. One or two nucleoli were evident. Also, binucleated cells were common. Mitochondria, which filled the cytoplasm, were normal in shape and size. They were round or elliptical with well visualized cristae. Abundant rough endoplasmic reticulum was distributed close to the mitochondria. Glycogen deposits were also observed and were typically arranged and formed clusters of electron-dense particles (Figure 1, 2).

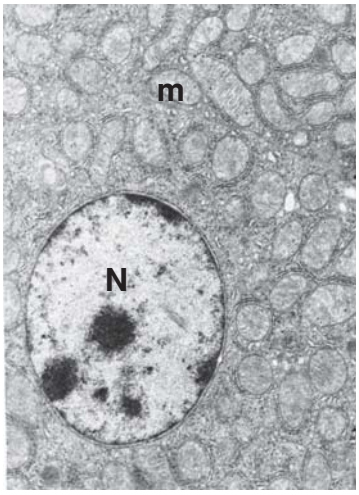

Figure 1. Electronomicrograph of hepatocytes from the control group. Nucleus (N) and mitochondria (m). (10 000 x)

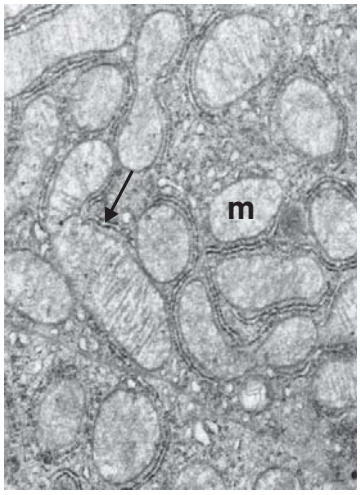

Figure 2. Electronomicrograph of hepatocytes from the control group. Mitochondria were orderly arranged, mitochondrial membranes were intact (m). Rough endoplasmic reticulum (arrow). (30 000 x)

In the Cd group, obvious ultrastructural changes of hepatocytes were found (Figures 3 and 4). Some nuclei were deeply invaginated, irregular, with condensed chromatin. The morphologic changes of the mitochondria were clearly observed under EM. Mitochondria were swollen, with membranes that were vague, and cristae were loose and dissolved. The rough endoplasmic reticulum (RER) was dilated and swollen thus appearing as free space around the mitochondria. Occasionally, in the cytoplasm, there were a few round or elliptic lipid droplets. The content of glycogen was decreased when compared to the control liver cells.

Upon Cd/Zn treatment animals, the cells structure almost returned to normal. Some changes were clearly evident. Mitochondria were almost kept in a normal shape and size, but cristae were affected. The nuclei of hepatocytes showed a marked condensation of chromatin around the edge of the nuclei,

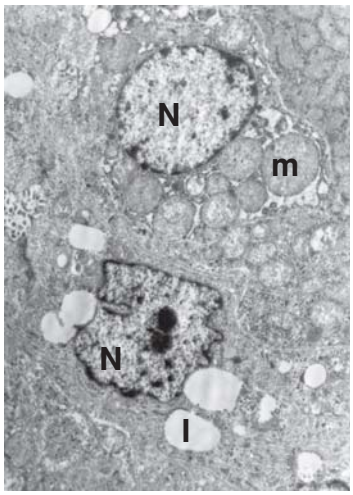

Figure 3. Electronomicrograph of hepatocytes from Cd group. Nucleus (N) irregular shape, swollen mitochondria (m), and lipid droplets (l). (6 400 x)

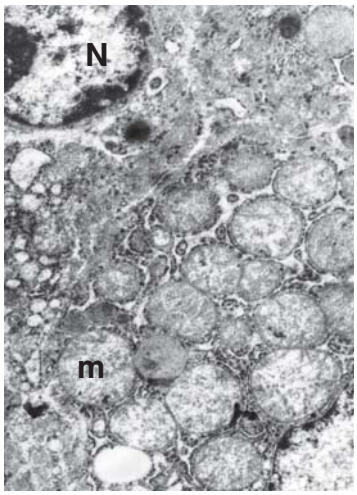

Figure 4. Electronomicrograph of hepatocytes from Cd group. Nucleus (N), mitochondria (m), and dilated endoplasmic reticulum (arrow). (18 000 x)

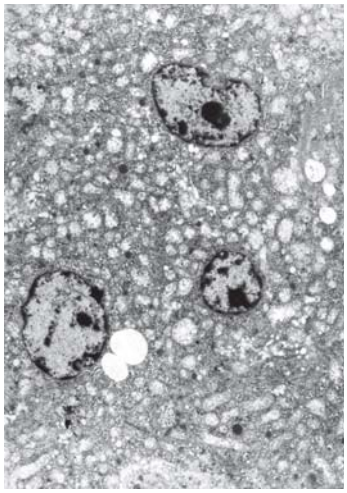

Figure. 5. Electronomicrograph of hepatocytes from Cd/Zn group. Nuclei (N), mitochondria almost normal shape and size (m), and lipid droplets (l) are present. (4 000 x)

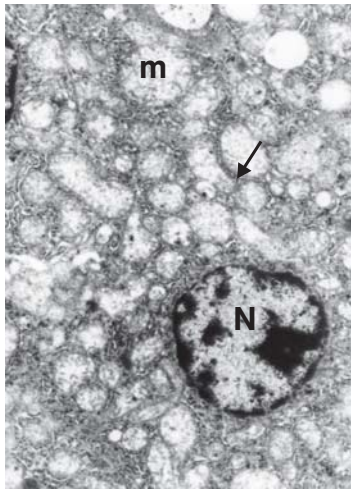

Figure 6. Electronomicrograph of hepatocytes from Cd/Zn group. Nucleus with condensed chromatin in the edge (N), mitochondria (m), and rough endoplasmic reticulum (arrow). (7 000 x)

which showed as large sharply marginated electron dense masses that abutted on the nuclear envelope. Scarce lipid droplets were occasionally scattered in the cytoplasm of these cells. Cisternae of rough endoplasmic reticulum were not dilated as in the previous group (Figure 5, 6).

Our results showed that 71 days of cadmium administration affected the activity of SOD. The specific activity of SOD was significantly increased in the Cd group. Zinc co-administration showed a protective effect in the Cd/Zn group (Figure 7).

One of the markers of oxidative damage of membrane lipids is the TBARS content in tissues. The content of TBARS showed no significant increase in the experimental groups compared with the control (Figure 8).

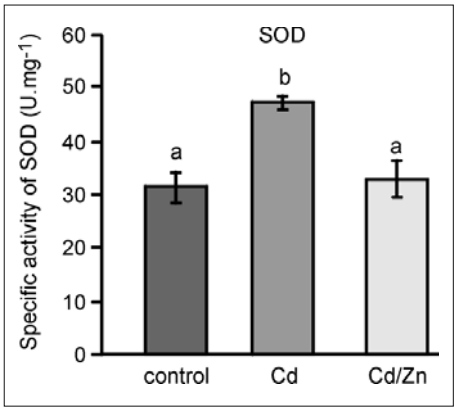

Figure 7. Specific activity of SOD in the liver of turkeys after exposure to cadmium.
Values are means ± SD (n=6). Distinct letters above columns mean significant differences (P<0.05)

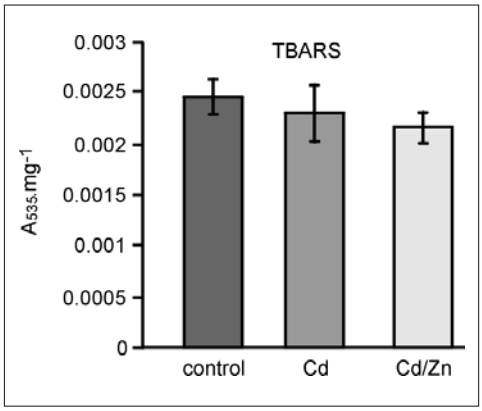

Figure 8. Content of TBARS in the liver of turkeys after exposure to cadmium.
Values are means ± SD (n=6)

DISCUSSION

Cadmium represents a dangerous environmental and industrial pollutant. Soluble cadmium salts accumulate and result in toxicity in various organs (Stohs and Bagchi, 1995; Massányi, 1996; Toman and Massányi, 1997; Sokol *et al*., 1998; Massányi *et al*., 2003; Cigánková *et al*., 2004; Danko *et al*., 2005; Kramárová *et al*., 2005; Lukáč *et al*. 2007; Massányi *et al*., 2007; Nad *et al*., 2007). Absorption following oral exposure to cadmium is likely to depend on the physiological status, as well as on the presence and levels of ions and other dietary components ingested together with the cadmium compound.

The liver plays a key role in most metabolic processes, especially detoxification. It is connected with the position of the liver in the circulatory system

that is optimal for gathering, transforming, accumulating metabolites and for neutralizing and eliminating toxic substances.

In our experiment, hepatocytes showed different submicroscopical structures in both experimental groups. In the Cd group, ultrastructural changes included swollen mitochondria with cavitation, dilated cisternae of rough endoplasmatic reticulum, and depletion of glycogen granules. Similar ultrastructural alterations were observed in the liver of birds treated with cadmium (Chishti and Rotkiewicz, 1993). It has been reported that mitochondria are the most important producers of reactive oxygen species (ROS). The electron transport chain on the inner mitochondrial membrane produces superoxide radicals and they can be converted to hydrogen peroxide. Hydrogen peroxide can then diffuse out of mitochondria into the cytoplasma. In the presence of metals in high concentrations, highly reactive hydroxyl radicals can form (Szeto, 2006). ROS can cause damage to all cellular macromolecules. Excessive ROS could directly react with unsaturated fatty acids on the surface of the mitochondrial membrane, thus resulting in the destruction of its structure and function.

The extent to which ROS yield oxidative stress depends upon the effectiveness of the antioxidant defence and significant damage occurs only if antioxidant defence is overwhelmed (Sies, 1986). SODs as a scavenger of superoxide radicals in biological tissues keep the concentration of superoxide radicals in low limits and therefore play an important role in the protection against free radical damage (Fridovich, 1997). It has been reported that SOD evoked varied responses to cadmium that are concentration-, cell type- and frequency of exposure- dependent (Ikediobi *et al*., 2004). Our results showed that cadmium affected the activity of SOD. The specific activity of SOD was significantly increased in the Cd group. The observed increase in SOD activity is a response to accumulation of ROS. Mitochondria try to decrease intracellular ROS levels by decreasing the consumption of oxygen via the formation of "extremely swollen mitochondria" or "megamitochondria" (Wakabayashi, 2002). Zinc co-administration shows a protective effect in the Cd/Zn group. The hepatocytes in the Cd/Zn group showed morphological features similar to those from the control group. Adequate amount of zinc in the diet affords some protection from exposure to cadmium. Our results suggest that dietary zinc in food sources might play a vital role in protecting cadmium induced oxidative damage in the liver.

One of the markers of oxidative damage of membrane lipids is the TBARS content in tissues. Cadmium induced ultrastructural changes in hepatocytes were not accompanied by a significant increase in the tissue content of TBARS. It is possible that the TBARS content did not increase due to the function of the other protective systems. The liver is one of the major sites where the metal-binding protein metallothionein is synthesized in response to metals exposure. Exposure to metals can induce the synthesis of metallothioneins, low molecular weight proteins with high sulfhydryl content,

which bind up to 90% of the metal accumulated in the liver and is redistributed slowly in the other organs especially in the kidneys (Massányi *et al*.,

2003). Metallothionein may be a compensatory protective mechanism. The liver controls the availability of cadmium to other target organs.

The results showed that cadmium induced morphological changes in the turkey's liver. The alteration in the activity of superoxide dismutase showed that the toxic effect of cadmium is not only in the high affinity for the thiol group, but also in ROS production.

ACKNOWLEDGEMENTS:
This work was supported by the VEGA No. 1/3495/06 and No. 2/6175/6.

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ULTRASTRUKTURNE I BIOHEMIJSKE PROMENE U JETRI ĆURAKA HRONIČNO IZLAGANIH KADMIJUMU

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SADRŽAJ

Cilj ovih ispitivanja je bio da se prouči uticaj kadmijuma ili kadmijuma i cinka na promene u hepatocitima ćurki pomoću elektronske mikroskopije i da se ispita uticaj ovih mikroelemenata na aktivnost superoksi-dismutaze (SOD) i nivo peroksidacije lipida u tkivu jetre. Ultrastrukturne promene su ispitivane nakon izlaganja navedenim mikroelementima tokom perioda od 71 dana.

U hepatocitima ćurki izlaganim kadmijumu, uočena su zadebljanja mitohondrija koje su poprimale okruglast oblik uz oštećenja kristi, proširenja granulisanog endoplazmatskog retikuluma i depleciju granula glikogena. U grupi izlaganoj kadmijumu i cinku, hepatociti su imali slične morfološke karakteristike kao i u kontrolnoj grupi sa izuzetkom mitohondrija. Ove organele su bile ovalne ili eliptične (kao i kod kontola) ali su njihove kriste bile oštećene.

Aktivnost SOD je bila značajno veća u grupi izlaganoj kadmijumu dok je cink ispoljavao protektivan efekat. Promene u aktivnosti SOD ukazuju da toksičnost kadmijuma nije samo posledica njegovog visokog afiniteta za tiolne grupe već i povećanog stvaranja reaktivnih radikala kiseonika. Istovremeno, kod jedinki izlaganih kadmijumu nisu uočene promene u nivou lipidne peroksidacije.