

EFFECTS OF ACUTE CADMIUM TOXICITY ON OXIDATIVE DAMAGE IN NERVOUS TISSUE

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Cadmium is a wide-spread environmental pollutant, redox-active metal that mainly accumulates in the liver and kidney, bones, pancreas and adrenals, rarely in the brain. The mechanisms of Cd toxicity are poorly understood, although some studies indicate that cellular damage results from an increase in production of reactive oxygen species and inhibition of antioxidant enzymes. We tested the hypothesis that acute Cd exposure would lead to increased oxidative damage in various brain regions (prefrontal cortex, hippocampus, nc.caudatus). Adult male rats were treated either with a single dose of 50 ppm of CdCl₂ (LD50), or a single dose of 100 ppm CdCl₂ (LD50), against control group not exposed to Cd. The enzymatic activity of total and mitochondrial SOD, as well as GR was significantly and dose-dependent decreased, but MDA content has shown only a moderate increase. Results demonstrated the harmful effects of acute Cd exposure, in the nervous tissue, through mechanisms of oxidative damage.

Key words: brain, cadmium, free radicals, oxidative stress

INTRODUCTION

Cadmium is a nonessential trace element, and its toxicity may be due to induced alterations in cellular homeostasis of essential metal ions, such as copper, zinc, and calcium. The absence of homeostatic mechanisms for toxic metals such as cadmium also suggests the absence of selective, mediated transport processes. To traverse plasma membranes, cadmium must therefore utilize transport systems that normally carry endogenous metals or gain entry via nonselective pathways.

Industrial and environmental exposure to cadmium (Cd) is well known to produce multiorgan toxicity in humans. Although cadmium represents one of widely spread toxic metals in the environment, little is known about its nervous system toxicity (Jarup *et al.*, 1998). Until recently attention has been focused mainly on cadmium storage in soft tissues, especially in the liver and kidneys,

rarely in the brain (Pope *et al.*, 1995). The mechanisms of cadmium toxicity are not completely understood, but it has been already shown that some of the cellular effects are related to chromosomal damage (Fowler, 1978). Some of the specific changes that lead to tissue damage and death in cadmium exposure have been related to oxidative stress and thiol depletion (Ercal *et al.*, 2001). Acute exposures in humans are rarely described, almost exclusively in the form of occupational accidents among metal workers, presenting chest pain, dyspnoea, dysuria, headache and dizziness (Wittman *et al.*, 2002). Although the environmental source of cadmium remains unknown, we speculate that acute cadmium toxicity led to brain intracellular accumulation with resulting cellular dysfunction, blood-brain barrier disruption, and lethal cerebral oedema.

Cadmium distributes to tissues rapidly and has a high volume of distribution. In general, transition metals, act as catalysts in chemical processes of metal-induced oxidative stress that finally results with oxidative tissue damage. Contrary to redox-active metals (iron, chromium and copper) that clearly enhance production of reactive oxygen species, like hydroxyl radical (HO·), superoxide radical (O₂⁻) and hydrogen peroxide (H₂O₂), the mechanism of tissue damage of redox-inactive metals remains controversial. Recently, the depletion of major cellular antioxidant defence system is proposed as the possible mechanism involved in toxic effects of cadmium in various tissues. In order to test the previous hypothesis, we analyzed several biological markers of the antioxidant defence system (superoxide dismutase - SOD; glutathion reductase - GR; malonyldialdehyde -- MDA) within the prefrontal cortex, hippocampus and nc. Caudatus, following acute exposure to cadmium in sublethal concentrations.

MATERIALS AND METHODS

Animals and Experimental Design

Adult Gerbil rats (*Meriones unguiculatus*), of both sex and average body mass of 75 g, were used in this study. They were maintained in a room with a controlled photoperiod (14h light/10h darkness) and temperature (22 ± 2°C), and were supplied with rat's chow and water "*ad libitum*".

The study design was approved by local ethics committee and the protocol conforms to the guidelines of the Laboratory Animal Care Committee.

Three groups of ten animals each were used. Group 1 received water from public supply served as the control. Group 2 was treated with a single dose of cadmium chloride (CdCl₂, M.T. 228.36. Carlo Erba Milan) at a dose of 50 ppm of CdCl₂ in drinking water, and Group 3 with CdCl₂ at a dose of 100 ppm of CdCl₂. This doses of cadmium was chosen, considering that 50 ppm of CdCl₂ represents LD₂₅, and 100 ppm of CdCl₂ LD₅₀. Twelve animals was in each group.

Tissue preparation

After thawing, the prefrontal cortex, hippocampus and nc. caudatus were immediately homogenized in saccharose medium (0.25 M/L saccharose, 10 mmol/L K/Na phosphate buffer, pH 7.0 and 1 mmol/EDTA) and centrifuged at 3000 rey/min for 15 min, at room temperature (Beckman J-21, rotor J-20). The

supernatant was removed and the sediment was mixed with additional 1.5 mL of saccharose medium. The whole procedure was repeated once more and supernatants from two successive procedures merged and kept frozen at -20° C pending SOD, GR activity and MDA content determination.

Enzymatic activity and MDA content measurements

1. *SOD activity* was measured as the inhibition of epinephrine autoxidation at 480 nm. Kinetics was followed up in the sodium carbonate buffer (50 mmol/L, pH 10.2) that contained EDTA (0.1 mmol) after the addition of 0.1 mL epinephrine (0.5 mmol/L) (Sun *et al.*, 1978).

2. *GR activity* was measured in Tris-HEPES buffer (110 mmol, pH-7.2) containing NADPH (1 mmol/L), EDTA (1 mmol/L) and oxidized glutathione (10 mmol/L). Reaction was started by sample addition (0.01 mL) and stopped after 15 min with HCl (1 mmol/L) that destroyed NADPH. Fluorescence of formed NADP⁺ was measured after the treatment with NaOH (6 mmol/L at 340/360 nm) (Lowry *et al.*, 1974).

3. *Lipid peroxidation (MDA content)* was determined as the content of thiobarbituric acid-reactive substances (TBARs) formed during *in vitro* stimulation by Fe²⁺ salts, while n-butanol was used for extraction (Andreeva *et al.*, 1988).

Statistical analysis

Enzymatic activity of SOD was expressed as mIU/mg protein, GR activity as nmol/mg protein/h and MDA content as mmol MDA/mg protein. The results were tested for variance homogeneity through the Snedecor test. As all results follow a homogenous variance, the Student's t-test was applied for comparisons between groups. The results were considered significant at $P \leq 0.05$. All values represent the mean \pm S.E.M.

RESULTS

After cadmium exposure, enzymatic activity of total and mitochondrial SOD (mSOD), as well as GR in tissue preparations decreased as compared with the values found in the control group, while MDA content slightly increased, but without relevant statistical significance.

SOD activity. Cadmium decreased total SOD content (LD₂₅ for 26.3 %, $p < 0.05$; LD₅₀ for 36.5%, $p < 0.01$) and a much more prominent drop of mSOD content (LD₂₅ for 68.5 %, $p < 0.01$; LD₅₀ for 69.5%, $p < 0.01$) in cortical slices. Similar changes were observed in the hippocampal (total SOD in LD₂₅ group dropped by 47.2 %, LD₅₀ for 41.6%; mSOD in LD₂₅ group decreased for 61.3%, and in LD₅₀ for 80.9%) and caudatus slices (total SOD in LD₂₅ group dropped for 53.2 %, LD₅₀ for 28.6%; mSOD in LD₂₅ group decreased for 25.1%, and in LD₅₀ for 62.8%) as shown in Fig. 1 and 2.

GR activity. Enzymatic activity of GR was decreased in a very similar fashion as shown for SOD activity ($p < 0.01$). The data in Fig. 3 illustrates an equal distribution of reduced GR activity among different neural structures (GR

decrease in prefrontal cortex for 54.4 % in LD₂₅ group, 67.2% in LD₅₀ group; in hippocampus for 67.7 % in LD₂₅ group, 76.1% in LD₅₀ group; and in nc. caudatus for 69.8 % in LD₂₅ group, 70.4% in LD₅₀ group).

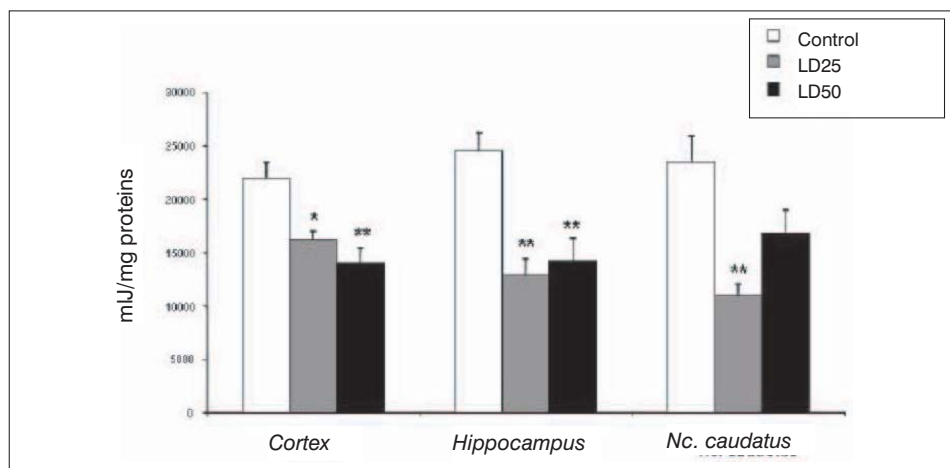


Figure 1. Mean total SOD in tissue preparation (mJ/mg proteins) of rat cortex, hippocampus and nc. caudatus. The symbols (*, **) denotes significant group separation ($P < 0.05$ and $P < 0.01$, respectively)

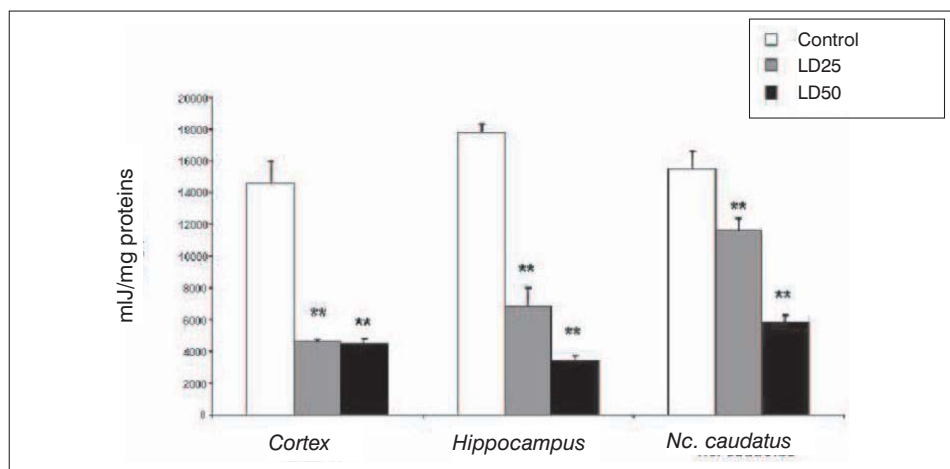


Figure 2. Mean mitochondrial SOD in tissue preparation (mJ/mg proteins) of rat cortex, hippocampus and nc. caudatus. All graphs follow the same conventions as in Fig 1.

Contrary to previous findings MDA contents has shown only a moderate increase, prevalently in the hippocampus and nc. caudatus (Fig. 4).

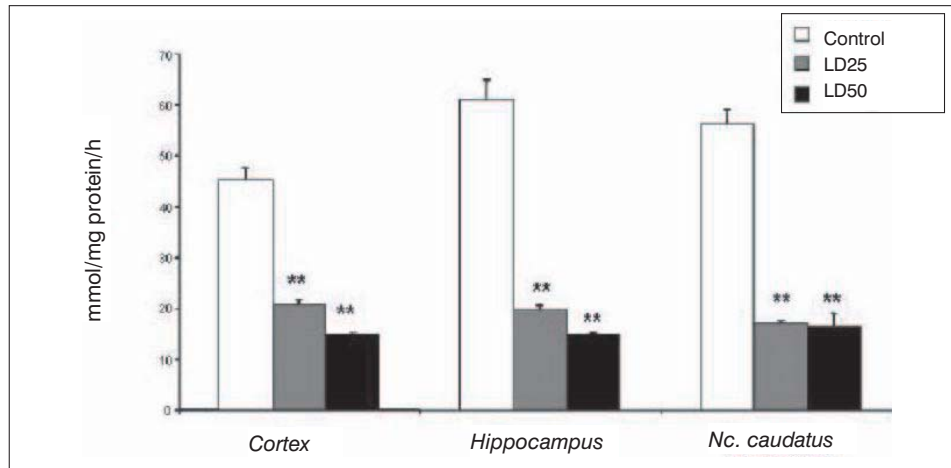


Figure 3. Mean enzymatic activity of glutathione reductase in tissue preparations (mmol/mg proteins/h) of rat cortex, hippocampus and nc.caudatus. All graphs follow the same conventions as in Fig 1.

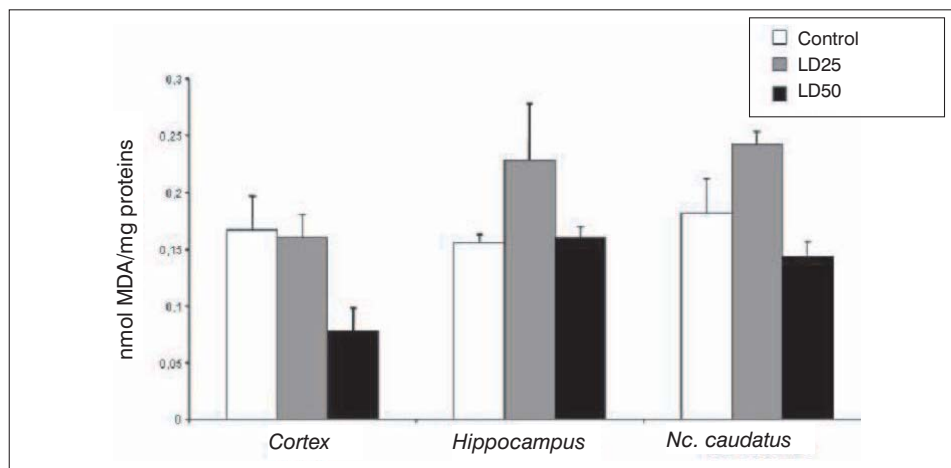


Figure 4. Mean MDA content in tissue preparations (nmol MDA/mg proteins) of rat cortex, hippocampus and nc. caudatus. All graphs follow the same conventions as in Fig 1.

DISCUSSION

The most relevant findings of our study are increased activity of two synergistic enzymatic systems (SOD and GR) as a response of acute exposure to cadmium in sublethal concentrations in various brain regions. Those effects were substantially prominent in comparison with the index of lipid peroxidation, MDA content, suggesting a relatively efficient antioxidant defence system.

Cadmium is considered one of the most toxic substances in the environment due to its relative wide range of organ toxicity and presence in elevated concentrations in fruits, vegetables and grains. The uptake from the soil and the underlying mechanisms by which cadmium induces cellular damage in the nervous tissue, are not completely understood. Some of the specific changes that lead to tissue damage in chronic exposure have been related to free radical production, resulting in oxidative deterioration of lipids, proteins and DNA, and the depletion of glutathione, as well as inhibition of antioxidant enzymes, including manganese-superoxide dismutase, and copper-zinc-superoxide (Casalino *et al.*, 2002). It has been reported, that Cd predominantly increases inhibition of complexes II and III of the mitochondrial electron transfer chain in the liver, brain and heart tissue. Thus suggesting further transfer of one electron from semiquinone to molecular oxygen to form superoxide (Wang *et al.*, 2004).

A recent study has indicated that rats treated with CdCl₂ for thirty days (alternate day administration) showed a significant increase of TBARs formation, and decrease in the activity of acetylcholinesterase (AChE) in the brain (El-Demerdash *et al.*, 2004). Although different methods and duration of Cd-exposure, were employed in El-Demerdash's and our study, in order to demonstrate oxidative stress induction, those findings suggest common mechanisms. Acute exposure to CdCl₂ in our study caused the decrease of enzymatic activity, with a trend toward increased lipid peroxidation, most probably the first step in consecutive chain reaction leading to cellular damage by highly toxic reactive oxidative species. Similar findings to ours, have been reported from El-Missiry and Shalaby, who reported that Cd decreased the activity of glutathione S-transferase (GST) in the brain of male rats (El-Missiry *et al.*, 2000). Possible explanation of enzymatic activity decline may be due to the high affinity of Cd on glutathione, regarding its specific molecular structure that represents a reactive site for metals, and subsequent formation of complexes or the oxidation of glutathione.

Acute exposure to cadmium in sublethal concentrations in adult male rats induces oxidative stress in various brain regions, modifying the MDA content and disrupting antioxidant enzymatic reactions. In summary, the data provided in this paper support the concept that one mechanism underlying the nervous tissue toxicity of cadmium is excessive tissue oxidative damage.

Cellular damage in cadmium intoxication can be indicated by inhibition of adenosine triphosphatase (ATPase) activity and increased lactate dehydrogenase (LDH) activity in brain and testicular tissues. Chronic Cd administration, as some studies show, can result in a decline in glutathione (GSH) content and a decrease of superoxide dismutase (SOD) and glutathione S-transferase (GST) activity in the brain (El-Missiry *et al.*, 2000).

It may be concluded that an early exposure of Cd may produce alterations in the development of different lipids, which may produce CNS dysfunctions with a possibility of being manifested even in later life (Gupta *et al.*, 1996). Similarly, acute cadmium exposure can induce an increase in vascular permeability in the sensory ganglia, but without ischemic changes. Acute administration of cadmium, can also, induce cerebral edema with protein leaks and apparent disruption of the

blood-brain barrier. Petechial hemorrhages in the parietal cortex occur within 2 h of cadmium exposure, accompanied by thinning and vacuolization of capillary walls and widening of interendothelial gaps.

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EFEKTI AKUTNOG TROVANJA KADMIJUMOM NA OKSIDATIVNI STATUS MOZGA

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SADRŽAJ

Kadmijum je u prirodi široko rasprostranjen polutant, redoks aktivan metal koji se uglavnom akumulira u jetri, bubregu, kostima, pankreasu i nadbubrežnoj žlezdi, a ređe u mozgu. Mehanizmi intoksikacije kadmijumom nisu baš najjasniji. Neke studije ukazuju na ćelijsko oštećenje koje nastaje kao posledica povećanja produkcije reaktivnog kiseonika kod nekih vrsta i inhibicije antioksidantnih enzima. Naša hipoteza je bila da će akutna ekspozicija kadmijumu voditi ka povećanom oksidativnom oštećenju u mnogim regionima mozga (korteksu, hipokampusu, nukleusu kaudatusu). Odrasli Džerbili su tretirani pojedinačnim dozama od 50 ppm CdCl₂ (LD₅₀), ili pojedinačnim dozama od 100 ppm CdCl₂ (LD₅₀), dok kontrolna grupa nije bila izložena kadmijumu. Aktivnost enzima ukupne i mitohondrijalne superoksi-dismutaze, kao i glutation reduktaze su značajno i dozno zavisno opadale, dok je sadržaj malonil-dialdehida samo neznatno rastao. Rezultati ukazuju na oštećujući efekat akutne ekspozicije kadmijumu, u nervnom tkivu, preko mehanizama oksidativnog oštećenja.