

Effects of six-week intoxication on cadmium and zinc distribution in internal organs and blood and on the mitotic activity of liver cells

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The aims of this study were to detect the distribution of Cd and Zn in mouse internal organs and blood over six weeks of intoxication with Cd and exposure to Zn, and to estimate the effect of Zn²⁺ and Cd²⁺ on the mitotic activity of liver cells. Administration of Zn led to a significant increase of Cd concentration in the organs and blood, while intoxication with Cd caused a significant increase of Zn concentration in the liver and kidneys.

Zn supplementation led to a decrease of Cd concentration in the heart, spleen, kidneys and blood. Intraperitoneal injection of Cd significantly increased the mitotic activity of liver cells, while Zn supplementation slightly decreased it. Our results demonstrate a close relationship between Zn and Cd distribution in mouse organs and blood. These data validate the hypothesis that the metabolism and action of Cd may be modulated by Zn administration.

Key words: cadmium, zinc, mice, mitotic index, atomic absorption spectroscopy

INTRODUCTION

The heavy metal cadmium (Cd), a well-known environmental hazard, exerts a number of toxic effects in humans and animals. Prolonged exposure to Cd can result in various pathologies including neoplasia, osteoporosis, irreversible renal tubular injury, anemia, etc [1]. Chronic exposure of Cd²⁺ results in accumulation of the metal mainly in the liver and kidneys [2]. The basis of Cd toxicity is its negative influence on enzymatic systems of cells, resulting from substitution of other metal ions in metalloenzymes and its very strong affinity to biological structures containing –SH groups, such as pro-

teins, enzymes, and nucleic acids [3]. Many effects of Cd²⁺ action result from interactions with necessary micro- and macroelements [4, 5]. These interactions can take place at different stages of the absorption, distribution and excretion of the bioelements and Cd²⁺. Hill and Matrone [6] first suggested that biologically important interactions could occur among bioelements and toxic metals with similar physical and chemical properties.

Zinc (Zn) is a ubiquitous element essential for a number of cellular processes, including DNA synthesis, transcription and translation, but in excess it can be toxic [7].

This study investigated the distribution of Cd and Zn in the internal organs and blood of mice over a 6-week intoxication with Cd and Zn administration and the effect of these trace elements on the mitotic activity of liver cells.

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MATERIALS AND METHODS

Outbred mice weighing 20–25 g were used in the study. All experiments were performed according to the regulations defined by European convention for the protection of vertebrate animals used for experimental and other scientific purposes (License No 0135). Mice were randomly assigned into four groups: three experimental and one control. Each group included 7–10 mice. Mice of the experimental group 1 were injected intraperitoneally with CdCl_2 solution (0.16 mg Cd / kg body mass). Mice of the experimental group 2 received intraperitoneal injection of ZnSO_4 solution at a dose level 0.53 mg Zn^{2+} / kg body mass. Mice of the experimental group 3 were injected intraperitoneally with ZnSO_4 solution and CdCl_2 solution in an aforementioned dose. Control animals received injection of the same volume of physiological solution. The injections were done into the peritoneum of the mice for six weeks three times per week.

The concentration of Cd and Zn in blood and tissue specimens was determined by using a Perkin–Elmer/Zeman 3030 electrothermal graphite furnace atomic absorption spectrophotometer. The venous blood was obtained by single-use syringes using an anticoagulant. Tissue specimens were resolved with 0.125 M NaOH upon 90 °C, and the digests were diluted to the appropriate volume with twice-distilled water. The modified analysis method [8] for heavy metal concentration detection in biological samples was used.

Samples from liver tissue were fixed in 10% neutral buffered formalin for 48 h and then processed for routine paraffin embedding. Five-micron-thick sections were routinely stained with hematoxylin and eosin. Histological slides were examined by light microscopy (objective $\times 40$). Mitotic activity of liver cells was evaluated by counting the number of mitotic cells (Fig.1) in ten randomly-selected reference areas (0.04 mm²) for each specimen.

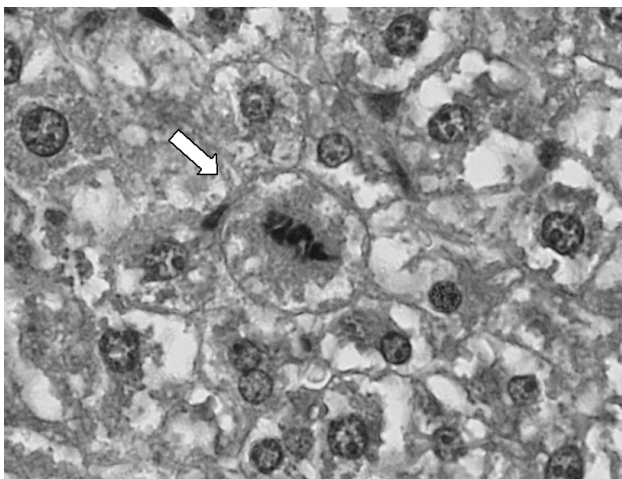


Fig. 1. Histology of mouse liver sections. Mice were injected intraperitoneally with 0.05 LD₅₀ of cadmium chloride solution for 6 weeks. Arrow indicates mitotic liver cells. (Haematoxylin and eosin, original magnification $\times 40$)

Histological slides were examined by light microscopy (objective $\times 40$). For each specimen, the number of mitotic cells was counted in ten randomly selected reference areas (0.04 mm²). Their histological images were taken using a DP-11 Olympus Digital Camera.

Nonparametric Kruskal–Wallis and Mann–Whitney tests were applied to evaluate the difference among mitotic cell counts in different groups. The Student t test with Bonferroni correction was applied for comparison of geometric means of cadmium concentration. 25–75 percentiles were calculated for evaluation of data dispersion. Statistical significance was set at $p = 0.05$.

RESULTS AND DISCUSSION

Zn^{2+} concentration in blood (Fig. 2 A) and all investigated internal organs (Fig. 2 B, 2 C) of mice injected only with ZnSO_4 solution was higher than in the control group.

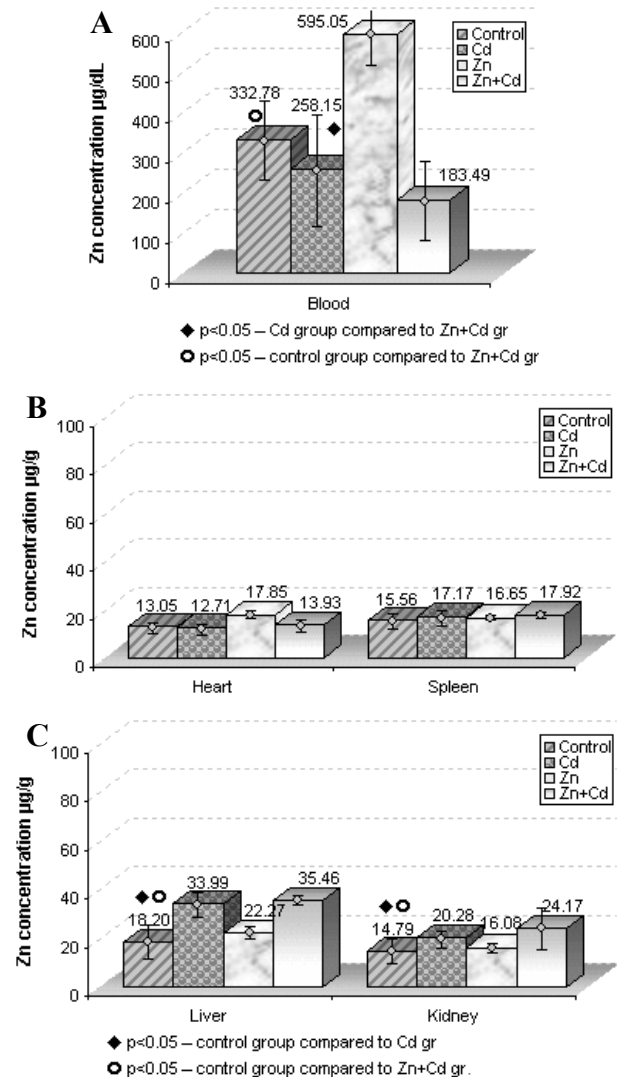


Fig. 2. Cadmium concentration: A – in blood; B – in heart and spleen; C – in liver and kidney of mice 6 weeks after CdCl_2 and ZnSO_4 injections

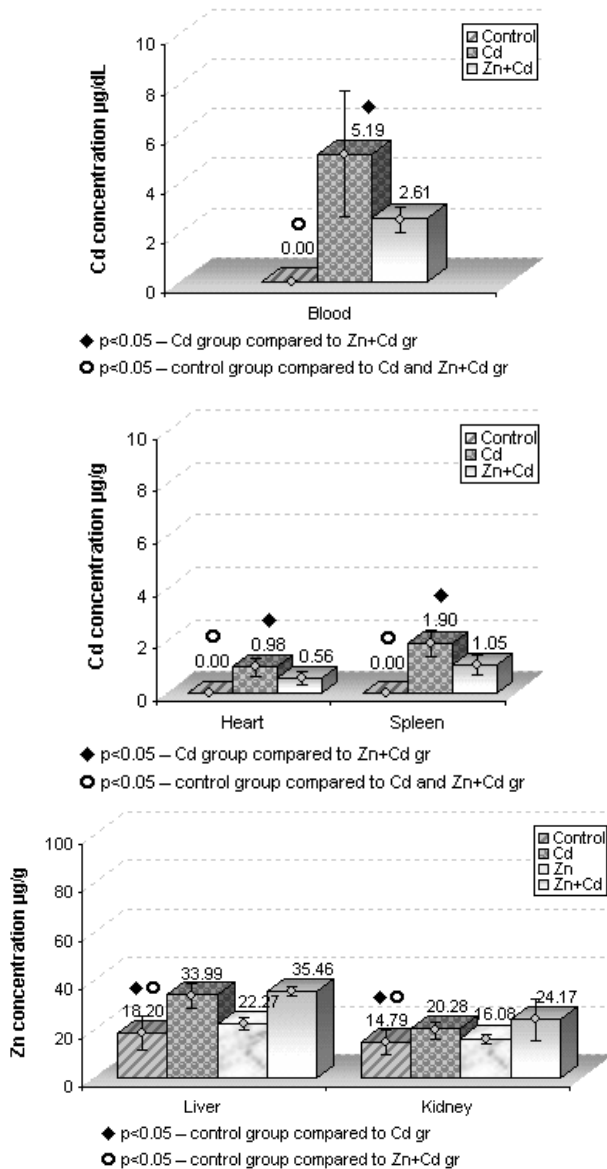


Fig. 3. Zinc concentration: A – in blood; B – in heart and spleen; C – in liver and kidney of mice 6 weeks after CdCl₂ and ZnSO₄ injections

Periodical injection of CdCl₂ for six weeks led to a significant increase of Cd²⁺ concentration in blood and all organs of mice in comparison to controls. It also caused a significant decrease of Zn concentration in blood (Fig. 2 A) and its slightly increase in internal organs (Fig. 2 B).

The injection of Zn + Cd caused a significant decrease of Cd²⁺ concentration in the blood (Fig. 3 A) and spleen, heart (Fig. 3 B) and kidney (Fig. 3 C) as compared to the Cd group.

A highly positive correlation between Cd and Zn concentrations in liver and kidneys has been noted by Oishi et al. [9]. Similar results were demonstrated also in our study. We showed that administration of Zn led to a significant increase of Cd concentration in internal organs and blood. Intoxication by Cd caused a slight increase of Zn concentration in the heart, spleen, liver and

kidneys (Fig. 2 B). It is of interest that Zn administration together with Cd led to a decrease of Cd concentration in the heart (1.7-fold), spleen (1.8-fold), kidneys (1.4-fold), and blood (8.5-fold) (Fig. 3 A, 3 B, 3 C).

It can be speculated that such interaction between Cd and Zn concentrations in the organs is caused by metallothionein (Mt). Mt is a low-molecular-weight, thiol-rich, metal-binding protein, which was first identified as a Cd-binding protein. Later its ability to bind Zn and Cu was detected. Mt naturally binds bivalent ions such as Zn, Cu, and Cd. A molecule of Mt is composed of two domains. The α-domain binds four atoms of metals, usually Zn, while the β-domain binds three atoms, usually Cu [10]. It is well known that Mt functions in absorption, metabolism, homeostasis and storage of both essential and non-essential trace metals [11].

In physiological conditions, Mt is first of all saturated with Zn or Zn and Cu. So the replacement of Zn in pre-existing Mt by Cd with a subsequent synthesis of new molecules of this protein results in an increase of Cd concentration in the internal organs. This phenomenon explains the increase of Cd concentration in experimental mice from Zn group.

Metallothionein expression can vary from tissue to tissue. The parenchymous tissues such as liver, kidney, etc. exert the highest level of metallothionein expression [12]. We demonstrated a Cd-induced retention of Zn in the liver and kidney. According to various researchers, it occurs due to Cd accumulation and Mt induction in these organs [13, 14].

It is well known that kidneys are target organs for Cd toxicity and a major site of antagonistic interactions between Zn and Cd. It was demonstrated that Zn pretreatment reduced the Cd–Mt-induced excessive deposition of Cd in the renal cortex as well as proteinuria and calcuria [15] Friberg et al. [16] indicate that such renal protection takes place without any decrease in Cd concentration in this organ. On the contrary, our results revealed that intraperitoneal Zn + Cd administration for six weeks 1.4-fold diminished the concentration of Cd in the kidneys as compared to the Cd group alone. A similar effect was observed in the spleen, heart, and blood.

The opposite tendency was observed in the liver – Cd²⁺ concentration slightly increased after Zn + Cd injections. Nordberg et al. [17] demonstrated a significant increase in the distribution of Cd in the liver following Zn pretreatment. It was shown that Zn induced (Mt) synthesis in the liver [18]. In animals receiving Cd alone, the majority of the metal localized within the cytosol was bound to high-molecular-weight proteins, while in Zn-pretreated ones the majority of Cd was bound to Mt. It has been shown that Zn administered prior to Cd protected against Cd-induced liver toxicity, including lipid peroxidation and cell damage, even using otherwise lethal doses of Cd [19, 20].

Mitotic activity was evaluated by estimating the number of mitotic cells in the liver cells. The number of mitotic cells estimated in the Zn group did not differ

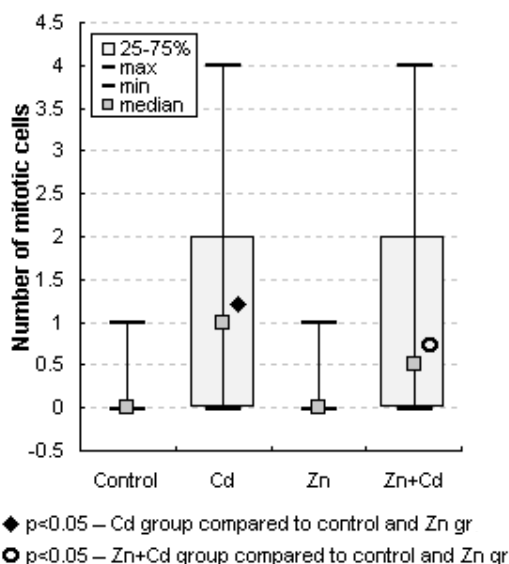


Fig. 4. The number of mitotic cells in liver tissue 6 weeks after CdCl_2 and ZnSO_4 injections

from the controls. Intraperitoneal injection of Cd led to a significant ($p < 0.001$) increase of the number of liver cells as compared to control and Zn group. Administration of Zn + Cd increased ($p < 0.001$) the number of mitotic cells in comparison to Zn exposure alone, but there was a tendency of a decrease of the number of mitotic cells in comparison to Cd group (Fig. 4).

It might be speculated that Zn administration can diminish the mitotic activity of liver cells increased by Cd toxicity. Coogan et al. [21] suggest that zinc pretreatment affects cadmium genotoxicity by inducing metallothionein production which may sequester cadmium from genetic material.

CONCLUSIONS

Our results demonstrate a close relationship between Zn and Cd distribution in mouse internal organs and blood. Injected Zn^{2+} didn't increase the mitotic activity of liver cells, but it was suppressed under Cd^{2+} influence which increased the mitotic activity of liver cells. These data validate the hypothesis that the metabolism and action of Cd may be modulated by Zn administration.

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ILGALAIKĖS INTOKSIKACIJOS POVEIKIS KADMIO IR CINKO PASISKIRSTYMU I VIDAUS ORGANUOSE IR KRAUJYJE BEI KEPENŲ LĄSTELIŲ MITOZINIAM AKTYVUMUI

Santrauka

Šio darbo tikslas – ištirti Cd ir Zn jonų pasiskirstymą vidaus organuose esant lėtinei Cd intoksikacijai bei ilgalaikiam Zn vartojimui, taip pat įvertinti šių jonų poveikį kepenų ląstelių mitoziniam aktyvumui. Nustatyta, kad 6 savaites švirkščiant ZnSO_4 į pelių pilvo ertmę statistiškai patikimai padidėjo Cd koncentracija visuose tiriuose organuose. Be to, išaugo ir Zn koncentracija: 1,9 karto kepenyse ir 1,4 karto inkstuose. Įšvirkštas ZnSO_4 sumažino Cd^{2+} koncentraciją širdyje, blužnyje, inkstuose ir kraujyje atitinkamai 1,7, 1,8, 1,4 ir 2,0 karto. Nustatyta, kad esant lėtinei Cd^{2+} intoksikacijai didėja kepenų ląstelių mitozinis aktyvumas. Keisdami Cd metabolizmą Zn jonai atlieka apsauginį vaidmenį: jie mažina Cd^{2+} sukeltą padidėjusį kepenų ląstelių mitozinį aktyvumą.