Effects of cadmium and zinc ions on mitotic activity and protein synthesis in mouse liver

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Key words: *cadmium, zinc, mitotic index, protein synthesis, mouse liver.*

Summary. *Objective. To evaluate the in vivo effects of cadmium and zinc ions on mitotic activity and protein synthesis in mouse liver.*

Material and methods. White outbred mice were injected intraperitoneally with cadmium chloride solution (14 μmoles cadmium per 1 kg of body mass) and/or with zinc sulfate solution (48 μmoles zinc per 1 kg of body mass). Histological slides were examined by light microscopy. For each specimen, the number of mitotic cells was counted in 10 randomly selected reference areas. Protein synthesis was evaluated by incorporation of [^{14}C]-labeled leucine into newly synthesized peptides and proteins.

Results. We found that the mitotic index of mouse liver cells was increased for periods of 2–8 h after cadmium chloride injection; after 24 h the mitotic index significantly diminished. These data indicate a possible increase in liver cell regeneration during the initial 8 h following acute cadmium exposure. Zinc ions did not affect liver mitotic activity, and, interestingly, decreased the mitotic index in the liver of cadmium-treated mice to control levels. An examination of the kinetics of protein synthesis in mouse liver over a 24 h period after cadmium chloride injection revealed that incorporation diminished by 38% at 2 h, then increased 51% by 8 h and again decreased by 32% at 24 h as compared to control. Zinc ions increased protein synthesis in mouse liver 8 h after zinc sulfate injection. In assessing the effects of cadmium and zinc ions in vivo, it appeared that zinc ions tended to protect protein synthesis in response to cadmium ions but only at 2 h after cadmium intoxication.

Conclusions. Zinc ions are capable of normalizing an increase in the mitotic index of liver cells at the early stage of cadmium poisoning (up to 8 h) and to protect the liver translation machinery against inhibition by cadmium.

Introduction

Heavy metal cadmium (Cd), a well-known environmental hazard, exerts a number of toxic effects in humans and animals. Cd affects cell proliferation, differentiation and other cellular activities (1). A number of Cd-induced effects including deterioration of cell-cell adhesion, DNA-related processes, cell signaling and energy metabolism can imply that this metal acts on the different molecular targets in human organism. It is shown that Cd can induce apoptosis in mouse live (2, 3). This occurs via different mechanisms including oxidative stress (4), Bax- and p53-dependent pathways (5). Cadmium-dependent induction of apoptosis is transient and is followed by necrosis of rat liver cells *in vivo* (6).

One of the targets of Cd is the system of protein synthesis. Activation of protein synthesis can be a consequence of Cd-induced gene transcription, as it was determined for metallothioneins (7), heat-shock proteins (8) and glutathione (9). It is shown that effect of Cd on the protein synthesis *in vivo* depends on intoxication duration and, probably, dose of this metal (10). According to the data of *in vitro* study, Cd in low concentrations can activate both the rate and the level of total protein synthesis but in high concentrations it inhibits those parameters (10).

Zinc (Zn) is essential for a number of cellular processes, including DNA synthesis, transcription, and translation, but its excess can be toxic (11). Intraperitoneal administration of Zn may result in increase in liver mass due to hypertrophy of the hepatocytes (12). Toxic effect of Zn ions in the lung can be caused by their inhibitory action on the pathways of RNA and protein synthesis (13). On the other hand, Zn is ubiq-

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uitous element essential for normal functioning of a number of enzymes in various metabolic pathways (14). Concentration of Zn ions required for enzyme activation is under tight control in cells. Metal-binding proteins known as metallothioneins are involved in this control. It is shown that in cultured keratinocytes Zn ions can induce expression of metallothioneins in time-dependent manner (15), and prevent cells from apoptosis (16).

The present study was designed to investigate *in vivo* the effects of Cd and Zn ions on mitotic activity and protein synthesis in mouse liver. We have found that Zn ions are capable to normalize increased mitotic index of liver cells at the early stage of cadmium poisoning (up to 8 h) and to protect liver translation machinery against inhibition by cadmium.

Material and methods

Experiments were made on outbred mice weighing 20–25 g. All experiments performed according to the rules defined by European convention for the protection of vertebrate animals used for experimental and other scientific purposes (License No. 0028). Experimental groups were used as follows:

1) Cd group. The mice were intoxicated by intraperitoneal injection of $CdCl_2$ solution (14 μmoles Cd per kg body mass) (n=24);
2) Zn group. The mice were injected intraperitoneally by $ZnSO_4$ solution (48 μmoles Zn per kg body mass) (n=15);
3) Zn+Cd group. The mice were injected intraperitoneally with $ZnSO_4$ solution and after 20 min – with $CdCl_2$ solution as described previously (n=17);
4) Control mice (n=12) were injected with the same volume of 0.9% saline.

For histological examination, samples from liver tissue were fixed in 10% neutral buffered formalin for 48 hours 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 40×) (Fig. 1). For each specimen, mitotic index was estimated as total number of mitotic cells in 10 randomly selected reference areas (0.04 mm^2). Their histological images were taken by Olympus Digital Camera DP-11.

For the measurement of protein synthesis, mice were injected i. p. with [^{14}C]-labeled leucine (7.4 MBq per kg of body weight) one hour before killing. Protein synthesis in mouse liver was evaluated by incorporation of [^{14}C]-labeled leucine into newly synthesized peptides and proteins as described in (10). Protein content in samples was determined by Lowry method (17).

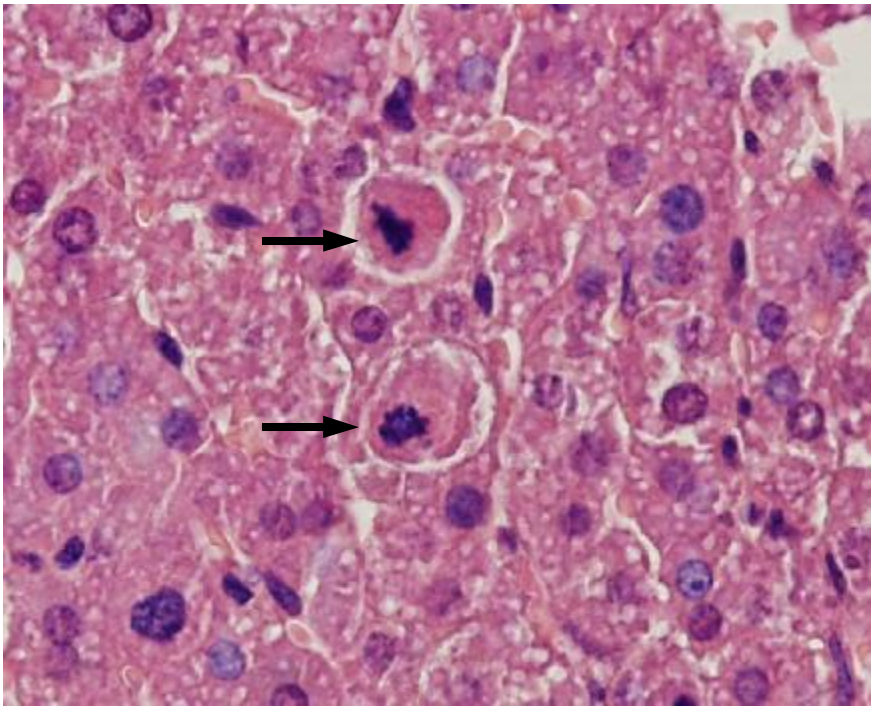

Figure 1. **Histology of mouse liver sections**
Mice were injected with 0.05 LD_{50} amount of cadmium chloride solution and 0.05 LD_{50} of zinc sulphate solution intraperitoneally for 6 weeks 3 times per week. Arrow indicates mitotic liver cells. (Hematoxylin and eosin, original magnification 40×).

Measurement of mitotic activity and protein synthesis in the liver cells was carried out 2, 8, and 24 hours following acute cadmium intoxication or zinc injection.

Results were expressed as the mean ± standard error of mean. Nonparametric Kruskal-Wallis and Mann-Whitney tests were applied for evaluation of mitotic index of liver cells. Statistical significance was set at $p<0.05$.

Results

The mitotic activity was evaluated by the calculation of the mitotic index of liver cells (Fig. 2). It increased gradually 2 h and 8 h after $CdCl_2$ injection. It is of interest that after 24 h following Cd exposure the mitotic index decreased and did not differ significantly from the control value.

The effect of Cd and Zn ions on protein synthesis in mice liver was evaluated *in vivo*. Results, shown in Figure 3, revealed a complex response of mouse liver translation system to the Cd administration. Primarily, liver protein synthesis decreased to 62% of the control level at 2 h after the $CdCl_2$ injection, later significant stimulation up to 151% took place by 8 h and decreased down to 68% by 24 h.

With the aim to determine the effect of Zn ions on total protein synthesis in different organs, mice were injected with $ZnSO_4$ solution. Data obtained showed that Zn ions increased protein synthesis in mouse liver by 67% at 8 h after the Zn administration (Fig. 3). In all other cases protein synthesis was as in liver of the control mouse.

To evaluate possible protective action of Zn ions on Cd-treated mice, we studied the protein synthesis in mouse liver after injection of both solutions – $ZnSO_4$ and $CdCl_2$. Data obtained showed that Zn ions protected liver protein synthesizing system from Cd action only at 2 h after injection (Fig. 3). At 8 h after injection Zn ions partly restored increased protein synthesis in liver. At 24 h after injection of both metals protein synthesis was at the levels of the liver of Cd treated mice (Fig. 3). Thus, our results showed that at early intoxication stage (up 8 h) Zn ions have a protective effect on mice liver protein synthesizing systems.

Discussion

Our study is one of the first attempts to evaluate the joint effects of Cd and Zn ions on the mitotic activity and on the translation system of experimental animals. Earlier we determined that the liver is primary target organ in acute Cd poisoning (18). The data of present study revealed that mitotic activity of liver cells depended on the time after $CdCl_2$ injection. A mitotic index of liver cells increased for periods of 2–8 h after administration. It indicates regeneration of liver cells, which starts early (2 h after in-

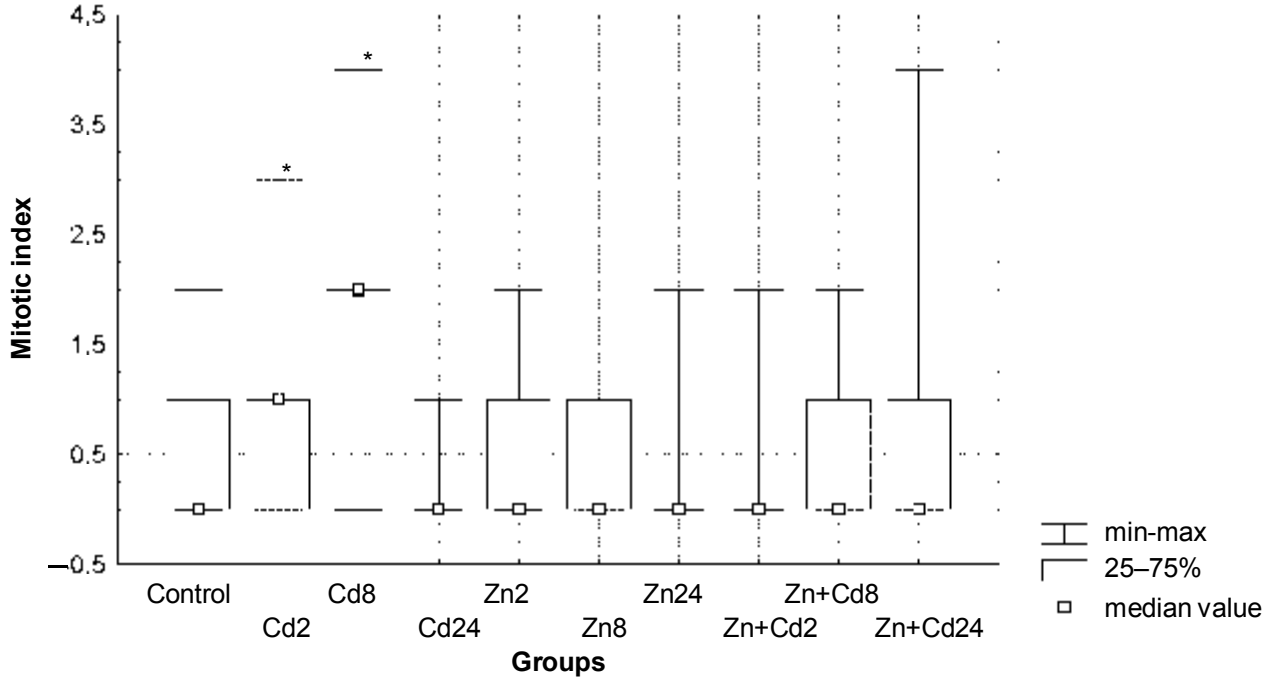

Fig. 2. **The number of mitotic liver cells 2, 8 and 24 h after intraperitoneal injection of cadmium chloride and/or zinc sulfate**

* – statistically significant differences between control and Cd2, Cd8 groups.

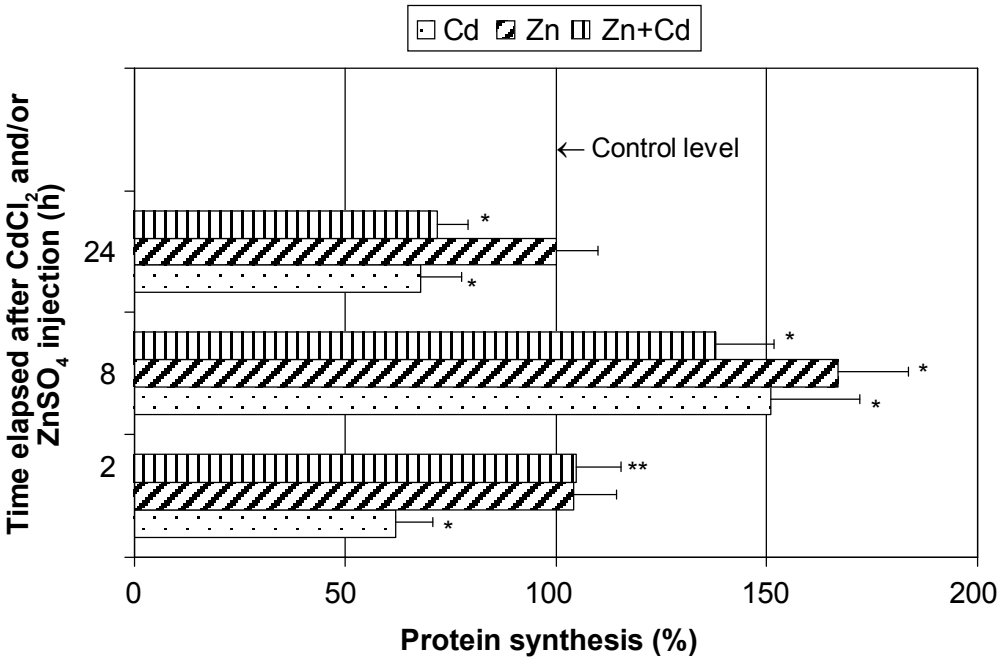

Fig. 3. **Dependence of protein synthesis on exposure time to Cd and/or Zn ions in mouse liver**
In the liver of control group animals protein synthesis was set at 100%. Data represent results of 8–10 separate experiments. * – differences are statistically significant in comparison to the control group mice; ** – differences are statistically significant in comparison to the group of Cd-treated mice.

jection) and reaches its peak after 8 h. The occurrence of early regeneration of liver cells following Cd administration was noted by other researchers (19, 20). According to Dudley *et al.* (19) it might be a direct effect of Cd on hepatocyte DNA. The mitotic index had significantly diminished after 24 h following Cd administration. It did not differ from control values. Increase in mitotic activity of liver cells does not coincide in time with an occurrence of Cd-induced necrosis or apoptosis. The highest apoptotic index is for 9–14 h periods after Cd administration and then it decreases; necrosis is most severe 14–48 h following injection of Cd ions (20, 21). It is of interest that injection of $CdCl_2$ leads to the increase of protein synthesis in mice liver 8 h after intoxication, after 24 h it decreases (Fig. 2). So, it seems that Cd-induced changes of protein synthesis in liver cells correspond to their mitotic activity.

Cd is known to induce synthesis of metallothionein, which is involved in the detoxification of heavy metals (7, 22), and of heat shock (stress) proteins (8) that stabilize the structure of newly synthesized proteins and facilitate perturbed protein synthesis. Observed by us severe inhibition of translation 2 h following Cd administration could reflect switching of Cd intoxicated mice liver to the synthesis of these rescue proteins. In our experiments organism fails to stabilize protein synthesis, and after temporary stimulation of protein synthesis at 8 h it slowly becomes inhibited again.

Zn is essential for normal enzymatic function in multiple metabolic pathways (14). Therefore, we examined possible protective action of small doses of Zn ions on mitotic activity and protein synthesizing system of Cd-treated mice. Our results showed that Zn ions normalize increased mitotic index of liver cells and protect liver protein synthesizing systems from Cd toxicity.

It is shown that Zn significantly protected endothelial cells from Cd-induced inhibition of DNA and protein synthesis (23). Treatment with Cd salts followed by Zn salt injection can induce further synthesis of metallothionein in liver, kidney and pancreas with subsequent binding of both Zn and Cd to the intracellular metallothionein (24). Pretreatment with Zn protected against acute Cd nephrotoxicity (25). Interestingly that Zn caused apoptosis in mammalian cell lines at high concentrations (>150 μM) whereas it protected against Cd-induced apoptosis at low concentrations (10–50 μM) (26).

It may be suggested that major preventive effect of Zn against Cd-induced increase of mitotic index and inhibition of protein synthesis in mice liver is due to its ability to reduce the toxicity of Cd by decreasing accumulation of this metal in liver cells.

Conclusions

1. Cadmium ions induce an increase in mitotic activity of liver cells and significant fluctuations of liver protein synthesis at the early stages of intoxication *in vivo*, which includes both inhibition and stimulation.

2. Zinc ions are capable to normalize increased mitotic index of liver cells at the early stage of cadmium poisoning (up to 8 h) and to protect liver translation machinery against inhibition by cadmium.

Kadmio ir cinko jonų poveikis pelės kepenų mitoziniam aktyvumui ir baltymų sintezei

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Raktažodžiai: kadmis, cinkas, mitozinis indeksas, baltymų sintezė, pelės kepenys.

Santrauka. *Darbo tikslas.* Įvertinti kadmio ir cinko jonų poveikį pelės kepenų mitoziniam aktyvumui ir baltymų sintezei *in vivo*.

Tyrimo medžiaga ir metodai. Baltosioms laboratorinėms pelėms į pilvo ertmę sušvirkštėme kadmio chlorido tirpalo (14 µmolių kadmio 1 kg kūno masės) ir (arba) cinko sulfato tirpalo (48 µmolių cinko 1 kg kūno masės). Histologinius preparatus tyrėme šviesiniu mikroskopu. Kiekviename preparate mitozines ląsteles skaičiavome 10 atsitiktinai pasirinktų nepriklausomų regėjimo laukų. Baltymų sintezės aktyvumą vertinome pagal [^{14}C]-leucino įsijungimą į naujai sintezuotus peptidus ir baltymus.

Rezultatai. Nustatyta, kad, praėjus 2–8 val. po kadmio chlorido tirpalo sušvirkštimo, pelės kepenų ląstelių mitozinis indeksas padidėja, o po 24 val. reikšmingai sumažėja. Remiantis šiais duomenimis, per pirmąsias 8 val. po ūminio kadmio jonų poveikio suaktyvėja kepenų ląstelių regeneracija. Cinko jonai neturi įtakos kepenų ląstelių mitoziniam aktyvumui, o kadmio jonais paveiktų pelių kepenų mitozinį indeksą sumažina iki kontrolinio dydžio. Tiriant pelės kepenų baltymų sintezės intensyvumą, praėjus 24 val. po kadmio chlorido sušvirkštimo, nustatytas 38 proc. jo sumažėjimas po 2 val., 51 proc. padidėjimas po 8 val., 32 proc. sumažėjimas po 24 val. Cinko jonai padidina baltymų sintezės intensyvumą pelės kepenyse praėjus 8 val. po cinko sulfato sušvirkštimo. Tiriant bendrą kadmio ir cinko jonų poveikį *in vivo,* nustatyta, kad cinko jonai apsaugo baltymų sintezės sistemą nuo slopinančio kadmio jonų poveikio tik 2 val. po kadmio chlorido tirpalo sušvirkštimo.

Išvados. Cinko jonai gali sumažinti padidėjusį kepenų ląstelių mitozinį indeksą ir apsaugoti kepenų transliacijos sistemą nuo slopinančio kadmio poveikio pirmąsias 8 valandas.

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