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Induction of apoptosis by cadmium chloride in mouse liver

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The aim of the work was to evaluate the effect of Cd^{2+} on the synthesis of proteins in mouse liver as well as to determine whether the ions induce liver cell apoptosis. The results showed a 30% diminution of protein synthesis intensity in 24 h after a single-dose injection of $CdCl_2$ solution (1.6 mg of Cd^{2+}/kg of body mass; the dose corresponds to 0.5 LD_{50} of Cd^{2+}). Examination of the kinetics of protein synthesis intensity within 24 h after 0.5 LD_{50} of $CdCl_2$ injection revealed its diminishing at 2 h followed by an increase until the maximum intensity was reached at 8 h and then by a slow decrease over the period left. Agarose gel electrophoresis did not identify oligosomal DNA ladder in preparations of chromosomal DNA isolated from the liver of cadmium intoxicated mouse (0.5 LD_{50}). Morphological examination of liver sections revealed the number of TUNEL+ (apoptotic) cells to depend on the duration of liver exposure to Cd^{2+}. In the sections, the number of TUNEL+ cells was significantly higher ($p < 0.05$) after 8 h than after 2 h of liver exposure to $CdCl_2$.

Key words: protein synthesis, cadmium, mice liver, apoptosis

INTRODUCTION

Cadmium is highly toxic to the living organisms. Poisoning with this metal leads to the disturbance of various biological systems of all living organisms, human beings included [1–5]. Some studies suggest that the protein synthesis system of a cell is a target of cadmium action [6]. Cadmium detoxification is closely related to the synthesis of protein metallothionein [3] and heat shock proteins [7]. Recently it was been shown that cadmium specifically induces over-expression of the translation initiation factor 3 [8] and of the translation elongation factor 1-delta [9]. Cd^{2+} can bind leucyl-tRNA synthetase at strict stoicheometry of two ions per polypeptide chain of the enzyme [10].

Apoptosis is a programmed cell death, which manifests in response to a number of factors that can be either internal to an organism, *e.g.*, impaired blood flow followed by reperfusion [11], or external ones, *e.g.*, toxins [12]. Different mechanisms are exploited in apoptosis development. They all, however, include caspases as the central executioners of cell death [13]. Heat shock proteins also take part in the pathways of biochemical events under apoptosis [13]. Although there are some facts to show a link between Cd^{2+} and apoptosis [4], the mechanism of those ions action is uncertain.

The present study deals with the effects of Cd^{2+}on both protein synthesis and apoptosis in mouse liver.

MATERIALS AND METHODS

Experiments were done on the liver of white laboratory mice of 20–25 g body mass. For the studies of Cd^{2+}, mice were injected i. p. with appropriate amounts of $CdCl_2$ in physiological solution. The concentrations of cadmium ions injected were expressed as a fraction of LD_{50}. LD_{50} was considered to be equal to 3.2 mg of Cd^{2+} per 1 kg of mouse body mass. Control animals were also injected the same volume of physiological solution. Upon anaesthesia, the mice were killed according to the rules defined by the European convention for the protection of vertebrate animals used for experimental and other scientific purposes (License No 0028).

Protein synthesis intensity was measured by the incorporation of [^{14}C]-leucine into newly synthesized protein and peptides as described earlier [14]. DNA extraction and agarose gel electrophoresis were carried out as in [15]. Protein content in samples was determined by the Lowry method [16].

Apoptosis of liver cells was histochemically detected by a TUNEL assay using an *in situ* cell death detection kit (AP, Roche). Sections of formalin-fixed

and paraffin–embedded liver tissue were dewaxed and rehydrated according to a standard procedure. Proteinase K permeabilised sections were subjected to enzymatic in situ labelling of DNA strand breaks using a TUNEL technique. After counterstaining with eosin, the sections were analysed on a light microscope (objective 20X). The number of positively stained (TUNEL+) nuclei of liver cells was determined by counting in the randomly selected 10 histological fields per section. The nonparametrical Kruskall–Wallis test was used for comparison between the groups.

The results were expressed as mean ± standard error of mean. Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

The data summarised in Table 1 show the effects of Cd^{2+} at various concentrations on the protein synthesis intensity in mouse liver *in vivo*. These results indicate that single doses of Cd^{2+} at the amount equal to 0.05 LD_{50} (0.16 mg/kg) slightly (19%) decreased protein synthesis intensity in liver 24 h after $CdCl_2$ administration. A low Cd^{2+} dose (0.025 LD_{50}) did not affect liver protein synthesis. 0.5 LD_{50} Cd^{2+} (1.6 mg/kg) reduced protein synthesis by approximately one third.

Protein synthesis intensity in liver at different time intervals upon administration of Cd^{2+} (0.5 LD_{50}) was determined in the subsequent experiments. The data presented in Table 2 revealed a rather complex response of the mouse liver translation system to Cd^{2+} poisoning *in vivo*. Primarily, liver protein synthesis decreased to 62% and 76% of the control level at the 2nd and 4th h, respectively. Then, a significant stimulation up to 151% took place at the 8th h after the cadmium administration, which proceeded into gradual decrease down to the 68 % at 24th h.

Table 1. **Dependence of protein synthesis intensity in mouse liver on the concentration of cadmium ions**

Cd^{2+} concentration (mg/kg body mass)	Protein synthesis intensity (cpm [^{14}C]-leucine / mg of protein)
0 (control)	614 ± 38
0.08	509 ± 37
0.16	497 ± 41*
1.60	417 ± 57*

Protein synthesis intensity was measured 24 h after cadmium chloride injection. Every point was calculated from the results of 8–12 separate experiments. *Differences are statistically significant in comparison to the control group.

Table 2. **Dependence of protein synthesis intensity in mouse liver on the time of exposure to cadmium ions**

Time after $CdCl_2$ administration (hours)	Protein synthesis intensity (cpm [^{14}C]-leucine / mg of protein)
0 (control)	614 ± 38
2	379 ± 65*
4	464 ± 36*
8	925 ± 71*
16	676 ± 66
24	417 ± 57*

Mice were injected with cadmium chloride (1.6 mg Cd^{2+}/kg body mass). Every point was calculated from the results of 8–12 separate experiments. *Differences are statistically significant in comparison to the control group.

Cadmium is known to induce synthesis of both metallothionein, which is involved in the detoxification of heavy metals [3, 17], and heat shock (stress) proteins [18], which stabilise the structure of newly synthesised proteins and facilitate perturbed protein synthesis. A severe inhibition of translation 2–4 h after cadmium administration observed by us could reflect switching of the cadmium-intoxicated liver to the synthesis of these rescue proteins. Interestingly, time point (2–4th h) of the initial inhibition of translation in our experiments coincides with the reported period (4th h) of cadmium-induced maximum synthesis of heat shock proteins [7]. Time-dependent fluctuations with the maximum at the 3rd h and with a subsequent decrease have been reported for the amount of metallothionein mRNA [17]. However, in our experiments the organism failed to stabilise protein synthesis in liver and after a temporary stimulation of protein synthesis at the 8th h it became slowly inhibited again. These data indicate that liver protein synthesis *in vivo* can resist poisoning with lower concentrations of cadmium. At sublethal concentrations of the ions, activated synthesis of rescue proteins tends to remove Cd^{2+} from the liver cells, diminishing their harmful effect.

For examination of Cd^{2+} effect on the programmed cell death, apoptosis, we used conditions of cadmium treatment in which changes of protein synthesis were maximal (8 h after injection when liver protein synthesis increases, and 2 h after injection when protein synthesis is suppressed). Agarose gel electrophoresis revealed no oligosomal DNA ladder – the hallmark feature of apoptosis – in the preparations of chromosomal DNA isolated from the liver under the conditions mentioned above (Fig. 1). A possible explanation for these negative results could be a low index of apoptotic cells, which was de-

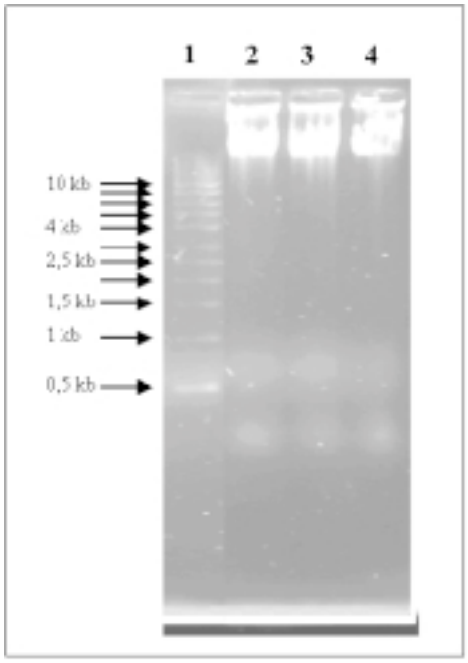

Fig. 1. Agarose gel electroforesis (1%) of chromosomal DNA isolated from mouse liver in norm (lane 4) and 2 h (lane 2) and 8 h (lane 3) after $CdCl_2$ (0.5 LD_{50} Cd^{2+}) injection. Lane 1 contains fragments from 1 kb DNA standard

monstrated by the TUNEL method. The TUNEL reaction did not reveal liver cells with apoptotic hallmarks in the sections of the liver specimens taken from the control group. Two hours following $CdCl_2$ administration (0.5 LD_{50} Cd^{2+}) the number of TUNEL+ liver cells in randomly selected histological fields ranged from 0 to 3, median score 1. After 8 h the number of TUNEL+ liver cells significantly ($p = 0.0093$) increased (range 1 to 3, median score 2) (Fig. 2).

The detailed mechanism of Cd^{2+} action in apoptosis is uncertain. Since sublethal concentrations of Cd^{2+} exerted two opposite effects on protein synthesis (either the inhibition within 2 h and 4 h or the activation within 8 h after injection), we can suppose that cellular amounts of ATP were high enough to maintain that energy-costly process. It is also believed that ATP is essential to complete the whole programme of apoptosis [19]. In this regard, partly damaged cells whose number had progressively increased depending on the duration of liver exposure to Cd^{2+} were "safely" eliminated by apoptosis. Thus, both stimulation of protein synthesis and induction of apoptosis can be regarded as the compensatory mechanisms designated for rescuing an organism in the conditions of acute cadmium intoxication.

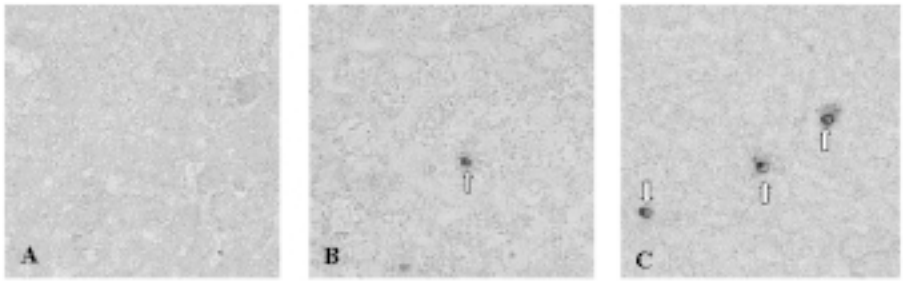

Fig. 2. Detection of apoptotic cells by *in situ* TUNEL-reaction in mouse liver specimens. A – negative TUNEL reaction for apoptosis in control mouse liver specimen. TUNEL+ liver cells after 2 h (B) and 8 h (C) upon intra-peritoneal injection of 0.5 LD_{50} Cd^{2+}. The arrows show TUNEL+ apoptotic cells

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KADMIO CHLORIDO SUKELTA PELIŲ KEPENŲ LĄSTELIŲ APOPTOZĖ

S a n t r a u k a

Darbo tikslas – įvertinti, kaip Cd^{2+} veikia baltymų sintezę pelės kepenyse, ir nustatyti, ar šie jonai sukelia kepenų ląstelių apoptozę. Iš rezultatų matyti, kad praėjus 24 val. po Cd^{2+} dozės, atitinkančios 0,5 LD_{50} (1,6 mg vienam kg kūno masės), sušvirkštimo, baltymų biosintezės intensyvumas pelės kepenyse sumažėjo apie 30%. Nustatyta, kad po 2 val. jis sumažėja, vėliau ima didėti ir po 8 val. tampa didžiausias; po to vėl mažėja. Atlikus chromosominės DNR preparatų elektroforezę agarozės gelyje, „kopėčių“ pavidalo oligosominių DNR fragmentų, būdingų apoptozei, neaptikta. Morfologiniai kepenų pjūvių tyrimai parodė, kad TUNEL+ ląstelių skaičius priklauso nuo laiko, praėjusio po Cd^{2+} injekcijos (0,5 LD_{50}): po 8 val. TUNEL+ ląstelių skaičius buvo patikimai didesnis ($p < 0,05$) negu po 2 valandų.