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Cadmium ions inhibit activity of translation components and induce cell death in mouse liver

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The aim of this study was to determine a relationship between activity of $tRNA^{Leu}$ and leucyl-tRNA synthetase and cell death in cadmium-intoxicated mouse liver. 24 h after intoxication of mice with a sublethal dose of cadmium ions (1.6 mg per 1 kg of body mass) the activity of leucyl-tRNA synthetase decreased by 30%, whereas the acceptor activity of mice liver $tRNA^{Leu}$ decreased by 54% as compared to the control. Experiments *in vitro* showed that the activity of mouse liver $tRNA^{Leu}$ and leucyl-tRNA synthetase were reduced by 98% and 70%, respectively, when a 20 μM concentration of cadmium ions was present in the reaction mixtures. It is proposed that tRNA molecule is more sensitive than aminoacyl-tRNA synthetase to cadmium action. According to data of a TUNEL assay, the number of apoptotic (TUNEL+) cells was the same in both Cd-intoxicated livers and control. Agarose-gel DNA electrophoresis revealed a diffusional pattern typical of randomly degraded DNA fragments. It is possible to assume that not apoptosis but rather necrosis is a principal mode for elimination of irreversibly damaged liver cells 24 h following cadmium intoxication.

Key words: cadmium, tRNA, leucyl-tRNA synthetase, apoptosis, necrosis

INTRODUCTION

The heavy metal cadmium (Cd), a well-known environmental hazard, exerts a number of toxic effects in humans and animals. It is recognized that an exposure to Cd can result in various pathologies including neoplasia, osteoporosis, irreversible renal tubular injury, anemia, etc. [1]. In Cd-affected cells, the system of protein synthesis (translation) is one of the targets of Cd ions. *In vivo*, the effects of Cd on protein synthesis can be masked by this ion-induced expression of heavy metal binding proteins such as metallothioneins and heat-shock proteins [2, 3]. It has been shown that toxic effects of Cd are dose- and time-dependent [4]. Recent investigations on the mechanisms of Cd-induced alterations of translation have been focused on both initiation and elongation stages of translation [5]. Almost no attention has been paid to the effects of those ions on the stage of aminoacylation. Thus, the influence of Cd on activities of aminoacylation components needs more detailed examination. Acting on the different sites of cellular metabolism, Cd can severely diminish cell viability and lead to cell death [6]. Interfering with death signal transduction pathways, Cd can induce apoptosis and necrosis, two extreme types of cell death [7]. The mechanisms that are behind Cd-induced cell death strongly depend on the type of cell. Experiments aimed to elucidate these mechanisms have been done on different types of tissues and cells. Therefore, their comparison can be misleading. The present study was designed to investigate the relationship between the effect of 24-h Cd intoxication on protein synthesis and cell-death induction in mouse liver.

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MATERIALS AND METHODS

White laboratory mice (20–25 g) were used for the study (License of government veterinary service for working with laboratory animals No 0028). Intoxication with Cd was performed by injection of a sublethal dose of $CdCl_2$ (1.6 mg Cd^{2+} per 1 kg of body mass) dissolved in physiological solution into the abdominal cavity of mice. Control animals received injection of the same volume of physiological solution. Isolations of total tRNAs and post-mitochondrial supernatant (source of leucyl-tRNA synthetase) from mouse liver has been described elsewhere [8].

The acceptor activity of $tRNA^{Leu}$ was determined in 100 µl of a reaction mixture containing 100 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 10 mM KCl, 4 mM ATP, 0.2 mM [^{14}C]-leucine, 250 µg protein of post-mitochondrial supernatant (source of leucyl-tRNA synthetase), and 50 µg total tRNAs. The reaction mixture was incubated at 37 °C for 20 min. The reaction was stopped by adding 0.2 ml ice-cold 10% trichloroacetic acid. Test tubes with reaction mixture were kept in an ice bath for 20 min to allow precipitate formation. The precipitate was collected on nitrocellulose filters and washed with 25–30 ml of ice-cold 5% trichloroacetic acid. Radioactivity was measured with a liquid scintillation counter. The acceptor activity of $tRNA^{Leu}$ was evaluated by the formation of [^{14}C]-leucyl-$tRNA^{Leu}$. Activity of leucyl-tRNA synthetase was measured in the post-mitochondrial supernatant according to the initial rate of $tRNA^{Leu}$ aminoacylation with [^{14}C]-labelled leucine [8]. Apoptosis of liver cells was immunohistochemically detected by the TUNEL assay using *in situ* a cell death detection kit, AP (Roche). Dewaxed, rehydrated and proteinase K permeabilised liver tissue sections were subjected to enzymatic *in situ* labeling of DNA strand breaks according to the manufacturers' instruction. DNA-se pretreated tissue sections were used as a positive control for TUNEL-assay validity. After counterstaining with eosin, the sections were analyzed with a light microscope (objective 20X). The number of positively-stained (TUNEL+) nuclei of liver cells was counted in randomly selected ten histological fields per section. The nonparametrical Kruskall–Wallis test was used for comparison between the groups. Results were expressed as mean ± SEM. Statistical significance was set at $p < 0.05$. Agarose-gel electrophoresis of nuclear DNA was carried out as described in [9].

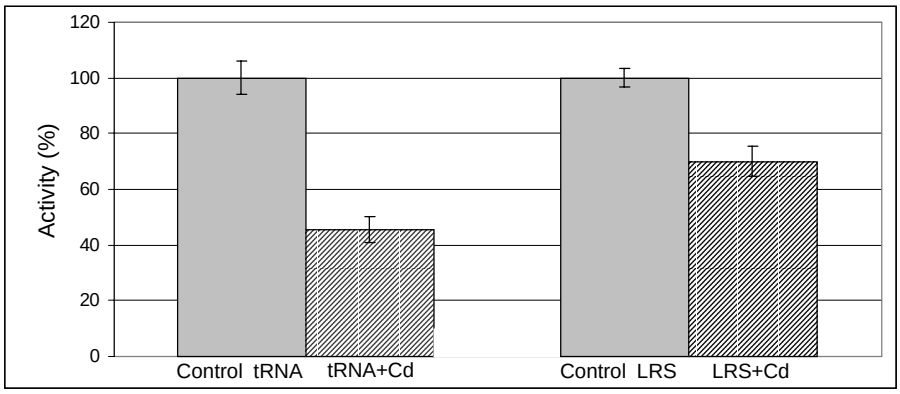

Fig. 1. Activity of $tRNA^{Leu}$ (tRNA) and leucyl-tRNA synthetase (LRS) isolated from mouse liver in norm (controls) and 24 h after injection of $CdCl_2$ solution (1.6 mg Cd^{2+} per 1 kg of body mass). The data represent results of 8–14 separate experiments. Differences between control and experimental groups are statistically significant. In control, the acceptor activity of $tRNA^{Leu}$ was as high as 1.45 ± 0.09 nmol Leu/mg tRNA, whereas the specific activity of leucyl-tRNA synthetase was equal to 200±7 pmol Leu/min/mg of protein

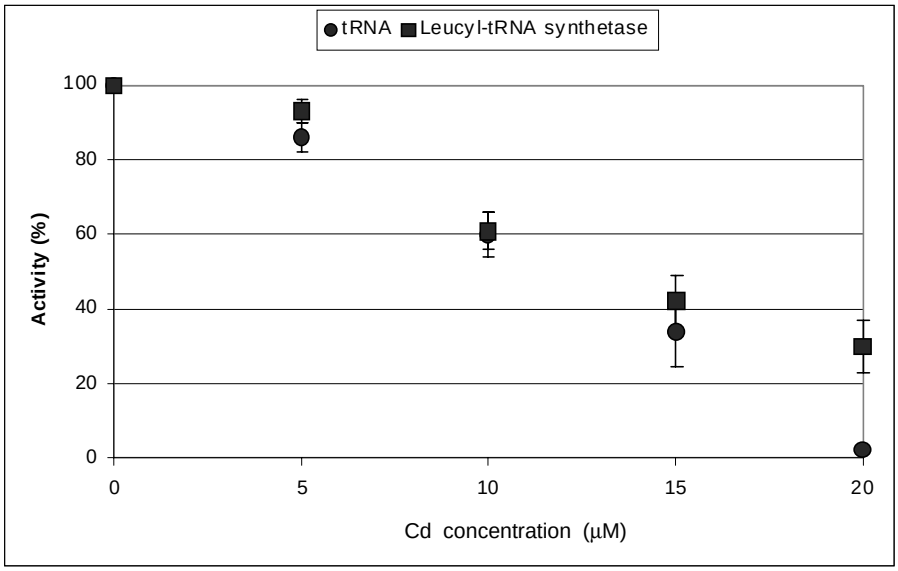

Fig. 2. Dependence of the effect of both mouse liver $tRNA^{Leu}$ and mouse liver leucyl-tRNA synthetase on the concentration of Cd ions *in vitro*. Data represent results of 6–8 separate experiments

RESULTS AND DISCUSSION

Activity of tRNA, one of the key components of translation machinery, isolated from normal mouse liver (control) and Cd-intoxicated mouse liver was compared. The effect of Cd ions on acceptor activity of $tRNA^{Leu}$ as a representative of all tRNA families is shown in Fig. 1. 24 h following injection of $CdCl_2$ solution, the acceptor activity of $tRNA^{Leu}$ decreased by 54% in respect to the control. Next, we examined the influence of Cd on activity of one of 20 aminoacyl-tRNA synthetases, namely leucyl-tRNA synthetase. The results indicated that activity of leucyl-tRNA synthetase was suppressed down to 70% of the control level 24 h after injection of $CdCl_2$ solution (Fig. 1).

Experiments *in vitro* were performed to evaluate the direct effect of Cd ions on the activity of

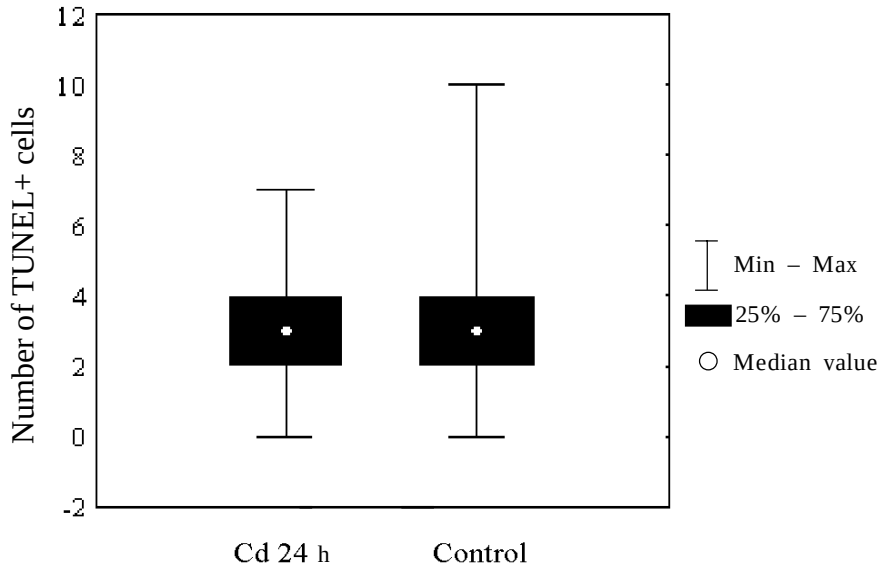

Fig. 3. Minimal, maximal, and median number of TUNEL+ cells in mouse liver 24 h after injection of $CdCl_2$ solution (1.6 mg Cd^{2+} per 1 kg of body mass). TUNEL+ cells were counted in 10 histological fields in each of 3 sections taken from experimental and control liver. Data represent results of 3 separate experiments

both $tRNA^{Leu}$ and leucyl-tRNA isolated from mouse liver. $CdCl_2$ solution was added into the reaction mixture containing either preparation of total tRNA or aminoacyl-tRNA synthetases. There were 5 μM, 10 μM, 15 μM, and 20 μM final concentrations of Cd ions in the reaction mixture. The effects of those ions on the acceptor activity of $tRNA^{Leu}$ and activity of leucyl-tRNA synthetase are shown in Fig. 2. According to our data, a 10 μM concentration of Cd caused a 40% diminution of both the acceptor activity of mouse liver $tRNA^{Leu}$ and activity of leucyl-tRNA synthetase *in vitro*. The acceptor activity of $tRNA^{Leu}$ was almost totally inhibited by a 20 μM concentration of Cd ions, whereas leucyl-tRNA synthetase activity made up 30% of the control level (Fig. 2). Thus, the results of our *in vivo* and *in vitro* studies have indicated that tRNAs are more susceptible to Cd-induced inhibition in comparison to aminoacyl-tRNA synthetase.

Whether the Cd-induced decrease of translation components' activity *in vivo* can correlate with the liver cell death we assessed according to the number of TUNEL+ cells detected in paraffin-embedded liver tissue sections. 24 h after $CdCl_2$ injection, the number of TUNEL+ cells did not differ from the control range (Fig. 3). Agarose-gel electrophoresis did not reveal DNA-laddering characteristic of apoptosis. The diffusional pattern observed in DNA electrophoresis (data not shown) can be considered as an evidence of extensive degradation of nuclear DNA, which occurs under necrosis of cells [10]. As reported in our recent study [4], after 24 h of Cd intoxication the level of total protein synthesis was significantly decreased *in vivo*. These data coincide with the time point of Cd-induced necrotic damage to liver tissue found in the present study. It can be assumed that cell-defense mechanisms against Cd such as expression of cytoprotective proteins [2, 3] and ability to withstand oxidative stress by maintaining glutathione reduced [6] are severely deteriorated by a 24-h intoxication with $CdCl_2$. As a consequence, irreversibly damaged cells are subjected rather to a necrotic than apoptotic mode of elimination.

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References

1. Waisberg M, Joseph P, Hale B et al. Toxicology 2003; 192(2–8): 95–117.
2. McKenna IM, Gordon T, Chen LC et al. Toxicol Appl Pharmacol 1998; 153(2): 169–78.
3. Goering PL, Fisher BR, Kish C. Toxicol Appl Pharmacol 1993; 122(1): 139–48.
4. Ivanov L, Sadauskiene I, Viezeliene D et al. Trace Elements and Electrolytes 2003; 20(3): 149–53.
5. Joseph P, Lei YX, Whong WZ, Ong TM. J Biol Chem 2002; 277(8): 6131–6.
6. Kim SC, Cho MK, Kim SG. Toxicol Lett 2003; 144(3): 325–36.
7. Habeebu SS, Liu J, Klaassen CD. Toxicol Appl Pharmacol 1998; 149(2): 203–9.
8. Rodovièius H, Vieþelienë D, Stanevièienë I et al. Medicina 2003; 39(7): 683–8.
9. Lesauskaite V, Epistolato MC, Ivanoviene L et al. Am J Clin Pathol 2004; 121(1): 108–16.
10. Hengartner MO. Nature 2000; 407(6805): 770–6.

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KADMIO JONØ POVEIKIS TRANSLIACIJOS KOMPONENTØ AKTYVUMUI IR SÀSAJOS SU PELËS KEPENØ LÀSTELIØ ÞÛTIMI

Santrauka

Ðio darbo tikslas yra nustatyti, ar kepenø làsteliø þûtis gali bûti siejama su transliacijos komponentø – $tRNA^{Leu}$ ir leucil-tRNR-sintetazës aktyvumo pokyèiu, praëjus 24 val. nuo peliø intoksikacijos kadmio jonais. Pagal tyrimo rezultatus, praëjus 24 val. po subletalios kadmio jonø dozës injekcijos (1,6 mg/kg kûno masës) pelës kepenø leucil-tRNR-sintetazës aktyvumas sumaþëjo 30%, o akceptorinis $tRNR^{Leu}$

aktyvumas – net 54% lyginant su kontrole. Remiantis i*n vitro* eksperimentø duomenimis, 20 mM kadmio jonø koncentracija inhibavo pelës kepenø $tRNR^{Leu}$ akceptoriná aktyvumà ir leucil-tRNR-sintetazës aktyvumà 98% ir 70% atitinkamai. Taigi tRNR inhibuojanèiam kadmio jonø poveikiui yra jautresnë negu aminoacil-tRNR-sintetazë. Kepenø làsteliø apoptozæ ávertinus pagal TUNEL reakcijos rezultatus, nustatyta, kad TUNEL teigiamø làsteliø skaièius kontroliniø ir kadmio jonais intoksikuotø peliø kepenø pjûviuose buvo vienodas. Atlikus kepenø DNR elektroforezæ agarozës gelyje, apoptozei bûdingø DNR „kopëèiø“ neaptikta, taèiau stebimas difuziðkas DNR fragmentø vaizdas. Tai bûdinga chaotiðkam DNR suirimui làsteliø nekrozës metu.

Raktaþodþiai: kadmis, tRNR, leucil-tRNR-sintetazë, apoptozë, nekrozë