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Influence of cadmium ions on the reduced glutathione and lipid peroxidation in the liver and red blood cells of mice

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The present investigation was undertaken to evaluate the influence of Cd on the reduced glutathione (GSH) and lipid peroxidation in liver and in red blood cells of mice. Experiments were done on outbred white laboratory mice using intraperitoneal injections of $CdCl_2$ solution (14 μmol Cd/kg body mass). The exposure-time was 2 h, 8 h, 24 h and 14 days. GSH was measured by reaction with 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) to give a compound that absorbs 412 nm light wavelength. Lipid peroxides were estimated by measuring thiobarbituric-acid-reactive substances and were expressed as malondialdehyde (MDA). Our results indicate that at the 8^{th} h the content of GSH in mice liver significantly increased by 35%, meanwhile in red blood cells the content of GSH exceeded the control level at the 2nd h, 8^{th} h and 24^{th} h by 19%, 35% and 18%, respectively. After 14 days of injections of $CdCl_2$ solution the content of GSH in liver and in red blood cells of mice decreased by 32% and 29% respectively, compared to the control group of mice. Further experiments were carried out in order to examine the influence of Cd ions on the content of MDA in liver and in red blood cells of mice after 2 h, 8 h, 24 h and 14 days of injections of $CdCl_2$ solution. The results indicate that at the 8^{th} h and 24^{th} h MDA content in mice liver was significantly increased by 236% and 118%, respectively. Meanwhile MDA content in mice red blood cells in all time points was at the control level.

Key words: oxidative stress, cadmium, glutathione, lipid peroxidation

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INTRODUCTION

Contamination of the environment with heavy metals and their potential toxicity, have received increasing interest. Concern about health effects of cadmium (Cd) mainly relates to the carcinogenic, teratogenic and mutagenic action of this metal. Cd has been recognized as one of the most toxic environmental and industrial pollutants (Shadi et al., 2001). The biological half-life in humans of this metal is more than 20 years. Progressive accumulation occurs, and there is no effective endogenous Cd elimination mechanism. The main storage organs of this metal are the liver and kidneys (Waisberg et al., 2003). The exposure to Cd results in various pathologies including neoplasia, osteoporosis, irreversible renal tubular injury, anemia, etc. (Kazantzis et al., 2004). Cd is a ubiquitous toxic metal that may induce oxidative damage by disturbing the prooxidant-antioxidant balance in tissues (Ognjanovic et al., 2008). This heavy metal stimulates free radical production, resulting in oxidative deterioration of lipids, proteins and DNA in humans and animals (Alam et al., 2000). Exposure to Cd initiates the cellular defense mechanism by upregulating the synthesis of sulfhydryl compounds such as glutathione (GSH) and metallothioneins (MT) (Bernotiene et al., 2012; Eybl et al., 2006).

The present study was conducted to evaluate the effect of Cd ions on the content of reduced glutathione and malondyaldehide (MDA) (marker of lipid peroxidation) in the liver and red blood cells of mice.

MATERIALS AND METHODS

Experiments were done on 4–6-week old outbred white laboratory mice weighing 20–25 g. All experiments were performed according to Law of the Republic of Lithuania on the Care, Keeping and Use of Animals (License of State Veterinary Service for Working with Laboratory Animals No. 0136).

We have chosen the model of acute single-dose injection (2 h, 8 h and 24 h) and the model of sub-acute prolonged 14-day injections of $CdCl_2$ solution (14 μmol Cd/kg body mass).

Determination of reduced glutathione in mice liver and in red blood cells

Determination of GSH concentration was carried out according to Moron (Moron et al., 1979). Removed mice livers were weighed and homogenized in 6 volumes (weight: volume) of 5% trichloracetic acid (TCA) solution. The homogenate was centrifuged at 10 000 × g for 7 min to obtain GSH-containing supernatant. 0.2 ml of the supernatant was combined with 2 ml of 0.6 mM DTNB in 0.2 M phosphate buffer (pH 8.0) and 0.8 ml of phosphate buffer were added to make the final volume of 3 ml. Light absorbance of the solution was detected at 412 nm of wavelength. A mixture of buffered DTNB solution containing 0.2 ml of 5% TCA was used as reference. GSH concentration was expressed as μmol/g of wet liver weight.

GSH in red blood cells was determined by method of Sedlak (Sedlak et al., 1968). For measurement 1 ml sample of the supernatant was added to 3 ml 0.4 M Tris-HCl buffer (pH 9.2) and 0.5 ml DTNB solution in ethanol (3.7 mg/1 ml). Color intensity was determined spectrophotometrically at wave 412 nm. GSH content in red blood cells was expressed in μmol/l.

Determination of malondialdehyde in mice liver and red blood cells

Lipid peroxides were estimated by measuring thiobarbituric-acid-reactive substances and were expressed as MDA content in nmol/g of wet liver weight (Uchiyama et al., 1978). The liver was removed and homogenized with 9 volumes (weight: volume) of cold 1.15% KCl to make 10% homogenate. 3 ml of 1% H_3PO_4 and 1 ml of 0.6% thiobarbituric acid aqueous solutions were added to 0.5 ml of this homogenate. The mixture was heated for 45 min in a boiling water bath. After cooling, 4 ml of n-butanol were added and mixed vigorously. The butanol phase was separated by centrifugation. The light absorbance of supernatant was determined at 535 and 520 nm wavelength.

For MDA determination in red blood cells the samples were combined of 2 ml H_2O, 0.1 ml red blood cells mass, 1 ml 10% trichloracetic acid, 2 ml of 0.5% TBA and mixed well with a glass rod. The samples were placed in the boiling water bath for 30 min. After cooling, the samples were centrifuged at 3 000 × g for 15 min; the supernatant absorbance was evaluated at 540 nm. MDA in red blood cells expressed as μmol/l (Stalnaja et al., 1977).

Statistical analysis

All results were expressed as the mean ± standard error of mean. Statistical significance was set at $p < 0.05$. Statistical analysis was performed using a statistical software package (Statistica 6.0).

RESULTS

Specific functions of GSH may be elucidated by studies in which the cellular levels of GSH are experimentally decreased or increased. In the present work we have used this approach to learn whether this tripeptide protects against Cd toxicity. GSH forms complexes with several heavy metals and thus might function in protection of cells against metal toxicity (Kowalewska et al., 2001).

The effects of Cd ions on the content of GSH in mice liver and red blood cells after 2 h, 8 h, 24 h and 14 days of injections of $CdCl_2$ solution are shown in Fig. 1. Our results indicate that at the 8th h the content of GSH in mice liver significantly increased by 35%, meanwhile in red blood cells the content of GSH exceeded the control level at the 2nd h, 8th h and 24th h by 19%, 35% and 18%, respectively. After 14 days of injections of $CdCl_2$ solution, the content of GSH in the liver and red blood cells of mice decreased by 32% and 29% as compared to the control mice group, respectively.

Some toxic effects of Cd seem to be indirect and due, at least in part, to oxidative stress promoted in response to this ion. As a result, lipid peroxidation can occur. This is a chain reaction in which polyunsaturated fatty acids of cell membranes are oxidized through C- and O-centered radicals and hydroperoxy-intermediates to yield various products, including epoxy-fatty acids, alkanes, alkenes and aldehydes (e. g. MDA). Determining the level of

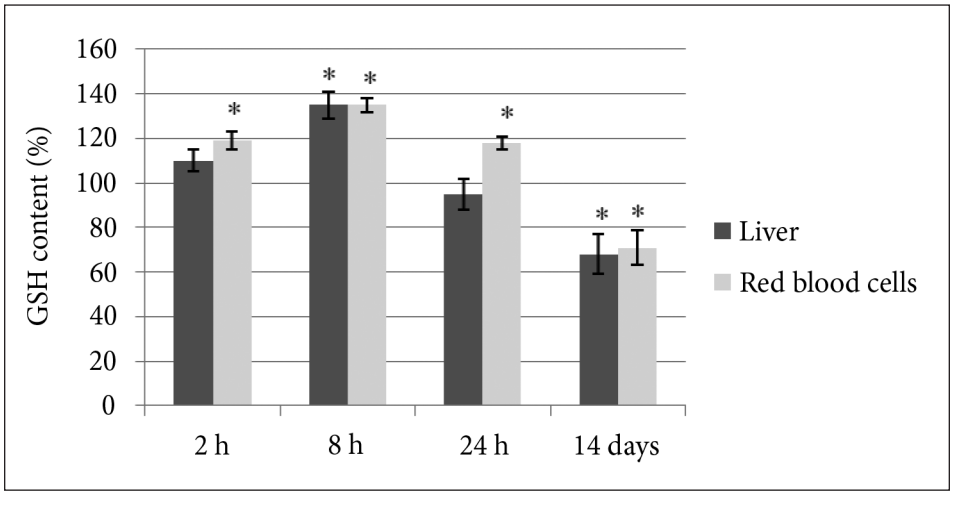

Fig. 1. Dependence of reduced glutathione content in liver and red blood cells of mice on the time of exposure to Cd ions. The content of GSH after single injection and 14 days of exposure in the liver (4.5 μmol/g and 4.9 μmol/g, respectively) and in red blood cells (604 μmol/l and 721 μmol/l, respectively) of control mice group was set at 100%. * = $p < 0.05$ compared to the control mice group. The data represent results of 8–10 separate experiments

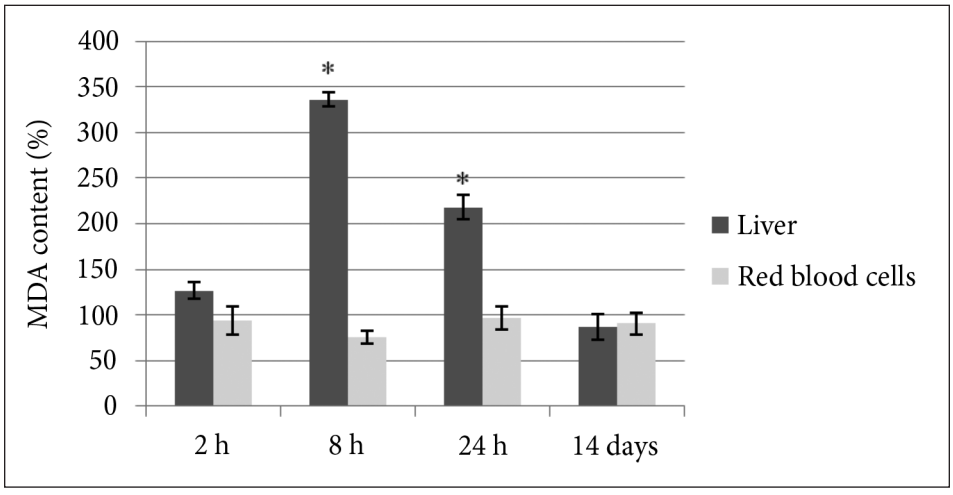

Fig. 2. Dependence of malondialdehyde content in liver and red blood cells of mice on the time of exposure to Cd ions. The content of MDA after single injection and 14 days of exposure in the liver (77 nmol/g and 124 nmol/g, respectively) and in red blood cells (287 μmol/l and 244 μmol/l, respectively) of control mice group was set at 100%.* = $p < 0.05$ compared to the control mice group. The data represent results of 8–10 separate experiments

MDA is usually the most practical and reliable method for detecting and screening oxidative stress (Nair et al., 2008).

Further experiments were carried out in order to examine the influence of Cd ions on the content of MDA in the liver and red blood cells of mice after 2 h, 8 h, 24 h and 14 days of injections of $CdCl_2$ solution (Fig. 2).

The results indicate that at the 8th h and 24th h, MDA content in mice liver was significantly increased by 236% and 118%, respectively. Meanwhile MDA content in mice red blood cells in all time points was at the control level.

DISCUSSION

Our experiments showed that Cd ions increased the content of GSH in mice liver (at the 8th h) and red blood cells (at the 2nd h, 8th h and 24th h) after injection of $CdCl_2$ solution. GSH content increase may be linked to increased activities of GSH-dependent enzymes, including glutathione peroxidase, glutathione reductase and γ-glutamylcysteine synthetase, which is the rate-limiting enzyme for the synthesis of GSH (Waisberg et al., 2003). After 14 days of injections of $CdCl_2$ solution, GSH content decreased in mice liver and red blood cells. Cd ions cause an increase in reactive oxygen species (ROS), which cause irreversible damage to various biomolecules. ROS cause a substantial decrease in GSH content along with depletion of other defenses, such as superoxide dismutase and catalase (Kowalewska et al., 2001; Waisberg et al., 2003). Thus, 14 days exposure to Cd ions is associated with depletion of hepatic and red blood cells content of GSH. The hepatic GSH depletion may also be associated with the protection against endogenous oxygen radicals and the synthesis of MT. Although MT synthesis requires cysteine, which may be derived from the breakdown of GSH, there is no evidence that GSH itself has an additional function in MT formation (Bernotiene et al., 2012). GSH depletion leads to cell death, and may be the ultimate factor determining vulnerability to oxidant attack (El-Marghy et al., 2001).

Lipid peroxidation is one of the main manifestations of oxidative damage and has been found to play an important role in the toxicity of many xenobiotics (Kara et al., 2005). The data obtained in our study (see Fig. 2) confirm

that acute Cd ions intoxication causes early oxidative stress in mice liver as demonstrated by increase in MDA content – at the 8th h and 24th h by 236% and 118%, respectively, and it may be linked to inactivation or decrease of levels of detoxifying antioxidant enzymes (catalase, superoxide dismutase, glutathione reductase and glutathione peroxidase) involved in antioxidant defense system by binding to their sulfhydryl groups, inducing an increased production of free radicals (hydroxyl radicals, superoxide anions, nitric oxide, hydrogen peroxide). Lipid peroxidation is, in turn, a source of ROS (Bagchi et al., 2000).

At acute Cd ions intake, the induction of lipid peroxidation has been reported to be the reason for Cd ions intoxication and has hence been demonstrated to cause generation of free radicals in early Cd ions intoxication, but if the metal effect is maintained for a longer period of time (2 weeks), the content of MDA decreases. It may be related to the adaptation of cells to Cd ions exposure (El-Maraghy et al., 2001; Ognjanovic et al., 2008). However, in our experiments we have failed to get statistically significant decrease in MDA content in mice liver and red blood cells (Fig. 2) after 14 days of Cd ions exposure. It may be that in our experimental conditions inhibition of lipid peroxidation starts at a later time.

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References

1. Alam J, Wicks C, Stewart D, Gong P, Touchard C, Otterbein S et al. Mechanism of heme oxygenase-1 gene activation by cadmium in MCF-7 mammary epithelial cells. J Biol Chem 2000; 275: 27694–702.
2. Bagchi D, Joshi SS, Bagchi M, Balmoori J, Benner EJ, Kuszynski CA et al. Cadmium- and chromium-induced oxidative stress, DNA damage, and apoptotic cell death in cultured human chronic myelogenous leukemic K562 cells, promyelocytic leukemic HL-60 cells, and normal human peripheral blood mononuclear cells. J Biochem Mol Toxicol 2000; 14: 33–41.
3. Bernotiene R, Ivanoviene L, Sadauskiene I, Liekis A, Ivanov L. Influence of cadmium ions on the antioxidant status and lipid peroxidation in mouse liver: protective effects of zinc and selenite ions. Trace Elem Electrolyt 2012; 29(2):113–6.
4. El-Maraghy SA, Gad MZ, Fahim AT, Hamdy MA. Effect of cadmium and aluminum intake on the antioxidant status and lipid peroxidation in rat tissues. J Biochem Mol Toxicol 2001; 15: 207–14.
5. Eybl V, Kotyzova D, Koutensky J. Comparative study of natural antioxidants – curcumin, resveratrol and melatonin – in cadmium-induced oxidative damage in mice. Toxicology 2006; 225:150–6.
6. Kara H, Karatas F, Canatan H. Effect of single dose cadmium chloride administration on oxidative stress in male and female rats. Turk J Vet Anim Sci 2005; 29: 37–42.
7. Kazantzis G. Cadmium, osteoporosis and calcium metabolism. Biometals 2004 Oct; 17(5): 493–8.
8. Kowalewska RS. Cadmium distribution and toxicity in tissues. Microsc Res Tech 2001; 55: 208–22.
9. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione s-transferase activities in rat lung and liver. Biochem Biophys Acta 1979; 582: 67–78.
10. Nair V, O'Neil CL, Wang PG. Malondialdehyde. Encyclopedia of reagents for organic synthesis. John Wiley & Sons, New York; 2008.
11. Ognjanovic BI, Markovic SD, Pavlovic SZ, Zikic RV, Stajn AS, Saicic ZS. Effect of chronic cadmium exposure on antioxidant defense system in some tissues of rats: protective effect of selenium. Physiol Res 2008; 57: 403–11.
12. Sedlak J, Lindsey RH. Estimation of total protein-bound and nonprotein sulphydryl groups in the tissue with Ellman reagent.

Analyt Biochem 1968; 25: 192–205.

13. Shadi AH, Minder S, Keith GJ, Ari MI, Janet TP. Cadmium accumulation in aortas of smokers. Arterioscler Thromb Vasc Biol 2001; 21: 863–7.
14. Uchiyama M, Mihara M. Determination of malondialdehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 1978; 86: 271–8.
15. Waisberg M, Joseph P, Hale B, Beyersmann D. Molecular and cellular mechanisms of cadmium carcinogenesis. Toxicology 2003; 192: 95–117.
16. Stalnaja ID, Garisvili TG. Metod opredelenija malonovogo dialdegida s pomoscju tiobarbiturovoi kisloty. Sovremenye metody v biochimii. M: Medicina 1977: 660–8.

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KADMIO JONŲ POVEIKIS REDUKUOTAM GLUTATIONUI IR LIPIDŲ PEROKSIDACIJAI PELIŲ KEPENYSE IR KRAUJYJE

Santrauka

Siekiant įvertinti kadmio (Cd) jonų poveikį redukuotam glutationui (GSH) ir lipidų peroksidacijai pelių kepenyse ir kraujyje, baltosioms laboratorijos pelėms į pilvo ertmę buvo suleistas $CdCl_2$ tirpalas (14 μmol Cd/kg kūno masės). Organizmas metalo jonais buvo veikiamas 2 val., 8 val., 24 val. ir 14 dienų. GSH koncentracija nustatyta reakcijos mišinyje su 5,5‘-ditiobis(2-nitrobenzoine rūgštimi) (DTNB) spektrofotometriškai matuojant sugertį ties 412 nm banga. Lipidų peroksidacija nustatyta vertinant lipidų peroksidacijos žymens malondialdehido (MDA) koncentraciją. Mūsų rezultatai rodo, kad po 8 val. GSH koncentracija pelių kepenyse statistiškai reikšmingai padidėjo 35 %, o kraujyje GSH koncentracija viršijo kontrolės lygį – po 2 val. – 19 %, po 8 val. – 35 % ir po 24 val. – 18 %. Praėjus 14 dienų po $CdCl_2$ tirpalo sušvirkštimo, GSH koncentracija, palyginti su kontrolinių pelių grupe, kepenyse ir kraujyje sumažėjo atitinkamai 32 % ir 29 %. Tolesniais eksperimentais siekta nustatyti Cd jonų poveikį MDA koncentracijai pelių kepenyse ir kraujyje praėjus 2 val., 8 val., 24 val. ir 14 dienų po $CdCl_2$ tirpalo sušvirkštimo. Gauti rezultatai rodo, kad po 8 val. ir 24 val. MDA koncentracija pelių kepenyse statistiškai reikšmingai padidėjo atitinkamai 236 % ir 118 %, o MDA koncentracija pelių kraujyje visais tirtais laiko tarpais buvo kontrolės lygio.

Raktažodžiai: oksidacinis stresas, kadmis, glutationas, lipidų peroksidacija