Spectrofotometrical determination of reduced glutathion and lipid peroxidation in liver of cadmium and zinc treated mice

K. Čijauskaitė, R. Bernotienė, L. Ivanov

Neurosciences Institute and Department of Biochemistry, Medical Academy, Lithuanian University of Health Sciences, Lithuania

E-mail: cijauskaite.kristina@gmail.com

Introduction. Contamination of the environment with heavy metals, and their potential toxicity, has received increasing interest. Cadmium (Cd) has been recognized as one of the most toxic environmental and industrial pollutants [1]. The biologic half-life in humans of this metal is more than 20 years. Cd is a ubiquitous toxic metal that may induce oxidative damage by disturbing the prooxidant-antioxidant balance in tissues [2]. This heavy metal stimulates free radical production, resulting in oxidative deterioration of lipids, proteins and DNA in humans and animals [3]. Exposure to Cd initiates the cellular defence mechanism by upregulating the synthesis of sulfhydryl compounds such as glutathione (GSH) and metallothioneins [4].

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 [5]. Zn is an essential catalytic and structural cofactor for many enzymes and other proteins. While Zn^{2+} is not redox active under physiological conditions, it has been known for many years that Zn deficiency causes increased oxidative stress and, consequently, increased oxidative damage to DNA, proteins, and lipids. These results have indicated that Zn plays an indirect antioxidant role and that dietary inadequacy may contribute to human diseases such as cancer [6].

The present study was conducted to evaluate the effect of Cd and Zn ions on the content of GSH and malondyaldehide (MDA) (marker of lipid peroxidation) in the mice liver.

Materials and methods. Experiments were done on 4-6-week-old outbred white laboratory mice weighing 20 - 25 g. All experiments performed according to the Republic of Lithuania Law 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 days injections of $CdCl_2$ (14 μmol Cd/kg body mass) or $ZnSO_4$ solution (24 μmol) Zn/ kg body mass). Control animals received i.p. injection of the same volume of physiological solution.

Determination of reduced glutathione in mice liver. Determination of GSH content was carried out according to Moron [7]. Removed mice livers were weighed and homogenized in 6 volumes (weight:volume) of 5%

trichloroacetic acid (TCA) solution. The homogenate was centrifuged at 10,000 xg 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. The supernatant absorbance was determined spectrophotometrically at wave 412 nm. The content of GSH in mice liver was calculated using the following formula (1):

$$C = A_{412} \cdot 13600 \ (\mu moles/g \ of \ wet \ liver \ weight), \qquad (1)$$

where A_{412} – supernatant absorbance; 13600 – the molar absorption coefficient.

Determination of malondialdehyde in mice liver. Lipid peroxides were estimated by measuring thiobarbituric-acid-reactive substances [8]. 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 was added and mixed vigorously. The butanol phase was separated by centrifugation and supernatant absorbance was determined at 535 and 520 nm. The content of MDA was calculated from the calibration curve (1.1.3.3-tetrahydroxypropane was used as a standard) and expressed as nmoles/g of wet liver weight.

Statistical analysis. The data are expressed as the mean ± SEM. Differences among means were analyzed by one-way ANOVA. $p < 0.05$ value was considered statistically significant (SPSS version 19.0, SPSS).

Results. GSH is the most important nonprotein thiol, and is involved in numerous biochemical pathways. GSH is important in protecting cells against damage from radiation, free oxygen radicals, heat, and sulfhydryl reactive agents, and provides the bulk of sulfhydryl groups for the detoxication of electrophilic xenobiotics [9].

The effects of metal ions on the content of GSH in mice liver after 2, 8, 24 h and 14 days of injections of $CdCl_2$ or $ZnSO_4$, solution(s) are shown in Fig. 1. Our results showed that at the 8th h the content of GSH in mice liver significantly increased by 35% after injection of $CdCl_2$ solution and 27% after injection of $ZnSO_4$ solution as compared to control mice group. After 14 days the content of GSH in mice liver was decreased by 32% after injection of $CdCl_2$ solution as compared to control.

The lipid peroxidation process 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 MDA is usually the most practical and reliable method for detecting and screening oxidative stress [10].

The effects of metal ions on the content of MDA in mice liver after 2, 8, 24 h and 14 days of injections of $CdCl_2$ or $ZnSO_4$, solution are shown in Fig. 2.

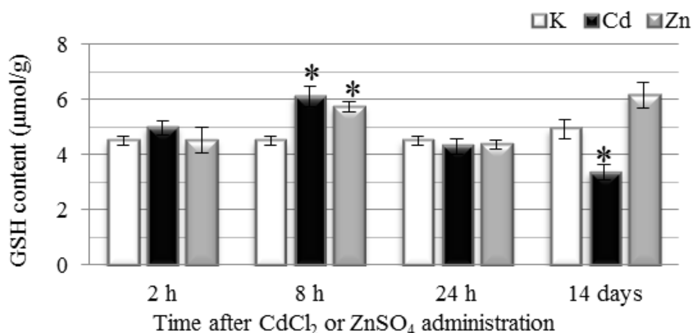

Fig. 1. Dependence of reduced glutathione content in liver of mice on the time of exposure to Cd ions, Zn ions and control group

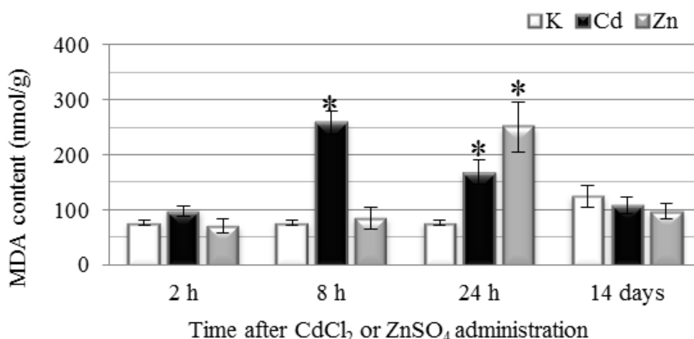

Fig. 2. Dependence of malondialdehyde content in liver of mice on the time of exposure to Cd ions, Zn ions and control group

Our results indicate that after 8 and 24 h of $CdCl_2$ injection, the content of MDA in mice liver was significantly increased by 236% and 118%, respectively, as compared to control mice group. Moreover, after 24 h of $ZnSO_4$ injection, MDA content was significantly increased by 226% as compared to the control.

References

1. Shadi A.H., Minder S., Keith G.J., Ari M.I., Janet T.P. Cadmium accumulation in aortas of smokers // Arterioscler Thromb Vasc Biol 2001;21:-P. 863-7.

2. Ognjanovic B.I., Markovic S.D., Pavlovic S.Z., Zikic R.V., Stajn A.S., Saicic Z.S. Effect of chronic cadmium exposure on antioxidant defense system in some tissues of rats: protective effect of selenium // Physiol Res. 2008; 57: -P. 403-11.

3. 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:-P. 27694-702.

4. 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 Elemnts and Electrolytes 2012;29(2):-P. 113-6.
5. Prasad A.S. Zinc: an overwiew // Nutrition 1995;11:-P. 93–9.
6. Eide D.J. The oxidative stress of zinc deficiency // Metallomics. 2011;3:-P. 1124-9.
7. Moron M.S., Depierre J.W., Mannervik. Levels of glutathione, glutathione reductase and glutathione s-transferase activities in rat lung and liver // Biochim Biophys Acta 1979;582:-P. 67–78.
8. Uchiyama M., Mihara M. Determination of malondialdehyde precursor in tissues by thiobarbituric acid test // Anal Biochem 1978;86:-P. 271–8.
9. Pompella A., Visvikis A., Paolicchi A., De Tata V., Casini A.F. The changing faces of glutathione, a cellular protagonist // Biochem Pharmacol 2003;66:-P. 1499–503.
10. Del Rio D., Stewart A.J., Pellegrini N. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress // Nutr Metab Cardiovasc Dis 2005;15:-P. 316–28.

Spectrofotometrical determination of reduced glutathion and lipid peroxidation in liver of cadmium and zinc treated mice

K. Čijauskaitė, R. Bernotienė, L. Ivanov
Neurosciences Institute and Department of Biochemistry, Medical Academy, Lithuanian University of Health Sciences, Lithuania

The present investigation was undertaken to evaluate the influence of Cd and Zn on the content of GSH and MDA. Experiments were done on outbred white laboratory mice using intraperitoneal injections of $CdCl_2$ or $ZnSO_4$ solution. The exposure-time was 2 h, 8 h, 24 h and 14 days. Our results showed that after 8 h the content of GSH was increased by 35% after exposure to Cd and 27% after exposure to Zn. After 14 days the content of GSH in mice liver was decreased by 32% after exposure to Cd. The content of MDA was increased after 8 h by 236% and 24 h by 118% after exposure to Cd. Moreover, 24 h after exposure to Zn, MDA content was increased by 226% as compared to the control.