ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXIII, N 4, 32 SECTIO DDD 2010

[1]Danylo Halytskyi Lviv National Medical University,
[2]Ivan Franko Lviv National University, Ukraine

OKSANA PERSHYN[1], ZYNOVIY VOROBETS[1], OKSANA KONOVART[1], HALYNA ANTONJAK[2]

Effect of Pb^{2+} upon antioxidant enzymes activity of rat lymphocytes

Wpływ Pb^{2+} na aktywność enzymów antyoksydacyjnych limfocytów szczurów

INTRODUCTION

High demand for Pb^{2+} in industry during recent years has cased the pollution of the environment by it. It has created a danger for Pb^{2+} human organism [10]. Lead, like other heavy metals, is immunosuppressive [2, 7]. Stimulation of generation of the reactive oxygen species (ROS) plays a very important role in the mechanisms of the toxic effect of Pb^{2+}. ROS stimulate the activation of lipid peroxidation of the biomolecules [1, 4]. As a result, oxidative stress is developed [4, 12]. It is an interesting problem because Pb^{2+} causes immunocompetent cells' abnormalities [2, 7].

The main aim of the present study was to investigate the effect of Pb^{2+} upon antioxidant enzymes activity of rat lymphocytes after the single dosing of lead acetate.

MATERIAL AND METHODS

*White m*ale rats (120 days old) were divided into control (10 animals) and experimental (15 animals) groups. Rats from the experimental group were single dosed 10 mg/ kg bogy mass, control rats were injected a corresponding volume of the physiological solution. Rats were killed by decapitation on the 1st, 3rd and 10th days after injection. Lymphocytes were removed from peripheral blood of the rats by Ficol and Verografin gradient (method of the differential centrifugation) [3].

Measurements were performed at the lysates, after the three times freeze-thaw process. Superoxide dismutase (SOD) activity was quantified by the level of inhibition of the process of reduction of 4-nitrotetrazolium chloride blau in the presence of NADH and phenazin methosulphate [5]. Glutathione peroxidase activity was performed by the glutathione oxidation rate in the presence of tertiary butyl hydroperoxide [9]. Glutathione reductase activity was measured by the reset rate in the presence of NADPH [14]. Measurements of the malondialdehyde (MDA) were determined by the

method that is based on the reaction MDA with tiohiobarbituric acid [8]. Lipid hydroperoxide was measured using ammonium tiocianate [11]. All data are presented as mean with standard error (SE).

RESULTS AND DISCUSSION

The results suggest intensification of the generation of ROS and lipid peroxidation under the influence of lead. The maximal effect occurred in the earlier period of the animals' intoxication. For the first tree days after injection, the concentration of MDA and lipid hydroperoxides rose steadily. On the 1st day their concentration increased up to 45.2% and 36.5% and on the 3rd day – up to 53.1% and 44%, correspondingly (the changes are significant). However on the 10th day, the concentration of the products of lipid peroxidation became normal.

The effect of Pb^{2+} upon the metabolic answer of the antioxidant enzymes of lymphocytes shows some peculiarities. Enzymatic activity of the peroxidation-defense enzyme, SOD, was significantly decreased on the 3rd and 10th days after injections of lead acetate (Table 1). This process was accompanied by the inhibition of glutathione reductase activity on the 3rd day ($p < 0.05$). Obviously, the continued activity of glutathione peroxidase depends on the regeneration of reduced glutathione by glutathione reductase. According to our results, glutathione peroxidase activity of lymphocytes was stable during the operation. What is more, this enzymatic activity was slightly increased on the 3rd day (the difference is not significant).

Table 1. Effect of Pb^{2+} upon antioxidant enzymes activity of rat lymphocytes (M ± m, n = 5)

Enzyme	Control	The period after injection of lead acetate		
		1	3	10
Superoxide Dismutase, U/mg of the protein	8.90 ± 0.50	7.32 ± 0.71	7.20 ± 0.40*	7.03 ± 0.25*
Glutathione Peroxidase, nmol of Glutathione per min per mg of protein	20.50 ± 1.34	20.11 1.92	24.78 ± 1.24	23.62 ± 2.41
Glutathione Reductase, nmol of NADPH per min per 1 mg of protein	14.85 ± 0.51	14.55 ± 1.57	12.30 ± 0.62*	12.60 ± 1.25

* Variation comparing to control group are significant ($p < 0.05$, M ± m, n = 5)

These observations show some features of the enzymatic systems that scavenge reactive oxygen species to prevent internal cellular damage, comparing to the other cells (erythrocytes, etc). As is well known, the rate of metabolism of glutathione in the lymphocytes is very intensive. For that reason, the intracellular concentration of the glutathione-defense enzymes remains on the fixed level, most of all for glutathione peroxidase [15]. Simultaneously, such a level of glutathione peroxidase activity has compensatory influence in conditions of inhibition of SOD activity in the intoxicational lymphocytes. Thus, it may explain the low sensitivity of lymphocytes to oxidative stress which was caused by Pb^{2+}, comparing to other blood cells.

Results of the study of the effects of lead acetate (single injection in a dose 10 mg/kg bogy mass) on the indices of lipid peroxidation and activities of enzymes of antioxidant system in lymphocytes

of white rats are presented in the article. It was established, that under the influence of lead cations the activities of superoxide dismutase and glutathione reductase decreased, while glutathione peroxidase activity did not change significantly. These effects were accompanied by accumulation of the products of lipid peroxidation (malondialdehyde and lipid hydroperoxides) in rats' lymphocytes during the initial period of intoxication with heavy metal and normalisation of their concentrations on the 10^{th} day after injection.

REFERENCES

1. Beckman K., Ames B.N.: The free radical theory of aging matures. Physiol. Rev., 78, 547, 1998.
2. Biser J.A., Vogel L.A., Berger J. et al.: Effects of heavy metals on immunocompetence of white-footed mice (*Peromyscus leucopus*). *J. Wildlife Dis.,* 40, 173, 2004.
3. Boyum A.A.: A one- stage procedure for isolation of granulocytes and lymphocytes from human blood. Scand. J. Clin. Lab. Invest., 21, 51, 1968.
4. Droge W.: Free radicals in the physiological control of cell function. Physiol. Rev., 82, 47, 2002.
5. Dubinina E.E., Salnikova L.A., Efimova L.F.: Activity and isoenzymatic spectrum of SOD of human erythrocytes. Lab. Affair, 10, 30, 1983.
6. Jurczuk M., Moniuszko-Jakoniuk J., Brzoska M.: Involvement of some low-molecular thiols in the peroxidative mechanisms of lead and ethanol action on rat liver and kidney. Toxicol., 219, 11, 2006.
7. Koller L.D.: The immunotoxic effects of lead in lead-exposed laboratory animals. In: Annals of the New York Academy of Sciences. G.T.Keusch, A.Cerami, and F.Takaku (eds.). New York Academy of Sciences, 587, 160, 1990.
8. Korobeinikov E.N.: Modification of the measurence. Lab. Affair, 7, 8, 1989.
9. Moin V.M.: Simple method of the measurence glutathione peroxidase activity of the erythrocytes. Lab. Affair, 12, 724, 1986.
10. Needleman H.: Lead poisoning. Ann. Rev. Med., 55, 209, 2004.
11. Orechovich V.N.: Modern biochemical methods. M: Medicine, 391, 1977.
12. Pagliara P., Carla E.C., Caforio S. et al.: Kupffer cells promote lead nitrate-induced hepatocyte apoptosis via oxidative stress. Comp. Hepatol., 2, 8, 2003.
13. Pershyn O.I., Antonyak H.L.: Effects of lead cations on lipid peroxidation and activities of enzymes of antioxidant system in erythrocytes of white rats. Exp. Clin. Physiol. Biochem., 3, 19, 2005.
14. Pinto R.E., Bartley W.: The effect of age and sex on glutathione reductase and glutathione peroxidase activities and on aerobic glutathione oxidation in rat liver homogenates. Biochem. J., 112, 109, 1969.
15. Umansky V., Rocha M., Breitkreutz R. et al.: Glutathione is a factor of resistance of Jurkat leukemia cells to nitric oxide-mediated apoptosis. J. Cell. Biochem., 78, 578, 2000.

SUMMARY

The main aim of the present study was to investigate the effect of Pb^{2+} upon antioxidant enzymes activity of rat lymphocytes after the single dosing lead acetate. It was established that under the influence of lead cations the activities of superoxide dismutase and glutathione reductase decreased,

while glutathione peroxidase activity did not change significantly. These effects were accompanied by accumulation of the products of lipid peroxidation (malondialdehyde and lipid hydroperoxides) in rats' lymphocytes during the initial period of intoxication with heavy metal and normalisation of their concentrations on the 10^{th} day after injection.
Keywords: Pb^{2+}, lymphocytes, lipid peroxidation, antioxidant system

STRESZCZENIE

Celem badań była ocena wpływu Pb^{2+} na aktywności enzymów antyoksydacyjnych limfocytów szczura po jednokrotnym podaniu octanu ołowiu. Wykazano, że pod wpływem kationów ołowiu aktywności dysmutazy nadtlenkowe i reduktazy glutationu zmniejszały się, podczas gdy peroksydazy glutationu nie zmieniały się istotnie. Tym efektom towarzyszyła akumulacja produktów peroksydacji lipidów (malondialdehydu i wodoronadtlenków lipidów) w limfocytach szczurów w trakcie początkowego etapu intoksykacji metalem ciężkim, a następnie normalizacja ich stężeń w 10 dniu po podaniu.

Słowa kluczowe: Pb^{2+}, limfocyty, peroksydacja lipidów, system antyoksydacyjny