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

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

A high demand for Pb^{2+} in industry in recent years has caused pollution of the environment with it. It created a danger of Pb^{2+} entering a human organism [10]. Lead, as other heavy metals, is immunosuppressive [2, 7]. Stimulation of generation of 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, the oxidative stress was developed [4, 12]. It is an interesting problem because Pb^{2+} causes abnormalities of immunocompetent cells' [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 a single dose 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 body mass, while 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 differential centrifugation) [3].

Measurements were performed at the lysates, after triple times freeze-thaw process. Superoxide Dismutase (SOD) activity was quantified by the level of inhibiton 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 malondialdehyde (MDA) were determined by the method that is based on the reaction of 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 three days after injection, the concentration of the MDA and lipid hydroperoxides was steadily rising. 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 metabolic answer of the antioxidant enzymes of lymphocytes shows some peculiarities. The enzymatic activity of the peroxidation-defense enzyme, SOD, was significantly decreased on the 3rd and 10th days after injections of lead acetate (Table).

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

Enzyme	Control	The term 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 is significant ($p < 0.05$, M ± m, n = 5)

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).

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 persists on a fixed level, most of all for glutathione peroxidase [15]. Simultaneously, such a level of glutathione peroxidase activity has a compensatory influence in conditions of inhibition of SOD activity in intoxicational lymphocytes. Thus, it may explain the low sensitivity of lymphocytes to oxidative stress that was caused by Pb^{2+}, comparing the other blood cells.

Results of the study of the effects of lead acetate (single injection in a dose 10 mg/kg body mass) on the indices of lipid peroxidation and activities of enzymes of antioxidant system in lymphocytes of white rats are presented in the article.

CONCLUSIONS

It was established that under the influence of lead cations in lymphocytes of white rats the activity 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 10th day after injection.

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SUMMARY

It was established that under the influence of lead cations in lymphocytes of white rats the activity 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.

STRESZCZENIE

Stwierdzono, że pod wpływem kationów ołowiu zmniejszała się aktywność dysmutazy nadtlenkowej i reduktazy glutationu w limfocytach szczurów, podczas gdy aktywność peroksydazy glutationu nie ulegała zmianie. Temu zjawisku towarzyszyła akumulacja produktów lipoperoksydacji (MDA i nadtlenków lipidów) w szczurzych limfocytach w okresie wczesnej intoksykacji, zaś w 10 dniu od podania metalu ciężkiego stwierdzano normalizację ich zawartości.