

Oxidative stress assessment in lung of lead-exposed rats

Ocena stresu oksydacyjnego w płucach szczurów narażonych na ołów

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ABSTRACT

Objective. Little is known about lead toxic effects on lung tissue. Therefore, the present study was undertaken to estimate the values of selected parameters related to oxidative stress in the lung of rats exposed to lead acetate.

Material and methods. Twenty male Wistar rats were randomly divided into two groups: control (n=10) and lead-treated (n=10). The lead-treated group was fed with regular rat chow and distilled water supplemented with lead acetate (1200 ppm) for 7 days. In lung homogenates, the level of malondialdehyde (MDA) and the activities of superoxide dismutase (SOD, CuZn-SOD, Mn-SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-S-transferase (GST) were determined.

Results. The activity of catalase was significantly higher in the lung of lead-treated rats than in controls by 98%. Similarly, the activities of GPx and GR were higher by 57% and 45%, respectively. The level of MDA was also higher in lead-exposed rats than in the control group by 52%. The remaining parameters did not differ between the studied groups.

Conclusions. Lead exposure stimulates antioxidant defense mechanisms in rat lung; however, these mechanisms are insufficient to prevent lead-induced oxidative stress development.

Key words: lead poisoning, oxidative stress, lung

STRESZCZENIE

Wstęp. Toksyczny wpływ ołowiu na tkankę płucną jest słabo poznany. W związku z tym, prezentowany eksperyment miał na celu ocenę zmian w wybranych parametrach związanych ze stresem oksydacyjnym w tkance płucnej u szczurów narażonych na octan ołowiu.

Materiał i metody. Dwadzieścia szczurów szczepu Wistar, podzielono na dwie grupy: kontrolną (n=10) i badaną z podawanym ołowiem (n=10). Szczury z grupy badanej były regularnie karmione granulatem dla szczurów i pojęone wodą destylowaną z octanem ołowiu (1200 ppm) przez 7 dni. W homogenatach z tanki płucnej oznaczany był poziom malonyldialdehydu (MDA) i aktywność dysmutazy ponadtlenkowej (SOD, CuZn-SOD, Mn-SOD), katalazy (CAT), peroksydazy glutationowej (GPx), reduktazy glutationowej (GR), oraz transferazy glutationowej (GST).

Wyniki. Aktywność katalazy była o 98% wyższa w grupie badanej aniżeli w grupie kontrolnej. Podobnie, aktywności GPx i GR były wyższe odpowiednio o 57% i 45%. Poziom MDA był również wyższy w grupie badanej, o 52% w porównaniu do grupy kontrolnej. Pozostałe parametry nie różniły się pomiędzy grupami.

Wnioski. Ekspozycja na ołów stymuluje mechanizmy obrony antyoksydacyjnej, jednakże mechanizmy te są niewystarczające by zapobiec stresowi oksydacyjnemu wywołanemu działaniem ołowiu.

Słowa kluczowe: zatrucie ołowiem, stres oksydacyjny, płuca

INTRODUCTION

It is well-established that lead is one of the most persistent environmental contaminants [1]. Lead is present in air, water, and soil. The main route of environmental exposure to lead is food and drink [2]. Due to many industrial applications of lead, occupational exposure to this xenobiotic is considered as an important health hazard as well. Occupational lead exposure occurs mainly through the respiratory tract [3].

There are many possible mechanisms of toxic lead action. One of these mechanisms is the proposed ability of lead to disrupt the prooxidant/antioxidant balance. It has been suggested that the production of reactive oxygen species (ROS) increases when lead ions are present in the microenvironment. Besides, lead has been shown to impair antioxidant defense system function. There are many studies reporting altered activities of antioxidant enzymes, such as superoxide dismutase, catalase, or glutathione peroxidase, due to lead exposure. Similarly, the levels of non-enzymatic oxidants have been shown to be influenced by the metal in question [4]. As a result, increased ROS levels lead to oxidative damage to critical biomolecules, such as lipids, proteins, and DNA [1].

The lung exists in a high-oxygen environment due to its large surface area and high blood supply. ROS in lung tissue are generated both endogenously and exogenously. The key endogenous sources of ROS include inflammatory cells, macrophages, as well as epithelial and endothelial cells. In these cells, the mitochondria are central to ROS production. Exogenous ROS exposure is related to the inhalation of many substances, including environmental gases, such as aldehydes/carbonyls, NO₂, SO₂, CO, cigarette smoke, and airborne particulate matters [5]. Lead is one of the most pervasive metals in urban particulate matter [6]. Consequently, ROS are suggested to play an important role in many pulmonary diseases, such as COPD, fibrosis, cancer [5].

In the available literature, the majority of studies concerning lead-induced oxidative stress focus on the blood, kidneys, liver, and brain. Little is known about lead toxic effects on other organs, such as the lung. In light of this, the present study was undertaken to estimate the values of selected parameters related to oxidative stress in the lung of rats exposed to lead acetate.

MATERIAL AND METHODS

Material

Twenty male Wistar rats, 4 months-old, weighing 200 ± 20 g, were purchased from the Center for Experimental Medicine, Medical University of Silesia, and kept in the Center for Experimental Medicine, Medical University of Silesia (Katowice, Poland). All animals were randomly divided into two groups: control (n=10) and lead-treated (n=10). The lead-treated group was fed with regular rat chow and distilled water supplemented with lead acetate (1200 ppm) for 7 days. The control group consumed distilled water as drinking water during the experimental period. Diets and drinking water were administered ad libitum. Animals were sacrificed after the experimental period by decapitation. After decapitation, lungs were quickly removed in saline. To obtain homogenates for analysis, lungs were homogenized and centrifuged at 4000 rpm for 10 minutes at 4°C. The supernatants were collected and stored at -80°C for further analysis.

Methods

The level of malondialdehyde (MDA) was determined as per Ohkawa et al. [7]. The results were recorded as micromoles per gram of protein ($\mu\text{mol/g P}$). The Oyanagui method [8] was used to measure the activity of superoxide dismutase (SOD). The enzymatic activity of SOD was expressed in nitric units. The activity of SOD is equal to 1 nitric unit (NU) when it inhibits nitric ion production by 50%. Activities of SOD were normalized to milligrams of protein (NU/mg protein). The catalase (CAT) was measured by the Aebi [9] kinetic method. Catalase activity was expressed as international units per milligram of protein (IU/mg protein). GPx activity was measured by the kinetic method of Paglia and Valentine [10] and expressed as micromoles of NADPH oxidized per minute per gram of hemoglobin (U/g Hb). The activity of GR was measured according to Richterich [11] and expressed as μmoles of NADPH utilized per minute, normalized to one gram of protein (IU/g P). The activity of GST was measured according to the kinetic method of Habig and Jakoby [12]. The activity of GST was expressed as μmoles of thioether produced per minute, normalized to one gram of protein (IU/g P). The amount of protein in the serum was measured by the biuret method by Richterich [11].

Statistical analysis

Statistical analysis was performed using Statistica 9.1 PL software program. Data were expressed as mean \pm standard deviation (SD) in tables. The Shapiro-Wilk test was used to verify the normality. Statistical comparisons were made using the Mann-Whitney U test. A value of $p < 0.05$ was considered to be significant.

RESULTS

The activity of catalase was significantly higher in the lung of lead-treated rats than in controls by 98%. Similarly, the activities of GPx and GR were higher by 57% and 45%, respectively (Fig. 1). The level of MDA was also higher in lead-exposed rats than in the control group by 52%. The remaining parameters did not differ between the studied groups (Tab. I).

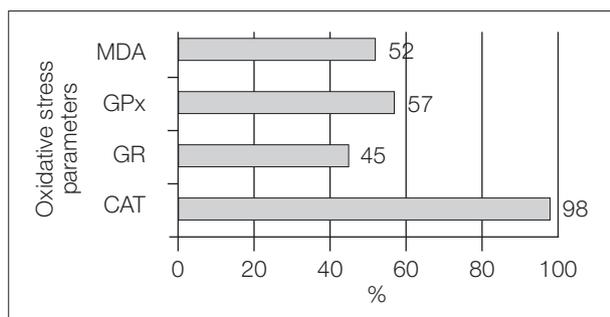


Fig. 1. Relative change (%) in selected parameters ($p < 0.05$) – between control and lead-exposed group. MDA – malondialdehyde; GPx – glutathione peroxidase; GR – glutathione reductase; CAT – catalase

Ryc. 1. Relatywna zmiana (%) w wybranych parametrach ($p < 0,05$) – pomiędzy grupę kontrolną, a eksponowaną na ołów. MDA – malonyldialdehyd; GPx – peroksydaza glutationowa; GR – reduktaza glutationowa; CAT – katalaza

DISCUSSION

SOD is the first line of the antioxidant defense by catalyzing the dismutation of superoxide anion to form H_2O_2 and molecular oxygen. There are several mechanisms that can explain the possible interactions between lead toxicity and SOD activity. It has been suggested that lead exposure may result in massive production of superoxide anions which override SOD enzymatic activity, leading to a fall in its concentration and activity. Besides, it is not excluded that lead-induced copper and zinc deficiency may disrupt synthesis of CuZn-SOD isoenzyme, while mitochondrial dysfunction may lead to decreased activity of the Mn-SOD isoenzyme [1]. How-

Table I. Measured biochemical parameters of oxidative stress in rat lung homogenate: malondialdehyde (MDA), superoxide dismutase (SOD, CuZn-SOD, Mn-SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST)

Tabela I. Oznaczane w homogenatach tkanki płucnej szczurów, parametry biochemiczne stresu oksydacyjnego: malonyldialdehyd (MDA), dysmutaza ponadtlenkowa (SOD, CuZn-SOD, Mn-SOD), katalaza (CAT), peroksydaza glutationowa (GPx), reduktaza glutationowa (GR), transferaza glutationowa (GST)

	LUNG				p
	control group		lead-exposed group		
	mean	SD	mean	SD	
CAT (IU/g protein)	23.79	6.50	47.03	14.06	<0.001
GR (IU/g protein)	42.60	6.91	61.79	18.51	0.021
GPx (IU/g protein)	14.74	2.49	23.08	7.57	0.014
GST (IU/g protein)	1.57	0.44	2.10	0.86	0.162
SOD (NU/mg protein)	72.70	17.89	92.31	37.45	0.186
MnSOD (NU/mg protein)	33.49	7.84	45.45	18.27	0.064
CuZnSOD (NU/mg protein)	39.21	11.74	46.86	20.18	0.521
MDA (μ mol/g protein)	1.90	0.71	2.89	0.96	0.017

$p < 0.05$

ever, in the present study, the activity of SOD, including its isoenzymes, did not differ between the examined and the control group. This observation may be due to the fact that the influence of lead on superoxide dismutase expression and activity may depend on antagonistic mechanisms. On the one hand, lead may decrease SOD activity via the above-described mechanisms. On the other hand, lead-exposure generates superoxide anions which upregulate the expression of SOD gene [4]. It can be speculated that this mechanism is only a component of a much more complex cellular stress response related to the cell injury caused by lead toxic action.

It is well-established that SOD works in conjunction with H_2O_2 -removing enzymes, such as CAT, GST, and GPx. CAT serves as a key enzyme catalyzing the decomposition of H_2O_2 to H_2O and O_2 [13]. The fact that H_2O_2 upregulates CAT gene expression [14] suggests that the increased activity of CAT due to lead action observed in the present study is a result of a cellular stress response, including the defense compensatory mechanism against lead-induced oxidative stress. On the other hand, lead decreases heme biosynthesis by inhibiting aminolevulinic acid dehydratase (ALAD) and ferrochelatase activity. Because CAT is a heme-containing enzyme, lead might be able to indirectly decrease its activity and overcome antioxidant defensive mechanisms [1].

The other measured parameters, GPx, GR, and GST, work together with glutathione (GSH) in the decomposition of H₂O₂ and organic hydroperoxides. GSH serves as a multifunctional intracellular non-enzymatic antioxidant. It is a major thiol antioxidant and protects cells from reactive oxygen species by conversion to its oxidized form, GSSG, by GPx and GST, while GR activity is needed for GSH resynthesis [15]. Significantly elevated activities of GPx and GR were simultaneously observed in the present study. Only the activity of GST did not differ between the studied groups. These observations suggest the presence of strong mechanisms of antioxidant defense in the lung. As in the case of SOD and CAT, the activities of GPx, GR, and GST may be a result of a sum of several opposite mechanisms triggered by lead action. On the one hand, the production of H₂O₂ upregulates the expression of GPx gene. On the other hand, lead-induced displacement of the selenocysteine group from the active site of the enzyme potentially decreases its activity [4]. Analogically, it is not excluded that lead may decrease the activities of GR and GST via binding to the thiol groups of their active sites. Simultaneously, lead may indirectly increase their activities via GSH pool depletion and accumulation of GSSG. Overproduction of GSSG needs a higher activity of GR, whereas a decreased GSH level induces elevation of the pi class of GST [16]. As in the case of CAT, it is not excluded that lead toxicity may trigger mechanisms of cellular stress response, which may lead to the upregulation of GPx and GR expression.

The elevation of CAT, GPx, and GR observed in the present study should be interpreted as a manifestation of antioxidant defense mechanisms triggered by lead toxicity. However, they seem to be insufficient because an increased level of MDA was simultaneously observed. MDA is the most well-known product of lipid peroxidation and a popular marker of oxidative stress. The ability of lead to elevate MDA level is well-established [17].

CONCLUSIONS

Lead exposure stimulates antioxidant defense mechanisms in rat lung, as demonstrated by elevated activities of some antioxidant enzymes; however, these mechanisms are insufficient to prevent lead-induced oxidative stress development.

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