ACTA ZOOLOGICA BULGARICA

Acta Zool. Bulg., 75 (3), September 2023: 343-349

Terrestrial Ecology and Behaviour
Research Article

Published online 26 September 2023
DOI: https://doi.org/10.71424/azb75.3.002667

Different Lead- and Cadmium-Induced Oxidative Stress Profiles in the Liver and Kidneys of Subchronically-Exposed Mice

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Abstract: The aim of the present study was to determine the oxidative stress profiles in the liver and kidneys of mice subchronically exposed to lead (Pb) and cadmium (Cd). Four 45-day experiments were conducted using laboratory mice. A control group was compared to three groups exposed to 0.00250M $Pb(NO_3)_2$ and 0.00125M $Cd(NO_3)_2$ solutions, applied in drinking water separately or in combination. As biomarkers of oxidative stress, the levels of malondialdehyde (MDA) and total glutathione were evaluated, respectively, by using the thiobarbituric acid reactive substances test (TBARS) and a colorimetric assay for total glutathione. The results demonstrated approximately 2-fold higher induction of MDA in the liver of Pb-exposed mice in comparison with Cd-exposed mice, combined with a slow but not statistically significant depletion of total glutathione. In the kidneys of the Cd-exposed mice, the highest accumulation of MDA was detected, accompanied by 1.5-fold glutathione depletion. Nevertheless, in groups exposed to Pb and Pb/Cd intoxication, an inducible increase in total glutathione was observed. These findings confirmed that Pb induces higher levels of oxidative stress in the liver while the kidneys suffered more oxidative damage due to Cd intoxication, attributable to the bioaccumulation profiles of the two toxic metals.

Key words: lead, cadmium, oxidative stress profiles, liver and kidneys, subchronically exposed mice

Introduction

Ecosystem contamination has been assessed as one of the most important environmental problems, raising serious concerns about human health and the state of populations. This is the reason for establishing strict legislation on the release and permissible levels of certain toxicants in nature. Soil contamination with heavy metals such as cadmium, lead, chromium, copper, zinc and mercury are worrying (Kabir et al. 2012). These elements are present in the soil naturally but their high concentrations are primarily due to industry (non-ferrous and ferrous metallurgy, energy, chemical industry), agriculture (irrigation with polluted water, use of mineral fertilizers), waste incineration, fuel burning, road transport, etc. (European Commission 2013, Tóth et al. 2016). The presence of Pb and Cd in increased amounts in organisms is perceived as toxic. Acute or chronic exposure to them may lead to health issues for animals and humans as they are able to accumulate, mainly in liver and kidneys (Ozimec et al. 2015). Cadmium is already known to cause several types of renal dysfunction as well as cancer (Dokmeci et al. 2009).

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The problem of environmental pollution with heavy metals and toxic elements is global and research questions addressing it are a priority. A number of studies have been performed, largely elucidating the pathways of bioaccumulation and distribution of toxicants in tissues and organs of organisms as well as the physiological and biochemical mechanisms of heavy metal elimination (Waalkes 2003, Rubino 2015, Puerto-Parejo et al. 2017). For this purpose, different species of rodents were used as bioindicators and convenient model organisms. In recent decades, a number of monitoring and ecotoxicological studies have been conducted in Bulgaria (Topashka-Ancheva et al., 2003, Metcheva et al. 2003, Mitkovska et al. 2012). Although serious measures have been taken in the last decades, there is still the problem of removing toxic substances already accumulated in the soil and water. Their slow excretion by animal organisms and high rate of accumulation at the higher levels of food chains are reasons to expect problems for human, animal and ecosystem health in the coming years.

Three main reasons for the toxicity of metals have been discussed: (1) the ability to interact directly with proteins (Jaishankar et al. 2014); (2) the displacement of elements necessary for metabolism (Cailliatte et al. 2009, Mao et al. 2018, Tandon et al. 2003); (3) enhancing the production of chemical compounds known as reactive oxygen species (ROS) (Szlacheta et al. 2020, Winiarska-Mieczan 2018). Although Pb and Cd are non-redox metals, exposure to them can cause disturbances in the oxidative status (Buege & Aust 1978). Previous studies have shown that cadmium and lead indirectly participate in the induction of oxidative stress (Arroyo et al. 2012). The possible mechanism of action could be increased peroxidation of lipids and a decreased glutathione (GSH) levels. Based on this, it has been hypothesised that the oxidative stress profiles would differ depending on the particular heavy metal or the target tissue.

The standard criterion for the normal homeostasis of the organism is the liver index (Wasmuth et al. 2010). The liver is not only an energy depot but also an organ accumulating proteins, including enzymes that detoxify xenobiotics of various origins. Therefore, the relative weight of the liver can be considered as an indicator of pathogenic changes in small mammals.

The aim of the present study was to evaluate the oxidative stress profiles in the liver and kidneys of mice subchronically exposed to lead and cadmium.

Materials and Methods

Experimental animals

Experimental animals were same-sex (male) and age-optimised ICR-strain laboratory mice. They were bred in a vivarium and housed in individual ventilated cages. The physical size of the cages was in accordance with European standards. The bedding material was obtained from an ISO 2000-accredited supplier. Mice were acclimatised for a 7-day period before starting the experiment. During the study, a standard temperature between 19°C and 23°C, humidity of 45–60% and a 12-hour day/night cycle were maintained. The food was in the form of pellets. All mice were allowed access to food and water *ad libitum*. The water, food and bedding material were inspected daily and changed when necessary. The animals were neither medicated nor vaccinated. The rearing of animals in a vivarium and the conduct of experiments are in accordance with Ordinance No. 20/01.11.2013 on the protection and welfare of animals and the Animal Protection Act (31.01.2008) of the Republic of Bulgaria. In the sampling process, a maximum of animal-friendly, low-invasive and non-invasive methods, fully compliant with the OECD guidelines of 17.12.2001 and 29.07.2016 were used.

Four variant toxicological experiments were conducted, each of 45 days. The administration of toxicants in certain concentrations was performed with drinking water in the form of dissolved salts. A control group was compared to three groups exposed to 0.00125M $Pb(NO_3)_2$, 0.00250M $Cd(NO_3)_2$ and 0.00125M $Cd(NO_3)_2$ + 0.00250M $Pb(NO_3)_2$ solutions applied as drinking water. Samples were collected on the 15th, 30th and 45th day from each experimental group. The body mass of each animal was recorded and the kidneys and liver were dissected for further analysis.

Morphophysiological parameters and oxidative stress markers

The assessment of morphophysiological parameters was carried out according to the indices of the kidneys and liver, which were calculated as the ratio of organ weight to body weight, expressed in per mille according to the following formula:

Organ mass index = (Organ Mass / Total body mass) * 1000,

which gives a standard index expressed in mg/g body mass.

The thiobarbituric acid reactive substances test (TBARS) was performed as described in Friberg & friberg (1984). Briefly, the mouse liver and kid-

neys were homogenized and subjected to the described procedure. The levels of malondialdehyde (MDA) were measured spectrophotometrically and expressed as nmol MDA/g fresh weight. Total glutathione was assayed colorimetrically following the protocol by Şehirli et al. (2008); glutathione levels were presented as mmol GSH/g tissue.

The statistical significance of differences between tested concentrations and the negative controls was calculated by one-way analysis of variances (ANOVA) with Bonferroni's post hoc-test. Calculations were done with GraphPad Prism software, version 6.04 (San Diego, USA). Asterisks provide information for the significance of the differences: $P > 0.05$ (non-significant); $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Heavy metal analysis

The determination of Cd and Pb concentrations was carried out using a Perkin Elmer SCIEX DRC-e ICP-MS system with a crossflow nebulizer. The spectrometer was optimized (RF, gas flow, lens voltage) to provide minimal values of the ratios CeO^+/Ce^+ and Ba^{2+}/Ba^+ and maximum intensity of the analytes. Working standard solutions of Cd and Pb in the concentration range from 0.01 to 500 µg L^{-1} were prepared from single-element calibration solutions (Merck), with initial concentrations of 1000 mg L^{-1}. External calibration was performed for $^{111,112,113,114}Cd$ and $^{204,206,207,208}Pb$. The calibration coefficients for all of the calibration curves were at least 0.99. The evaluation of the accuracy was done by the analysis of two certified reference materials: Horse Kidney IAEA-H-8 and NIES N6 CRM mussel. The experimental results were in very good agreement with the certified values.

Results

The results of the analysis of the heavy metals bioaccumulation in liver and kidneys of the studied animals are presented on Table 1. The results showed a substantial increase in the levels of bioaccumulation in both kidney and liver. The quantities of toxicants in the kidneys were up to six times higher than in the liver.

The changes of mice body mass (g) during each experimental variant are presented in Table 2. Significant weight gain was observed in the control and Pb-exposed groups ($p < 0.05$) during the entire 45-day experimental timeframe. The growth performance in Cd and Cd+Pb exposed groups was significantly reduced in the 45-day experimental period ($p < 0.01$) in comparison with the other two groups.

Table 1. Lead and cadmium bioaccumulation (mg/kg) in liver and kidneys of chronically intoxicated laboratory mice (mean ± SD).

Days	Control (x ± SD)				Cd (x ± SD)		Pb (x ± SD)		Pb+Cd (x ± SD)			
	Liver		Kidneys		Liver	Kidneys	Liver	Kidneys	Liver		Kidneys	
	Cd	Pb	Cd	Pb					Cd	Pb	Cd	Pb
0	0.05±0.01	0.02±0.01	0.14±0.01	0.82±0.02	0.05±0.01	0.14±0.01	0.22±0.01	0.82±0.02	0.05±0.01	0.02±0.01	0.14±0.01	0.82±0.02
15	0.06±0.01	0.08±0.02	0.19±0.02	0.98± 0.03	44.2±0.9	39.1± 0.4	24.1±0.2	175± 3	44.6±0.9	14.2±0.6	35.7± 0.2	65.6±0.9
30	0.05±0.01	0.07±0.02	0.60±0.06	0.29±0.03	78.8±0.8	74.6± 0.6	29.0±0.2	205± 4	47.2±0.8	12.9±0.2	75.2± 0.8	83.2±0.3
45	0.05±0.01	0.08±0.04	0.91 ±0.01	0.40±0.01	81.1±0.4	100.0± 0.6	27.2±0.2	157± 3	58.±2	15.0±0.8	116± 4	68.0±0.3

In the course of the experiment with cadmium, the value of the liver index decreased (Table 3). A similar trend was reported in the control group and, therefore, considered normal. Subchronic intoxication of mice with cadmium resulted in a considerably higher cadmium concentration in the kidneys than in the liver, despite similar absorption levels. Statistically significant differences were not obtained between Cd and Pb+Cd experiments. It was found only at the 45th day between Pb and Pb+Cd variants.

Results concerning the effect of the heavy metals on the oxidative stress in liver tissue demonstrated c. twofold higher induction of MDA in the liver of Pb-exposed mice in comparison with Pb/Cd and Cd-exposed mice (Fig. 1). Slow but not statistically significant depletion of total glutathione was observed after cadmium treatment (Fig. 2). Exposure to lead and cadmium/lead for 30 and 45 days led to GSH levels comparable with the control. On the other hand, in terms of MDA, the kidneys of exposed mice were more susceptible. Around 3-fold higher levels, in comparison with the control, were measured after 30- and 45-days exposure (Fig. 3). Cadmium was also determined to induce 1.5-fold steady glutathione depletion in the kidneys of the exposed mice (Fig. 4). Nevertheless, in groups exposed to lead and combined lead/cadmium intoxication, an inducible increase in total glutathione was observed.

Table 2. Body mass changes (g) of control and toxicant-exposed mice during the experimental period.

Day	Experimental groups			
	Control	Cd	Pb	Pb+Cd
0	25.4±3.4	25.4±3.4	25.4±3.4	25.4±3.4
15	27.1±2.6	23.0±1.2	24.8±2.2	23.4±3.5
30	33.1±2.5	27.3±4.4	30.2±5.9	26.9±2.9
45	35.3±3.6	266 ±2	30.5±1.3	25.8±2.4

Discussion

As shown above, the value of the liver index decreased during the experiments. Along the body growth, the mass of the liver increases relatively less than the total weight of the animals. Although the liver is a primary target organ for cadmium, it responds relatively slowly when exposed to cadmium nitrate concentrations. In the case of $Pb(NO_3)_2$, significant increase ($p<0.05$) was obtained in comparison with the control group. With oral exposure to chronic Cd intoxication, the liver is an organ that takes up the greatest quantity of Cd during the initial hours after exposure (Andjelkovic et al. 2019). After oral uptake, cadmium is transported to the liver (where it stimulates the synthesis of metallothionein) or is transported directly to the kidneys (Matović et al. 2015). This is the most probable reason for the statistically significant increase in the liver index in the Cd-exposed group in the first two weeks of the experiment. Over time, lead is accumulated in both liver and kidneys, while the largest amounts of cadmium accumulate in the kidneys.

Table 3. Liver mass (g) and liver index (mg/g) for control, Pb-exposed, Cd-exposed and Pb+Cd-exposed mice.

Day	Experimental groups							
	Control		Cd		Pb		Pb+Cd	
	Liver mass	Liver index	Liver mass	Liver index	Liver mass	Liver index	Liver mass	Liver index
0	1.53 ± 0.19	60.2	1.53±0.19	60.2	1.53 ± 0.19	60.2	1.53 ± 0.19	60.2
15	1.77 ± 0.24	65.3	1.56±0.38	68.0	1.41 ± 0.18	56.9	1.12 ± 0.10	47.9
30	2.03 ± 0.25	61.4	1.79±0.44	65.9	1.78 ± 0.37	58.8	1.68 ± 0.32	62.4
45	1.98 ± 0.29	56.2	1.4±0.23	53.2	1.95 ± 0.22	64.0	1.50 ± 0.11	58.2

Table 4. Kidneys mass (g) and kidneys index (mg/g) for control, Pb-exposed, Cd-exposed and Pb+Cd-exposed mice.

Day	Experimental groups							
	Control		Cd		Pb		Pb+Cd	
	Kidney mass	Kidney index	Kidney mass	Kidney index	Kidney mass	Kidney index	Kidney mass	Kidney index
0	0.47± 0.23	18.5	0.47± 0.23	18.5	0.47±0.23	18.5	0.47±0.23	18.5
15	0.49±0.05	17.9	0.49 ±0.07	21.6	0.50±0.08	20.2	0.46±0.05	16.6
30	0.55±0.07	16.5	0.47± 0.10	17.2	0.51±0.07	16.9	0.39±0.09	17.1
45	0.50 ±0.08	14.1	0.42±0.04	15.9	0.48±0.07	15.8	0.49±0.08	19.0

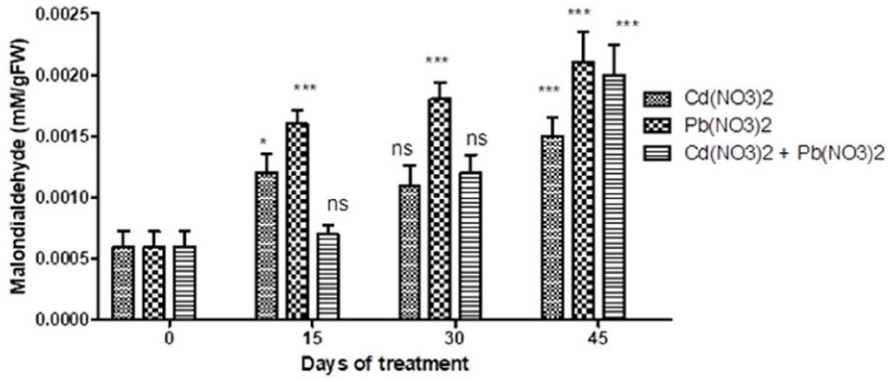

Fig. 1. Levels of MDA measured in liver tissue. If no error bars are visible, they are equal to or lower than the values.

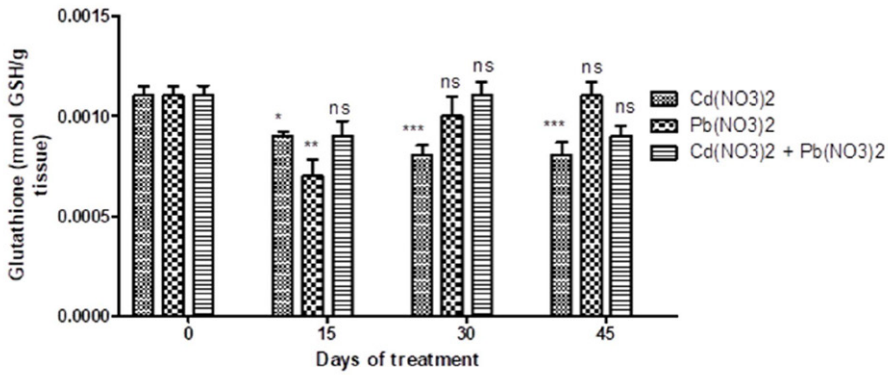

Fig. 2. Levels of glutathione measured in liver tissue. If no error bars are visible, they are equal to or lower than the values.

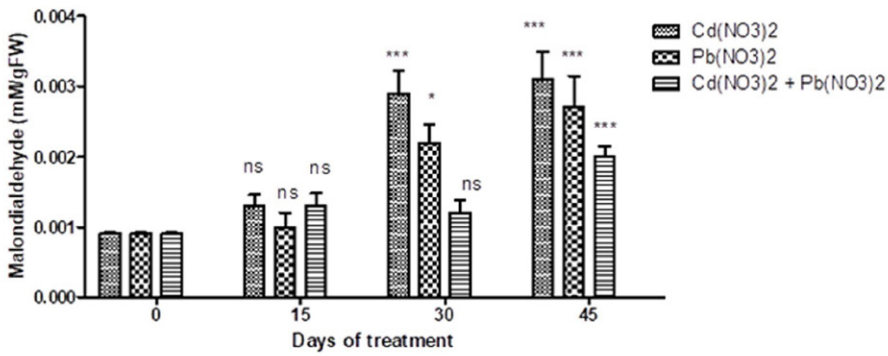

Fig 3. Levels of malondialdehyde measured in kidney tissue. If no error bars are visible, they are equal to or lower than the values.

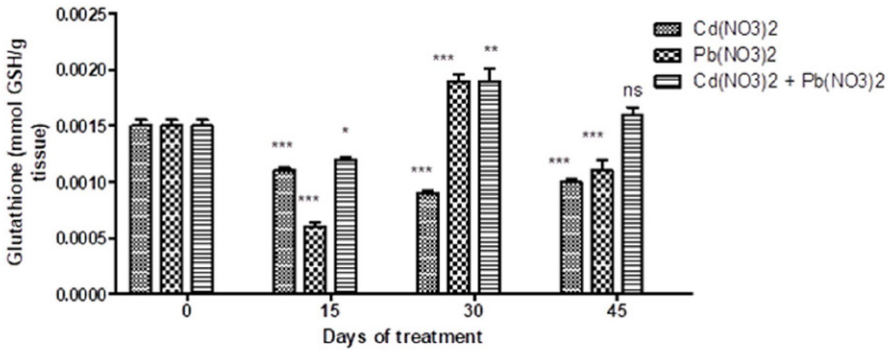

Fig 4. Levels of glutathione measured in kidney tissue. Where no error bars are visible, they are equal to or lower than the values.

Regarding oxidative stress in cells, the stronger effect of lead and the combination of lead/cadmium could be explained precisely by the presence of essential accumulation of lead in the liver (Patra et al. 2011). The obtained results are in accordance with the statement of Andjelkovic et al. (2019), concluding that the major consequence of lead-induced oxidative stress in the liver was the induction of high levels of MDA leading to the alteration of membrane integrity and fatty acid composition. Interestingly, exposure to lead and cadmium/lead for 30 and 45 days led to GSH levels comparable with the control. This may be explained by overexpression of glutathione, thus reducing the oxidative stress (Patra et al. 2011). The highest MDA-induction was measured after cadmium intoxication. This result confirms the observation concerning cadmium-induced lipid peroxidation in kidneys (Andjelkovic et al. 2019). The observed inducible increase in total glutathione in groups exposed to lead and combined lead/cadmium intoxication could be explained by compensatory mechanisms, including overexpression of glutathione following chronic exposures (Patra et al. 2011, Andjelkovic et al. 2019).

Conclusions

The results of the present study indicate an inhibition of mouse growth performance by cadmium, alone as well as after combined lead + cadmium subchronic exposure. No significant inhibition of growth performance has been induced by lead at the tested concentrations. The measurement of oxidative stress via MDA and glutathione indicates different toxicities of Pb and Cd in the liver and kidneys. The experimental findings confirm that Pb induces higher levels of oxidative stress in the liver; in contrast, the kidneys suffer more oxidative damage due to Cd intoxication, which attributable to the bioaccumulation profiles of the two toxic metals.

Acknowledgments: This work was supported by a grant from the National Science Fund, Ministry of Education and Science, Project No. KP-06-PN44/3.

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Received: 27.09.2022
Accepted: 12.07.2023