BIOPHYSICS AND BIOCHEMISTRY

Effects of Prenatal and Neonatal Cadmium Intoxication on the Intensity of Lipid Peroxidation and Activity of Glutathione System in Progeny of Albino Rats

O. V. Slyuzova, E. V. Stepanova, A. D. Temraleeva, R. A. Kireev, and V. V. Ignatov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 146, No. 7, pp. 46-49, July, 2008
Original article submitted May 28, 2007

Oral treatment of pregnant or lactating rats with $Cd(NO_3)_2$ in a dose of 2 mg/kg increased plasma and erythrocyte levels of MDA and reduced glutathione, activities of glutathione-S-transferase, glutathione reductase, and γ-glutamyl transferase in their 4-month-old progeny. Changes in the function of glutathione system and LPO intensity after cadmium intoxication during the neonatal were less pronounced than after prenatal exposure.

Key Words: *cadmium; lipid peroxidation; glutathione system; erythrocytes; plasma*

Today an appreciable part of the population lives in ecologically unfavorable zones (large cities) and is exposed to low-dose exotoxicants, for example, heavy metals. One of the most hazardous in this group of xenobiotics is cadmium. This metal is very slowly eliminated from the body, which leads to its accumulation predominantly in the kidneys, liver, and bone tissue. Cadmium compounds are characterized by a pronounced prooxidant effect [10], which is assumed to be responsible for their general toxic, nephrotoxic, carcinogenic, and embryotoxic effects [3]. Exposure to low-dose cadmium can lead to gradual changes in organs and tissues as a result of oxidative stress induction and reduction of the adaptation potential of the organism; these changes are unspecific, but can indicate initial stages of the disease [1]. Metal exposure of the mother—newborn system is regarded as a possible cause of health problems in children during the early ontogeny [4]. It was proven that cadmium penetrates through the placenta and is accumulating in it [3] and in fetal organs, it is also accumulated in the mammary glands and newborns receive it with breast milk [7,9].

We studied the prenatal and neonatal effects of cadmium ions on the LPO intensity and changes in glutathione system parameters.

MATERIALS AND METHODS

Experimental study was carried out in 2 stages. Stage 1 was devoted to studies of the progeny of albino rats, receiving orally 1 ml saline (control group) or $Cd(NO_3)_2$ (2 mg/kg) daily (experimental group) on days 6-15 of pregnancy. The progeny (n=10) was sacrificed on day 120 after birth. At stage 2 we examined the progeny of females, receiving orally $Cd(NO_3)_2$ in the same dose (experimental group) or saline (control group) on days 1-10 of lactation. The progeny (n=10) was sacrificed on day 120 of life by decapitation. The blood was collected in heparin-treated tubes, centrifuged for 10 min at 3000 rpm, and the plasma was col-

Department of Biochemistry and Biophysics, N. G. Chernyshevsky Saratov State University, Russia. ***Address for correspondence:*** slyuzova@mail.ru. O. V. Slyuzova

lected. Cell suspension for preparation of the erythrocyte mass was centrifuged in 0.9% NaCl (2×10 min at 2000 rpm); the supernatant was discarded.

The intensity of LPO was evaluated by accumulation of MDA in erythrocytes and plasma. The efficiency of the glutathione system function was evaluated by the level of reduced glutathione (RG), measured by the reaction with Elman's reagent, and by activities of glutathione reductase, glutathione-S-transferase (GST) in erythrocytes [2], and γ-glutamyl transferase (γ-GT) in the plasma, measured using a kit for clinical assays (Diacon-DC). The significance of differences in the means for independent variables was evaluated using Student's *t* test. The differences were considered significant at $p<0.05$.

RESULTS

Activation of LPO processes after prenatal and neonatal exposure to the ecotoxicant was detected. Adaptive activation of the erythrocyte glutathione system in 4-month-old progeny in response to the toxic agent differs in males and females.

The content of MDA in erythrocytes and plasma increased in experimental females and males in comparison with the control (Table 1). In males, the increase in MDA level was more pronounced: 7.8 times in the plasma and 1.8 times in erythrocytes. On the other hand, activation of all components of the glutathione system was observed in males: GST activity increased 1.5 times *vs.* control, glutathione reductase activity increased 2.1 times, erythrocyte RG content increased 1.3 times, and plasma γ-GT activity increased 1.5 times in comparison with the control group. In experimental females, activity of erythrocyte glutathione reductase and plasma γ-GT increased 2.1- and 2-fold, respectively, in comparison with the control, the level of RG and activity of GST were unchanged, while MDA content surpassed the control values 2.1 times in the plasma and 1.2 times in erythrocytes.

Neonatal exposure of females to cadmium led to a significant decrease in RG content (by 1.3 times), increase of GST activity (by 2.5 times) in erythrocytes against the background of activation of glutathione reductase (by 2.5 times) and γ-GT (by 1.6 times) in comparison with the control (Table 2). The level of erythrocyte MDA increased slightly (by 1.3 times) and plasma MDA by 2.1 times in comparison with intact females. In experimental males, activity of GST increased by 2.3 times, erythrocyte glutathione reductase activity increased by 1.5 times, RG level increased by 1.5 times, levels of erythrocyte MDA by 1.3 times and plasma MDA by 2.9 times, and activity of γ-GT increased 2.1 times compared to the control.

Cadmium exposure of females during pregnancy produced a direct toxic effect on the fetus: the toxicant penetrated through the placenta and elevated blood concentration of toxic LPO products. In studies of the neonatal effect of Cd ions the major attention was paid to indirect effect of this toxin entering the body with breast milk in a very low dose, because in these experiments cadmium content in tissues was below 0.05% of the dose received by lactating females [9]. That is why intensification of the glutathione system in experimental series 2 was aimed largely at utilization of cadmium-induced LPO, but not at detoxification of the toxic agent.

The GST family enzymes conjugate toxic products (including xenobiotics and LPO products) with RG promoting elimination of these products. Activation of GST indicates acceleration of detoxication processes. The increase in RG content and glutathione reductase activity indicate the development of compensatory mechanisms, aimed at elimination of toxic agents and destruction of free radicals. γ-GT is a membrane enzyme; increased plasma activity of this enzyme can confirm cell damage, primarily hepatocyte damage, which can be attributed to proven hepatotoxicity of cadmium [6]. Therefore, the increase in glutathione system parameters is a positive event indicating the formation of adaptation in experimental animals.

Oxidative stress caused by delayed effect of cadmium differs by the mechanisms of glutathione defense in females and males. In females, high activity of glutathione reductase maintains the RG pool within the normal level or its content decreases because of involvement in the conjugation by intensive GST work (after neonatal exposure), while in males, activities and levels of all studied glutathione system components increase. However, plasma MDA content and γ-GT activity in males increase more significantly in comparison with the control than in females after prenatal and neonatal exposure, the parameters in females also being higher than in the control, indicating total tissue damage. Hence, despite intensification of glutathione defense, males are more sensitive to Cd-induced oxidative stress. It was reported that activities of antioxidant systems changed under the effect of cadmium. Activities of SOD and catalase and content of vitamins C and E decrease in response to chronic oral Cd [8]. Presumably, in our study the progress of oxidative stress can be explained by insufficiency of these substances.

Based on published data indicating that antioxidant systems are fortified in females by the age

TABLE 1. Level of MDA, Parameters of Glutathione Defense, and γ-GT Activity in Erythrocytes and Plasma of 4-Month-Old Albino Rats after Prenatal Exposure to Cadmium Ions (*M*±*m*)

Parameter	Males		Females	
	control	cadmium	control	cadmium
Erythrocyte MDA, μmol/liter	4.28±0.24	7.76±0.79 $p<0.02$	5.00±0.24	6.39±0.39 $p<0.05$
Plasma MDA, μmol/liter	0.94±0.44	7.34±1.90 $p<0.04$	1.70±0.19	3.58±0.08 $p<0.001$
GST, μmol/g Hb/min	3.89±0.35	6.03±0.69 $p<0.04$	4.58±0.78	3.70±0.59
Glutathione reductase, μmol/g Hb/min	0.51±0.09	1.10±0.18 $p<0.01$	0.97±0.22	2.09±0.33 $p<0.02$
RG, mmol/liter	1.700±0.058	2.10±0.17 $p<0.01$	2.39±0.29	2.39±0.40
γ-GT, μmol/g protein/min	0.31±0.15	0.48±0.09 $p<0.05$	0.29±0.05	0.58±0.10 $p<0.05$

Note. Here and in Table 2: *p*: significant difference from the control.

TABLE 2. Level of MDA, Parameters of Glutathione Defense and γ-GT Activity in Erythrocytes and Plasma of 4-Month-Old Albino Rats after Neonatal Exposure to Cadmium Ions (*M*±*m*)

Parameter	Males		Females	
	control	cadmium	control	cadmium
Erythrocyte MDA, μmol/liter	1.23±0.22	1.64±0.65	1.06±0.61	1.46±1.07
Plasma MDA, μmol/liter	0.81±0.05	2.37±1.19 $p<0.009$	1.08±1.23	2.32±0.66 $p<0.04$
GST, μmol/g Hb/min	2.06±0.28	4.75±0.83 $p<0.001$	2.19±0.81	5.47±0.81 $p<0.02$
Glutathione reductase, μmol/g Hb/min	1.33±0.67	1.98±0.35 $p<0.03$	1.47±0.78	3.63±0.68 $p<0.05$
RG, mmol/liter	1.62±0.48	2.47±0.22 $p<0.05$	2.82±0.41	2.20±0.17 $p<0.02$
γ-GT, μmol/g protein/min	0.23±0.13	0.48±0.20 $p<0.05$	0.25±0.06	0.41±0.16 $p<0.04$

of 4 months due to increased expression of glutathione peroxidase and SOD genes, increased RG content, and positive effects of estrogens [5], we conclude that in our experiment females were also less sensitive to oxidative stress developing in response to cadmium intoxication.

On the whole, our experiment demonstrated aftereffects of cadmium during the critical period of organogenesis and the period of postnatal stress reaction. The effect of toxic exposure during pregnancy was more pronounced and was associated with intensification of free-radical oxidation, changes in thiol status and γ-GT activity. Despite less pronounced manifestations, aftereffects of neonatal intoxication were detected in animals reaching the adult age.

REFERENCES

1. N. L. Elaeva, E. V. Semenov, T. M. Ivanova, and G. V. Rutkovskii, *Toksikol. Vestn.*, No. 1, 27-32 (2005).
2. *Medical Laboratory Technologies. Handbook*, Ed. A. I. Karpishchenko [in Russian], St. Petersburg (2002), Vol. 2.
3. E. B. Mirzoev, V. O. Kobyalko, O. A. Gubina, *et al.*, *Toksikol. Vestn.*, No. 1, 35-38 (2005).
4. N. P. Setko and E. A. Zakharova, *Gig. San.*, No. 6, 65-67 (2005).

5. C. Borras, J. Sastre, D. Garsia-Sala, *et al.*, *Free Radic. Biol. Med.*, **34**, No. 5, 546-552 (2003).
6. A. Kawahara, M. Yoshizuka, T. Hirano, *et al.*, *Exp. Mol. Pathol.*, **53**, No. 2, 180-190 (1990).
7. H. Ohrvik, M. Yoshioka, A. Oskarsson, and J. Tallkvist, *Toxicol. Lett.*, **164**, No. 3, 207-213 (2006).
8. L. Pari, P. Murugavel, S. L. Sitasawad, and K. S. Kumar, *Life Sci.*, **80**, No. 7, 650-658 (2007).
9. K. Petersson Grawe and A. Oskarsson, *Arch. Toxicol.*, **73**, Nos. 10-11, 519-527 (2000).
10. S. Thijssen, A. Cuypers, J. Maringwa, *et al.*, *Toxicology*, **236**, Nos. 1-2, 29-41 (2007).