Effect of Administration of Lead Nitrate to Pregnant Rats on the Lungs in Their Offspring

O. A. Lebed'ko and B. Ya. Ryzhavskii

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 139, No. 6, pp. 621-623, June, 2005
Original article submitted June 16, 2004

Lead nitrate in a dose of 200 mg/kg was administered to female rats via a gartric tube on days 5 and 12 of pregnancy. The lungs of their offspring were examined on day 40 of life. We found a decrease in the ratio between the specific volumes of alveolar lumens and interalveolar septa and hypertrophy of lymphoid tissue in the bronchial wall (compared to the offspring of intact females). Chemiluminescent analysis revealed activation of lipid peroxidation and decrease in antioxidant antiradical activity of the lungs.

Key Words: *lungs; lead; offspring; free radical oxidation*

During early ontogeny the organism is highly susceptible to adverse effects of chemical factors. Therapeutic treatment is often accompanied by decompensated activation of free radical oxidation (FRO) and damage to the lungs in newborns (particularly in preterm infants). Delayed consequences include lung fibrosis and respiratory failure [13]. This mechanism of lung damage is of considerable importance, because activation of FRO results from the exposure to various pathogenic factors. For example, these changes are observed upon treatment with lead compounds [8-10,14]. We found no published data on the effect of prenatal treatment with lead nitrate ($Pb(NO_3)_2$), a prevalent technogenic toxicant on postnatal ontogeny of the lungs.

Here we studied the effect of $Pb(NO_3)_2$ administration to pregnant rats on FRO and morphological characteristics of the lungs in 40-day-old offspring.

MATERIALS AND METHODS

Experiments were performed with the offspring (n=18) of 3 female rats weighing 180-220 g. $Pb(NO_3)_2$ (4% solution, 0.9-1.1 ml, 200 mg/kg) was administered to females via a stomach tube on days 5 and 12 of pregnancy. According to previous data this dose of $Pb(NO_3)_2$ does not cause miscarriage, but produces pathological changes in organs [4,6]. The offspring of 3 intact animals (n=28) and 2 females (n=14) receiving an equivalent volume of distilled water in the same period of pregnancy served as the control. The animals were maintained in a vivarium and received food and water *ad libitum*. Forty-day-old rat pups were killed by decapitation. The right lung (part of the organ including its root) was fixed in Carnoy's fluid and embedded into paraffin. The sections (7 μ) were stained with hematoxylin and eosin. Volume density of alveolar lumens and interalveolar septa was measured using a morphometric grid [1]. The ratio between these values was calculated. The height of the epithelium in bronchi with a lumen of 150-200 μ was estimated on a MEKOS device. Each measurement was performed in 7-10 bronchi.

The intensity of FRO was determined by the method of chemiluminescence (CL). CL of the lung homogenate was measured on a LS 50B luminescent spectrometer (Perkin Elmer). Signal standardization and mathematical treatment of CL curves were performed by means of Finlab software. Spontaneous and Fe^{2+}-induced CL was evaluated as described elsewhere [3]. Total CL estimated over 1 min of spontaneous CL (S_{SP}) correlated with the intensity of FRO. The maximum flash amplitude (h) of Fe^{2+}-induced CL reflected

Clinical and Diagnostic Laboratory, Khabarovsk Branch, Far-Eastern Research Center for Physiology and Pathology of Respiration, Siberian Division of the Russian Academy of Medical Sciences, Institute of Maternity and Child Welfare; Department of Histology, Far-Eastern State Medical University, Khabarovsk

0007-4888/05/1396-0655

TABLE 1. Effect of $Pb(NO_3)_2$ Administration to Pregnant Rats on CL in Lung Homogenates from the Offspring (rel. units, *M*±*m*)

Parameter	S_{SP}	Induced CL				
		Fe^{2+}		luminol+H_2O_2		
		h	S_{IND}	H_1	t	H_2
Control	0.089±0.050	0.50±0.03	0.68±0.05	0.36±0.02	2.58±0.09	1.11±0.05
$Pb(NO_3)_2$	0.200±0.110*	1.46±0.08*	1.48±0.08*	0.96±0.06*	1.60±0.14*	2.06±0.07*

Note. *$p<0.05$ compared to the control.

the content of lipid hydroperoxides. Total CL recorded over 4 min of the post-flash period (S_{IND}) characterized the rate of peroxide radical formation. Kinetic parameters of H_2O_2-induced luminol-dependent CL [2,11] were analyzed by the maximum amplitude of the first flash (H_1) reflecting the intensity of radical generation in Fenton-like reactions, maximum amplitude of the second flash (H_2) correlating with antiradical protection, and interval (t) between H_1 and H_2 that depended on activity of the antioxidant system. The intensity of CL (mV) was calculated per 1 g wet tissue and expressed in relative units. Parameters of CL and morphometric indexes of the lungs practically did not differ in 2 control groups and were combined.

RESULTS

No morphological differences were revealed between the lungs in experimental and control rats. However, hyperplasia of the lymphoid tissue in the bronchial wall and peribronchial connective tissue was more often seen in experimental animals. Sometimes lymphoid follicles formed conglomerates of considerable size and were located the perivascular space. Morphometry revealed no significant differences in the height of the bronchial epithelium in control and experimental rats (12.8±0.5 and 14.5±1.2 μ, respectively). The ratio between volume densities of alveolar lumens and interalveolar septa differed in the control and experimental groups (1.61±0.10 and 1.36±0.05, respectively, $p<0.05$). Therefore, the relative volume of oxygen-containing respiratory compartment in the lungs decreased in experimental rats. These differences were associated with thickening of the interalveolar septa in the offspring of females receiving $Pb(NO_3)_2$. Morphological characteristics of the respiratory system in 40-day-old rats did not differ from those in adult animals. These data suggest that the observed characteristics of the lungs will not change during postnatal ontogeny.

Recording of CL showed that antenatal treatment with $Pb(NO_3)_2$ activates FRO in the lungs of 40-day-old rats. S_{SP} increased by 2.4 times compared to the control (Table 1). We revealed an increase in the concentration of lipid hydroperoxides (3-fold increase in h), accelerated formation of peroxide radicals (2.4-fold increase in S_{IND}), and accumulation of hydroxyl radicals (3-fold increase in H_1, Table 1). Enhanced generation of FRO product is related to decreased activity of the antioxidant and antiradical systems (1.7-fold decrease in t, 2-fold increase in H_2).

Oxidative stress in cells, tissues, and systems is a major mechanism for *in vivo* (direct effect on humans and animals) and *in vitro* toxicity of various compounds, including lead salts [8-10,14]. Recording of CL showed that antenatal exposure to $Pb(NO_3)_2$ contributes to decompensated activation of FRO in the tissue of mature rat lungs. Morphological changes in the lungs are mainly associated with free radical-produced damage and oxidative modification of the corresponding biological molecules. The delayed and immediate effects of $Pb(NO_3)_2$ are mediated by the same free radical mechanisms (overproduction of extremely toxic hydroxyl radicals and activation of lipid peroxidation) [8,9]. Various lead salts have different effect. For example, lead iodide is more mutagenic than lead nitrate [5]. However, all these compounds (iodide, fluoride, nitrate, and acetate) produce the same damage to organs and tissues [7,10,12].

REFERENCES

1. G. G. Avtandilov, *Medical Morphometry* [in Russian], Moscow (1990).
2. A. V. Arutyunyan, E. E. Dubinina, and N. N. Zybina, *Methods for Study of Free Radical Oxidation and Antioxidant System in the Body. Methodical Recommendations* [in Russian], St. Petersburg (2000).
3. Yu. A. Vladimirov, O. A. Azizova, A. I. Deev, *et al*., *Itogi Nauki Tekniki. Ser. Biofizika*, **29** (1991).
4. A. A. Korolev and B. P. Sukhanova, *Vopr. Pitaniya*, No. 3, 34-37 (1996).
5. N. V. Reutova, *Genetika*, **29**, No. 6, 928-934 (1993).
6. B. Ya. Ryzhavskii, V. I. Mikhailov, Yu. I. Fel'dsherov, *et al*., *Byull. Eksp. Biol. Med*., **129**, No. 1, 28-30 (2000).
7. S. A. Filin, A. I. Filin, and A. A. Sikaeva, *Gig. San*., No. 3, 28-29 (1992).

8. Y. Ding, H. C. Gonick, N. D. Vaziri, *et al*., *Am. J. Hypertens*., **14**, No. 2, 169-173 (2001).
9. G. H. El-Sokkary, E. S. Kamel, and P. J. Reiter, *Cell. Mol. Biol. Lett*., **8**, No. 2, 461-470 (2003).
10. G. J. Flora and P. K. Seth, *Cytobios*, **103**, No. 403, 103-109 (2000).
11. A. I. Kuzmenko, *Wis. Med. J*., **71**, No. 4, 63-66 (1999).
12. E. G. Moreira, G. B. Rosa, S. B. Barros, *et al*., *Toxicology*, **196**, No. 2, 145-151 (2001).
13. O. D. Saugstad, *Semin. Neonatol*., **8**, No. 1, 39-49 (2003).
14. N. D. Vaziri and Y. Ding, *Hypertension*, **37**, No. 2, 223-226 (2001).