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Morphological changes of feto-placental barrier during lead intoxication and under the condition of correcting zinc influence

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Abstract: The article presents results of experimental researches of zinc bioprotective action on the blood-placental barrier in conditions of lead intoxication.

Key words: lead, zinc, influence, placenta, experimental studies.

Negative changes in public health and demographic situation on the background of unfavorable ecological situation in Ukraine caused increased attention of scientists to the problem "environment - human health" [6, 7-22]. Among environmental pollutants lead is one of the most dangerous because it related to the poisons with polytropic action, capable to accumulation in the body, causing a wide range of adverse effects, including reproductive system [1, 34-37].

Long-term epidemiologic studies of scolars of general hygiene department of SI «DMA» found that in conditions of technogenic biogeochemical provinces biological substrates of the system "mother-placenta-fetus" contain lead in the increased concentrations [6, 50-70] that determines development of complications of pregnancy, childbirth and postnatal period and is potentiated by deficiency of essential micronutrients, primarily – zinc, this is caused both by its insufficient entry with food rations and its bio-antagonism with lead [6, 71-94].

Female reproductive system is highly sensitive to the effects of external and internal factors of the environment, especially during pregnancy [5, 464-465]. Due to the complexity of field research among female population and considering data of the structural similarity of the utero-placental relationship of women and that of female rats [2, 54-97], experimental investigations on laboratory animals are of particular importance to establish peculiarities of xenobiotics impact on the structural and functional features of formation and functioning of the placental barrier, maternal-fetal relationship [1, 5-9].

Thus, study of mechanisms of pathology development in pregnant women in modern ecologic conditions is an actual task of clinical and preventive medicine, having caused the purpose of this study.

Objective. To determine features of bioprotective action of zinc combinations on structural organization and state of the blood-placental barrier in conditions of lead intoxication in a laboratory experiment on rats.

Materials and methods. Experimental studies were conducted on female rats of Wistar line (nursery - "Dali-2001"). After 12-days' quarantine 40 animals with persistent rhythm of the estrous cycle aged 3-3.5 months weighing 170-200 g at the stage of pro estrus and estrus were matched with intact males according to the scheme 2:1 [2, 57-62] Studies on animal were conducted to the Law of Ukraine "On protection of animals from cruelty" (Kyiv, 2001), "General ethical experiments on animals "(Kyiv, 2009), which are consistent to the European Convention for the Protection of experimental animals (Strasbourg, 1985) [3, 1-53]. The animals were kept in optimal conditions of the vivarium on a standard diet with free access to food and water.

Female rates with dated gestation period were divided into 4 groups, one of which - the control (group N1), three ones - research (8-9 females in each group). Experimental groups were administered isolated lead acetate at the dose of 0.05 mg/kg (group N2), and in combination with zinc chloride at a dose of 1.5 mg/kg body weight (group N3) and zinc citrate obtained by nanotechnology [4, 1-4] at the dose of 1.5 mg/kg (research group N4) by means of daily intragastric tube from 1

to 19th day of pregnancy. At the same terms rats in the control group were injected solvent used in preparing agents of influence, i.e. distilled water. Doses of metals were increased by 10 times as compared with the level of their total daily entering in an organism of pregnant in industrial areas [1, 34-37]. The selection of female rats in control and experimental groups was performed in random order.

At the final stage of the study, animals were withdrawn from the experiment under thiopental anesthesia and sampling of biological materials for their further their studies was done. Uterus with horns was isolated, fetuses with placenta were isolated from the uterus, mass-metric parameters of placentas and fetuses were measured [2, 1-191]. For the histological examination placenta of rats was fixed in 10% solution of neutral formalin, followed by dehydrated of paraffinic blocks. Histological sections with 5 mm of thickness were prepared using a rotary microtome. Dying of sections released from paraffin was performed with hematoxylin-eosin.

Light microscopy was performed using microscope «Leika CM-E» (USA), lens × 10, × 20, × 40, × 100, × 200. With the digital camera Canon, microscopic data were recorded as digital images in JPEG format. Quantitative study of placenta area was performed using the software package ImageJ 1,47 v. In addition to histological, histomorphometrical analysis of total thickness of placenta, thickness of labyrinthine, spongiotrophoblast and decidual zones was conducted.

All data received were processed by licensed computer programs Microsoft Excel, Statistica 10. Statistical significance of differences was determined by Student t-test.

Results and discussion

Analysis of macroscopic study of the placenta, its mass-metric parameters in animals of the control and experimental groups (Table 1) show that the average weight of placentas in all groups of experimental animals varies between 0.43±0.01 – 0.59±0.02 g, making up 1.40±0.02-1.58±0.03% of mass of pregnant females. In combined administration of zinc chloride with lead acetate a significant

increase in weight and size of the placenta as compared with the group treated with lead acetate only is observed, but significant differences appeared only for diameter finding - by 9.7% ($p<0.05$).

Table 1

Mass-metric parameters of placenta in animals of the control and experimental groups (M±m)

Indexes	Groups			
	N1 control	N2 lead acetate	N3 lead acetate+ zinc chloride	N4 lead acetate+ zinc citrate
Weight of placenta, g	0,59± 0,02	0,57± 0,02	0,59± 0,02	0,57± 0,01
Diameter of placenta, sm	1,51± 0,04	1,44± 0,03	1,58± 0,03***	1,49± 0,03
Fetus-placenta coefficient	0,24	0,25	0,26	0,27
total placenta thickness, μm	2537,3± 163,7	2867,5± 185,4	2529,4± 194,2	2647,4± 185,8
thickness of labyrinthine zone, μm	1945,7± 184,2	2496,4± 203,7*	2075,1± 207,6	2127,4± 198,5
thickness of spongiotrophoblast,μm	452,6±48,2	221,8± 52,9**	279,7± 56,8*	386,6± 49,7
thickness of decidual zone,μm	139,4± 71,1	149,3± 74,5	174,6± 58,7	133,4± 73,2

Note. * - the differences with the control group significant ($p<0.05$), **- $p<0,01$,
*** - the differences with the research group N1 significant ($p<0.001$).

Despite the fact that macroscopic and weight-metric parameters of placenta in control and experimental groups were virtually identical, results of histological and histomorphometrical studies testify to the development of dystrophic-degenerative and discirculatory changes of histological structure of all placental zones of varying degree of manifestation, in maintaining the overall plan of the body structure.

The total placenta thickness in the group receiving lead acetate was not significantly different from the control group values and made up 2867.5±185.4 μm. At the same time thickness of the labyrinthine zone increased by 28.3%

($p<0,05$) as compared with controls (Fig. 1-A), due to the development of destructive-degenerative processes and edema. In the experimental group N2 number of fetal capillaries and degree of their development decreased, vessels contained a small amount of corpuscular blood cells with empty spaces, somewhere being devastated (Fig. 1-B). Quite often phenomenon of erythrocyte sludge syndrome was observed in capillaries. For dilated maternal lacunae stagnant plethora with formation of red blood cells stasis and thrombosis was characteristic. In the trophoblast of the labyrinthine zone significant accumulation of fibrin deposits around the villa comparing with control ones is observed and their micro-villa were shorter and less developed. Violations of cyto- and syncytiotrophoblastic structure of labyrinth beams was defined. Their thickness varied considerably and was the smallest near fetus blood vessels. In some cytotrophoblast areas along with degenerative changes necrotic changes were manifested as well. In syncytium on-site of glycogen granules storage vacuole-like cavities were formed.

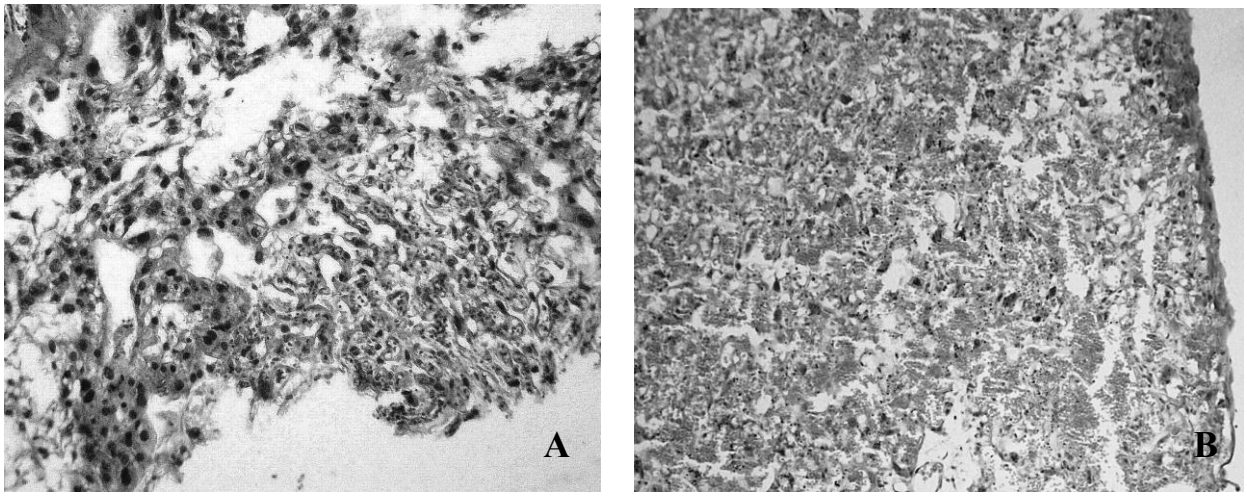

Fig. 1. Placenta at 20th day of pregnancy in control group(A) end group, which received lead acetate (B). Labyrinthine zone. Hematoxylin-eosin. ×200

In combined administration of lead and zinc compounds labyrinthine zone thickness was not significantly different from the control group. In the labyrinthine zone of placenta in experimental group N3 fetal capillaries were somewhat devastated, in some places phenomena of erythrocyte sludge syndrome was

observed (Fig. 2-A). The study did not reveal reliable differences from the norm. In trophoblast cells degenerative changes, focal accumulation of syncytiotrophoblast nuclei in beams, accumulation of fibrin deposits were found. Thickness of trophoblast beams varied considerably. A big number of glycogen cells corresponded to the normal values. Maternal lacunae were moderately dilated, phenomenon of red blood cells stasis, in the periphery of labyrinth - stagnant hyperemia, thrombosis were observed.

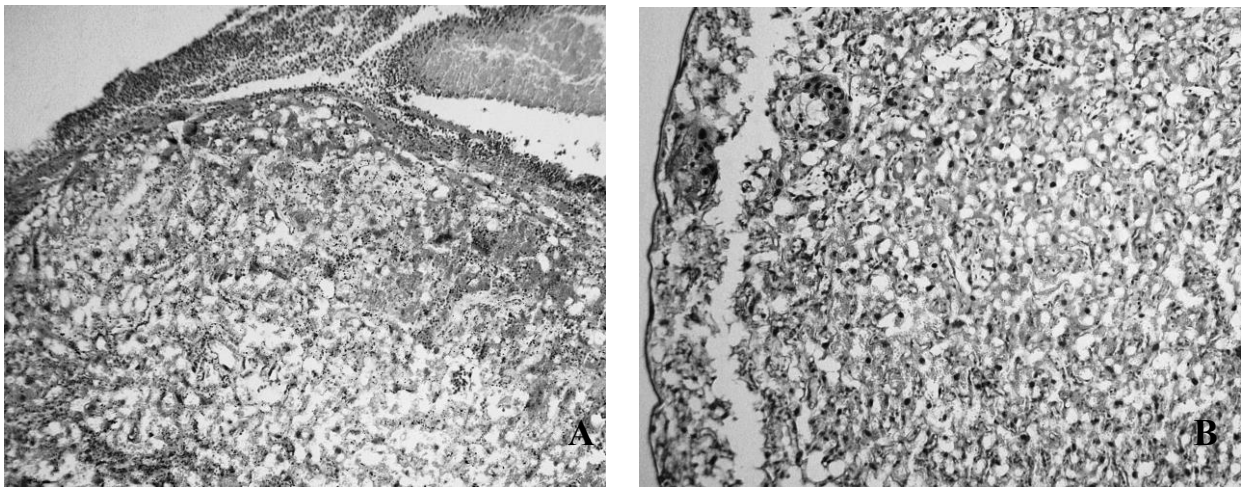

Fig. 2. Placenta at 20th day of pregnancy in experimental groups, which received combination lead acetate with zinc chloride (A) and zinc citrate (B). Labyrinthine zone. Hematoxylin-eosin. ×200

Introduction of lead acetate and zinc citrate (Fig. 2-B) led to the increase of trophoblast giant cells number with a large dense core. In some cytotrophoblast areas necrotic foci were revealed, but their number was not significant. Glycogen islet cells occurred as well. The degree of fetal capillaries development did not differ from the control group, maternal lacunae in the center and trophoblast periphery were moderately dilated, in some places phenomenon of erythrocyte stasis, accumulation of fibrin deposits was observed.

Thickness of placenta spongiotrophoblast of rats receiving lead acetate was 221.8±52.9 μm, being 50,9% ($p<0,05$) less than normal. There was marked enhancement of destruction in islands of glycogen cells, cavities were formed on their places. Number of their glycogen granules was lower than the control. A layer

of trophoblast giant cells was significantly narrowed and consisted mainly of one row of fine cells, in their cytoplasm numerous inclusions were present, this testified to the increased phagocyte activity of cells. Area of boundary maternal sinusoids and of peripheral maternal lacunae decreased, in their lumen thrombosis and erythrocyte aggregation phenomena were observed.

Introduction of zinc supplements reduced pathological manifestations. Thus, while administering inorganic zinc compounds thickness of spongiotrophoblast was less than the norm already by 38.2% ($p<0,05$), number spongiotrophoblast cells was reduced, they were located loosely, forming cavities and gaps often filled with fibrinoid. Strengthening of destruction of glycogen cells was also noted, but it was less expressed than in the experimental group N1, cavities were formed in their places. Peripheral maternal lacunae and sinusoids were dilated and plethoric and characterized by mild stagnation signs and aggregation of red blood cells, and in their lumen thrombosis phenomena were observed. Giant trophoblast cells were smaller in size than normal ones, they were characterized by the signs of phagocytic activity. Among leukocytes clusters necrotically changed cells with altered pyknotic nuclei were revealed.

Index of spongiotrophoblast thickness in the group receiving zinc citrate as a protective agent did not differ from the norm and was 386.6±49.7 μm. This area was represented by polygonal spongiotrophoblast cells that formed correct bands. Among them cluster of glycogen cells was revealed. A layer of trophoblast giant cells was characterized by normal development, cells were arranged in several rows and had inherent elongated, bean-like shape, rounded or oval nucleus. In the cells moderate phagocyte activity was revealed. Area of peripheral maternal lacunae and marginal sinusoids did not differ from the norm, moderate erythrocyte aggregation was observed, accumulation of glycogen cells occurred.

Thickness of the decidual zone in administration of isolated lead acetate and in combination with the compounds of zinc did not differ significantly from the findings of the control group, but light-optic investigation revealed increase of necrosis foci and fibrin deposits, reduce of a big number of lipid granules and

amount of lipids and glycogen in the decidual cells. Herewith pathological changes in the combined administration of lead and zinc compounds were significantly lower as compared with the isolated administration of lead acetate.

Conclusions

1. Introduction of lead acetate during pregnancy causes pathological changes in all placenta areas, this affects the state of the blood-placental barrier, results in distinct dystrophic-degenerative changes and discirculatory changes of histological structure of all placenta zones.

2. Application of zinc in macro- and nanoform decreases negative impact of lead acetate on the organ state. Herewith zinc chloride demonstrates protective effect on formation of labyrinthine zone, namely on the character of development of maternal lacounes and fetal capillaries. Protective effect of zinc in nanoaquachelate form is more expressed and lies in the activation of compensatory reactions both in the labyrinthine area and in spongiotrophoblast where dystrophic, necrobiotic and circulatory changes were minimal.

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