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TRANSLOCATION OF TRACE ELEMENTS IN THE SYSTEM "MOTHER-PLACENTA-FETUS" IN RATS WITH PHYSIOLOGICAL PREGNANCY AND UNDER CONDITIONS OF LEAD EXPOSURE

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Abstract. Translocation of trace elements in the system "Mother-placenta-fetus" in rats with physiological pregnancy and under conditions of lead exposure. Biletska E.M., Onul N.M. *Heavy metals are the second major environmental pollutants. Especially toxic is lead, increased content of which in the body of a pregnant determines the development of pregnancy, childbirth and postpartum period complications and is potentiated by deficiency of essential trace elements - zinc and copper. Article presents the results of impact of solutions of lead acetate and citrate in experimental models. Solutions of metals were injected into the stomach through a tube once a day during 19 days of pregnancy in the dose of 0,05 mg/kg in the form of inorganic compound - lead acetate and in organic form – lead citrate. The content of trace elements - lead, cadmium, copper and zinc were determined by stripping voltamperometry. In addition, indices of placental and fetal accumulation, as well as index of transplacental migration were calculated. Effect of lead during pregnancy in all biosubstrates is increased by 1,3-1,9 times as compared with the control group. This causes imbalance of essential trace elements due to significant reduction in zinc content in all the studied biological substrates. Prolonged contact with lead during pregnancy leads to disruption of placenta, inability of the placenta to fully protect the fetus from excessive intranatal influence of xenobiotics.*

Реферат. Транслокация микроэлементов в системе «Мать-плацента-плод» у крыс при физиологической беременности и при влияния свинца. Белецкая Э.Н., Онул Н.М. *Тяжелые металлы занимают второе место среди основных загрязнителей окружающей среды. Особенно токсичным является свинец, повышенное содержание которого в организме беременной детерминирует развитие осложнений беременности, родов и послеродового периода и потенцируется дефицитом эссенциальных микроэлементов - цинка и меди. В статье представлены результаты изучения влияния ацетата и цитрата свинца на элементный гомеостаз беременной и плода, особенности транслокации металлов в системе «мать-плацента-плод». Металлы вводили перорально ежедневно в течение 19 дней беременности в дозе 0,05 мг/кг. Содержание микроэлементов - свинца, кадмия, меди и цинка определяли методом инверсионной вольтамперометрии. Кроме того, рассчитывали индексы плацентарного и эмбрионального накопления, а также индекс трансплацентарной миграции. Установлено, что в результате воздействия свинца на организм беременной его уровень во всех биосубстратах повышается в 1,3-1,9 раза по сравнению с контролем, вызывает микроэлементный дисбаланс в организме самки и плода и, как следствие, обуславливает нарушение эмбриогенеза и повышение пренатальной гибели плодов. Длительный контакт со свинцом во время беременности приводит к нарушению фетоплацентарного комплекса и характеризуется неспособностью плаценты в полной мере защищать плод от чрезмерного интранатального влияния ксенобиотика.*

Heavy metals occupy second place among the basic contaminants of the environment, their concentration exceeds the background level by 30-600 times [3, 9]. Prolonged action of toxic metals on the organism leads to violation of adaptation, barrier-detoxication and excretory systems, which is accompanied by their active cummulation in the organs and tissues. Such a situation significantly increases the risk of development of normal biochemical and biologic fundamentals of vital activity [5].

Among all heavy metals and their compounds lead has the most harmful action [3, 8]. In the number of investigations a reproductive toxicity of

this xenobiotic has been proved, especially in conditions of technogenic biogeochemical provinces, as its increased content in the organism of a pregnant woman determines development of complications in pregnancy, labor and postnatal period, deterioration of findings of physical and intellectual development of a child and is potentiated by deficit of essential microelements – lead and cuprum [4, 9].

At the same time there exists a very small number of works dedicated to the impact of low concentrations of lead on the embrional development [3, 6, 7, 10]. Questions of etiopathogenesis of a negative impact of this metal on placenta and fetus remains poorly studied. It is known that a prolonged contact with lead during pregnancy, even at the level of threshold and sud-threshold values leads to violation of development and functioning of a feto-placental complex [3, 7]. That is why studying functional possibilities of defense systems, which prevent entry of harmful and metabolically active components and toxicants into fetus organism or favor their desactivation is extremely relevant, as it may serve as theoretical basis for the development of practical measures towards defense of intrauterine development of fetus in conditions of increased technogenic loading on pregnant woman's organism.

Aim of the research – to study peculiarities of metals translocation in the system «mother-placenta-fetus» in the action of inorganic and organism lead compound on the organism of a pregnant woman.

MATERIALS AND METHODS

Experimental investigations were carried out on female rats of Wistar line (nursery – «Dali-2001»). After 12-days' quarantine 30 animals with a stable rhythm of estral cycle aged 3-3,5 months weighing 170-200 grams in the stage of proestrus and estrus were paired with intact males by the scheme 2:1. Investigations on animals were carried out according to the Law of Ukraine «On defense of animals from brutal behavior» (Kyiv, 2001), «General ethical principles of experiments on animals » (Kyiv, 2009), which come to an agreement with European convention on defense of experimental animals [11].

Female rats with dated term of pregnancy were divided into 3 groups. One group (№1) - control one, animals receives distillated water. Two other groups – under investigation, by means of intra-intestinal probe they were introduced lead in the dose of 0,05 mg/kg daily from 1 till 19 day of pregnancy in the form of inorganic compound (lead acetate - group №2) and in organic from (lead citrate, obtained by aqua-nanotechnology UkrSRINanobiotechnology – group № 3). Selection of male rats to control and group under investigation was in randomized order.

At the terminal stage of the investigation animals were withdrawn from the experiment under thiopenthal narcosis and sampling of biological materials for further defining microelement content was done. Samples of blood, fetuses were prepared by means of dry ashing. Content of microelements – lead, cadmium, copper and zinc was defined by method of inversion volt-amperometry using the device ABA-2. As standard solutions there were used Interstate standard samples of contents of ions of lead, cadmium, zinc and copper of Physical-chemical institute of NAS of Ukraine, Odesa.

Besides, to study peculiarities of translocation of metals from mother's organism to fetus a number of indices were calculated. It should be mentioned, that in separate researches, concerning studying peculiarities of metals migration in the organism of a pregnant woman, there was calculated index of placental invasion of metals as ratio of metals concentration in the fetus up to their level in mother's organism. But, to our opinion such definition is somewhat false, as in developing embryonal organism, together with passive entry of metals there occurs their active accumulation [9]. Taking into account all listed we developed procedure of calculation of such informative indices of metals translocation:

Index of placental accumulation (IPA) – ratio of concentration of metal in the placenta to its content in the blood of a pregnant woman, conv. un.

Index of embryo accumulation (IEA) – ratio of concentration of metal in the embryo to its content in the blood of a pregnant woman, conv. un.

Index of transplacental migration (ITM)– ratio of index of embryo accumulation to index of placental accumulation, conv. un..

All the digital data obtained were processed by computed licensed Microsoft Excel, Statistica 10. Reliability of distinctions were defined by t-criteria [1].

RESULTS AND DISCUSSION

Analysis of the performed researches testify (таble), that content of lead in the blood of pregnant female rat in introducing metal in the form of acetate and citrate reliably grows by 1,6 and 1,8 times correspondingly as compared to control group and coincides with the results of other researches [7]. Such a situation reflects loading of the organism with lead, this, is probably, may cause definite changes in the metabolism, though without clinical symptoms of poisoning [4, 6].

It should be noted that in accumulation of lead in the organism there occurs change of elemental homeostasis of the blood, violation of physiological interrelation of essential microelements. So, while

introducing lead compounds there is observed diametrically opposite changes of concentrations of zinc and cooper in the blood – against background of decrease of zinc content by 2,9-3,1 times, increase of cooper concentration by 2,2-2,4 times ($p<0,001$) as compared with intact animals. Besides, there was revealed re-distribution of concentration of metals under investigation in the blood of pregnant rat. So, if in the control group content of metals decreases towards zinc>cooper>lead>cadmium, while introducing lead compounds this row gains a new aspect: cooper>zinc>lead>cadmium, that is there occurs increase of cooper level at the expense of a significant zinc concentration. While introducing lead both in inorganic and organic form a correlation of Cu:Zn makes up 1:0,5, at the same time in physiological course of pregnancy this is characterized as 1:3,3. It is an interesting fact that such a correlation of essential microelements is observed in pregnant women too [9], this confirms significance of experimental data for understanding of mechanisms of metal migration in the organism of a pregnant woman.

Concerning comparative aspects of metal content in the blood while introducing organic and inorganic compounds of lead, under the action of lead citrate there is observed tendency to a more higher concentration of lead and cadmium in a more significant decrease of cooper and zinc content, but these differences were reliable only for cadmium– by 51,6% ($p<0,05$).

Content of heavy metals in the biosubstrates of experimental animals in the system «mother-placenta-fetus», M±m

Group of animals	Concentration of metals, mg/kg			
	Pb	Cd	Cu	Zn
Blood				
control	0,061±0,005	0,0048±0,0009	0,88±0,06	2,89±0,19
lead acetate	0,101±0,011**	0,0031±0,0005	2,08±0,23***	1,01±0,07***
lead citrate	0,110±0,009***	0,0047±0,0004°	1,93±0,24***	0,94±0,12***
Placenta				
control	0,096±0,015	0,0155±0,0035	2,36±0,26	4,15±0,41
lead acetate	0,304±0,055***	0,0069±0,0014*	4,49±0,50***	1,39±0,18***
lead citrate	0,232±0,051*	0,0139±0,0024	3,07±0,39	2,11±0,26***
Fetus				
control	0,077±0,005	0,0054±0,0007	2,47±0,18	3,46±0,26
lead acetate	0,114±0,004***	0,0056±0,0006	3,14±0,34	1,39±0,10***
lead citrate	0,102±0,019	0,0044±0,0008	2,17±0,19	1,46±0,12***

N o t e s : * - $p<0,05$; ** - $p<0,01$; *** - $p<0,001$ compared with control; ° - $p<0,05$; °° - $p<0,01$; °°° - $p<0,001$ compared with group N 1.

Placenta in the organism of pregnant woman performs a number of important functions, barrier one including, defending fetal organism from excessive entry of toxic compounds, lead and cadmium belong to them. In parallel it provides embryo with necessary microelements – zinc and cooper. Analysis of the performed investigations testify, that in the placenta of female rats wxposed to lead in inorganic and organic form, metal content increased by 3,2 times ($p<0,001$) та 2,4 ($p<0,05$) correspondingly with intact animals. Herewith there was observed a reliable decrease of zinc concentration - by 3,0 and 2,0 times ($p<0,001$) correspondingly. Introduction of lead acetate is characterized by decrease of cadmium content by 2,3 times ($p<0,05$) and increase of cooper concentration by 1,9 times ($p<0,001$) in the placenta as compared with control group.

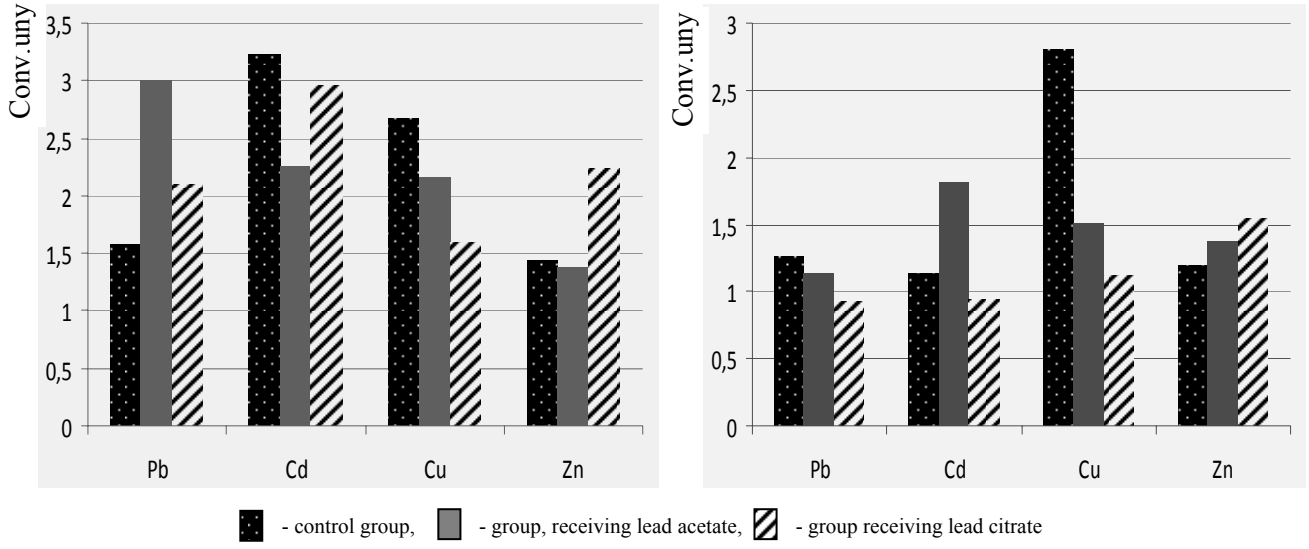
Concerning ratio of metals in the placenta, as well as in the blood when introducing lead there is observed decrease of their concentrations toward: cooper>zinc>lead>cadmium. Besides, ratio Cu:Zn is

disturbed – from 1:1,8 under conditions of physiological course of pregnancy to 1:0,3 and 1:0,7 times in case of impact of lead acetate and citrate correspondingly.

Introduction of lead into the organism of pregnant rat is characterized by accumulation of metal in the fetal organism as compared with control group, but it was reliable only for lead acetate - by 1,4 times ($p<0,001$). Herewith there was observed decrease of zinc content by 2,4-2,5 times ($p<0,001$) in the absence of reliable differences in concentration of cooper and cadmium for both groups under investigation. Ratio of metals in the blood and placenta changes analogously to research results. All listed above testify that despite sufficiently low level of cooper in mother's blood, its content in the placenta significantly rises with following active entry in embryo, this is marked on the ratio Cu:Zn in fetal organism. In case of physiological pregnancy this ratio makes up 1:1,4 correspondingly, in condition of introducing lead acetate and citrate, that is it is observed a more active intrauterine accumulation of cooper.

To our opinion such a situation is caused by inclusion of adaptation mechanisms in placenta for provision of normal trophism of fetus under conditions of lead intoxication. This is confirmed by calculated by the authors indices of placental, embryonal accumulation (fig.) under conditions of lead intoxicationи and index of trans-placental transition.

So, despite higher indices of absolute lead content in fetuses while introducing lead compounds, level of its embryonal accumulation decreases by 10,3-26,2% at the expense of intensification of intraplacental accumulation by 45,0-52,5% as compared with intact animals In parallel there occur changes of trans-placental migration of cadmium, that under condition of impact of lead acetate increases by 2,3 times, this favors its more intensive accumulation in fetal organism – by 1,6 times as compared with control group. However, level of cadmium in the blood while introducing lead acetate was somewhat lower than that of control group, this fact does not have meaningful impact on cadmium level in fetuses as compared with intact animals.



Indices of placental – IPA (a) and embryonal accumulation – IEA (b) of animals of control and group under investigation

Concerning peculiarities of trans-placental migration of essential microelements – zinc and cooper in case of lead compounds impact, there was revealed the following. Zinc under conditions of physiological pregnancy accumulates in placenta relatively not actively – its concentration in comparison with other metals increases only by 1,44 times ($p<0,01$). Herewith 83% of metal from the level of its intraplacental concentration transfers to fetus and significantly do not differ from its content in the blood of a pregnant woman. While introducing lead acetate, intensity of zinc accumulation in placenta and embryo is characterized by a more lower index– 1,38 conv. un. and, despite the fact that under conditions of toxic impact of lead, trans-placental migration of zinc increases by 20% in comparison with the control group; its concentration in the fetal organism reliably decreases. While introducing lead citrate, vice versa, intensity of accumulation of zinc in placenta and embryo increases as comparing with

control group, but at the expense of decrease of intensity of trans-placental migration by 14%, content of metal in fetal organism also decreases as compared with intact animals.

In physiological pregnancy level of intra-placental and intra-embryo accumulation of cooper grows by 2,68 and 2,81 times correspondingly ($p<0,001$) in comparison with content in rat's blood, herewith index of trans-placental migration was the highest of all metals – 1,05 conv. un. Such a situation confirms data on significant accumulation of cooper in embryo, this is extremely necessary for its normal formation and development, especially under conditions of physiological hypocooperemia in a pregnant. Lead introduction into organism of a pregnant rat leads to decrease both of level of intra-placental accumulation of cooper by 1,7-2,5 times, this is caused by decrease of ITM by 33%. So, despite reliable increase of cooper content in rat's blood, exposed to lead impact, its concentration in fetal organism reliably dos not differ from indices of intact animals at the expense of decrease of intensity of trans-placental migration of microelement.

So, entry of lead into organism of female rat during the whole period of pregnancy is accompanied with increase of its content both in the blood – by 1,6-1,8 times, and in placenta and fetal organism – by 1,3-1,9 times. Content of cadmium remains practically unchanged, excluding decrease of its concentration in placenta by 2,3 times in introducing lead acetate. Herewith, there is observed imbalance of essential microelements at the expense of reliable decrease of zinc content in all bio-substrates under investigation by 2,0-3,1 times and increase of cooper content by 1,9-2,4 times, fetus is an exception. Such a situation leads to disorder of physiological ratio Cu:Zn in all biosubstrates under investigation. Herewith, lead acetate causes more expressed changes of microelement balance both in the blood of a pregnant woman and in placenta and fetal organism, this leads to disorder of placental- and embryogenesis, causes increase of embryo lethality as compared with intact animals [2]. All above-mentioned testifies that, unfortunately, embryo's organism is unable to selective accumulation of essential microelements, treating toxic metals as functionally necessary ones, that is there is observed mimesis phenomenon [9].

Against a background of lead intoxication adaptation and barrier-detoxication processes in placenta are involved, this is manifested in the increase of level of intra-placental accumulation of xenobiotics – lead and cadmium for decreasing of level of their entry to fetus. At the same time intensity of intraplacenta accumulation of essential micro-elements – zinc and cooper decreases by 1,04-1,7 times, with exception of increase of level of zinc accumulation in introducing lead citrate. Herewith, essential decrease of zinc concentration in the blood of a pregnant female under condition of introducing lead is compensated by growth of its trans-placental migration, while hypercooperemia is compensated by decrease in intensity of trans-placental migration of cooper 33%. However, despite inclusion of above-mentioned compensatory mechanisms, placenta is unable to defend fetal organism from toxic action of lead in full; this ultimately leads to increase of its concentration and decrease of zinc content in fetal organism, especially under condition of inorganic form of lead. Such a situation may be caused by a number of factors: chemical peculiarities of metals, their initial concentration in pregnant organism, period of pregnancy – as it is known [9], that diffusion stage of metals depends on surface size and thickness of placenta, which are quite insignificant at early stages of placenta- and embryogenesis. In addition, active period of placentation in rats begins on day 4-5 of pregnancy, that is at early stages embryo practically is not defended from direct toxic impact of metals.

CONCLUSIONS

1. Impact of low doses of lead in inorganic and organic form causes increases of its accumulation in the system «mother-placenta-fetus» by 1,3-1,9 times as compared to physiological course of pregnancy and leads to microelement imbalance in the organism of female and fetus, this causes disorder of embryogenesis and increase of pre-natal death of fetuses.

2. Long-term contact with lead during pregnancy leads to disorder of fetal-placenta complex, causing intrauterine desadaptation processes, which are characterized by expressed disturbance of element homeostasis in fetal organism and reflect inability of placenta to defend fetus from excessive intra-natal impact of xenobiotic in full measure.

3. There were defined peculiarities of translocation of essential and toxic metals in the system «mother-placenta-fetus», this is an important theoretical basis of opening of mechanism of etiopathogenesis of embryo-toxic impact of lead and developing practical measures towards defense of intrauterine fetus development in conditions of increased technogenic loading on pregnant organism.

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