

**SEARCH OF NEW BIOANTAGONISTS OF EMBRYOTOXICITY OF CADMIUM CHLORIDE
IN A CHRONIC EXPERIMENT ON RATS**

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The problem of embryotoxicity of cadmium remains extremely important, because the work of women in harmful production conditions often comes into contact with the action of heavy metals, which is very dangerous for the fetus and, in general, for the offspring. The purpose of the study to search for possible bioantagonists of the embryotoxicity of cadmium chloride among zinc and copper succinates when combined with cadmium chloride in chronic experiments in rats. The study was carried out on pregnant female Wistar rats with a dated gestation period. The research groups, in addition to the control group, included a group of isolated injection of cadmium chloride and 2 groups of combined injection of cadmium chloride and zinc succinate or copper succinate. The influence of the studied factors was carried out daily from the 1st to the 19th day of pregnancy by injecting the solution intragastrically (by probing), once a day. The degree of embryotoxicity of the studied compounds was determined by the indicators: the number of embryos ($M \pm m$), pre-implantation, post-implantation and total embryonic mortality in comparison to the control. In the group of isolated influence to cadmium chloride, a significant decrease in the number of embryos was observed, and pre-implantation, post-implantation and total embryonic mortality compared to the control increased several times. In groups of combined injection of cadmium chloride with succinates of copper or zinc metals, the studied indicators demonstrate the modifying effect of succinates on the embryotoxicity of cadmium chloride. Conclusions: 1. Isolated chronic injection of cadmium chloride has a marked embryotoxic effect on indicators of the number and mortality of embryos. 2. The combined injection of zinc or copper succinates with cadmium chloride has a modifying effect on the embryotoxicity of cadmium chloride according to the main indicators of embryotoxicity, and the succinates themselves can be considered as potential bioantagonists of cadmium during chronic exposure in an experiment on rats.

Key words: rats, embryotoxicity, cadmium, zinc, copper.

Connection of the publication with planned research works.

The experimental study was carried out as part of the research work of the Department of Medical Biology, Pharmacognosy, Botany and Histology of the Dnipro State Medical University "Biological bases of morphogenesis of organs and animals under the influence of microelements and ultramicroelements in the experiment" (state registration number 0118U006635).

Introduction.

The problem of the occurrence and development of microelement dyselementosis in all countries of the world is extremely urgent, its solution, according to the definition of the WHO, is the main task in ensuring the health of the Earth's population in the 21st century [1, 2, 3]. Hypomicroelementoses, which are caused by a deficiency of essential microelements, cause the development of the "deficiency" disease, and the body's contact with toxic microelements provokes toxicopathy. Micronutrient deficiency is never isolated, because it is associated with an imbalance of micronutrients and is manifested in a metabolic disorder with corresponding morphological manifestations [4, 5, 6]. The modern development of new potential means for the correction or prevention of trace element imbalance is held back by the lack of knowledge about the peculiarities of the metabolism of trace elements in the body of healthy people and the norms of their daily need in conditions of increased technogenic load, as well as data on the balance, forms and types of interaction of trace elements and ultra-trace elements in the case of their simultaneous arrival [7, 8].

It is a well-known fact that the ecological disadvantage of society is gradually taking on the features of a planetary disaster. According to a number of authors, indicators of reproductive function in women living in polluted cities differ significantly from control indicators. They have the same orientation in the form of increasing the number of complications of pregnancy, childbirth and the postpartum period; an increase in the rate of early neonatal morbidity and mortality, the number of congenital malformations; deterioration of children's health in the distant periods of postnatal ontogenesis. All these changes lead to a decrease in the birth rate, as well as the birth of sick children with physical and intellectual defects [9, 10, 11].

Last years there has been an expansion of the scope of the use of various cadmium compounds and a significant increase in the anthropogenic contribution to their pollution of the environment, as a result of which the content of cadmium in atmospheric air, food products and objects of domestic and drinking water supply exceeds permissible standards in a number of regions of our country and abroad. The problem of embryotoxicity and fetotoxicity of cadmium remains extremely important, because the work of women in harmful production conditions often comes into contact with the effect of heavy metals, which is very dangerous for the fetus and, in general, for the offspring. Currently, the influence of microelementosis of the mother on the development of the embryo and fetus has been established, and all types of microelementosis of the mother's organism can have a teratogenic effect on the offspring [12]. All these circumstances present researchers with the task of carefully studying the impact of cadmium on the body dur-

ing intrauterine development. The prospects of chronic exposure of heavy metals to the course of embryogenesis with microelements that have bioantagonistic properties relative to the toxicity of heavy metals are poorly researched [13, 14, 15]. Thus, the actual direction of morphological experimental research is the search for new possible bioantagonists of cadmium toxicity among microelements capable of affecting dyselementoses, supporting the body's homeostasis, including during pregnancy.

The aim of the study.

To experimentally determine the indicators of the degree of embryotoxicity of cadmium chloride at a dose of 2.0 mg/kg with isolated intragastric administration and to search for possible bioantagonists among zinc or copper succinates with combined injection with cadmium chloride in a chronic experiment on pregnant female rats.

Object and methods of research.

The experiment was carried out in the vivarium of the Dnipro State Medical University on 32 young female rats of the Wistar line (nursery "Dali 2000", Kyiv), weighing 180-280 g. The study of the estrous cycle of females by the method of vaginal smears made it possible to determine the presence and duration of the cycle and individual phases, and the rhythmicity of their alternation in each female. To select experimental animals for the experiment, females with a steady rhythm of the estrous cycle at the stage of proestrus and estrus were determined, which were mated with intact males according to the generally accepted scheme of 2:1. Determination of the first day of pregnancy of females was carried out under the condition of the presence of spermatozoa in vaginal smears of females. Females with a dated gestation period were obtained by this method, which allowed them to obtain embryos with a certain date of development.

All pregnant females were divided into the following groups: the first group – control (8 females); the second group – isolated injection of cadmium chloride solution at a dose of 2.0 mg/kg (8 females); the third group – combined injection of cadmium chloride solution at a dose of 2.0 mg/kg + zinc succinate at a dose of 5 mg/kg (8 females); the fourth group – combined injection of cadmium chloride solution at a dose of 2.0 mg/kg + copper succinate 0.1 mg/kg (8 females). In each group, females were divided into 2 subgroups according to the terms of withdrawal from the experiment.

In our experiment, zinc succinate and copper succinate, namely their chelate compounds, which are widely used in chemistry, medicine (pharmacy) and agriculture, were used for the introduction of trace elements into food due to the high digestibility of chelate complexes compared to free metal ions (in our experiment – cadmium chloride). Solutions of succinates were nanosized, that is, we used nanoaquachelates of copper and zinc succinates.

The influence of the studied factors on female rats was carried out daily from the 1st to the 19th day of pregnancy by injecting the solution intragastrically (through a probe) once a day. On the 13th and 19th days of pregnancy, females were slaughtered. To achieve the goal, during surgery, the number of fetuses in each uterine horn and their correspondence to the number of corpora lutea in the ovary on the corresponding side

were counted. The number of corpora lutea in the ovaries of females was used to calculate pre-implantation mortality of embryos, and post-implantation mortality was determined by the difference in implantation sites (embryo resorption) in the uterine horns and the number of surviving embryos. These indicators are basic indicators for calculating the total embryonic mortality and determining the degree of embryotoxicity of the studied compounds in comparison to the control.

The embryotoxic effect of the studied substances was evaluated based on the results of calculating standard indicators of embryological development:

1. Average indicators of the number of embryos in the litter of each group (M±m)

2. Total Embryonic Mortality (TEM)

$$TEM = \frac{B-A}{B} \times 100\%$$

where A is the number of live embryos

B – the number of corpora lutea of pregnancy

3. Preimplantation mortality (PreIM)

$$PreIM = \frac{C-(A+B)}{C}$$

where A is the number of live embryos

B – the number of dead (resorbed) embryos

C – the number of corpora lutea of pregnancy

4. Postimplantation mortality (PostIM)

$$PostIM = \frac{B}{A+B}$$

where A is the number of live embryos.

B – the number of dead (resorbed) embryos.

5. Number of embryos per 1 female in the group (M±m).

The obtained results were processed by the method of variation statistics. The assessment of the probability of statistical studies was carried out using the Student's t-test.

Research was carried out in accordance with the principles of the Declaration of Helsinki, adopted by the General Assembly of the World Medical Association (2000), "General ethical principles of experiments on animals", approved by the First National Congress on Bioethics (Kyiv, 2001) in accordance with the provisions of the "European Convention on the protection vertebrate animals used in experiments and other educational purposes" (Strasbourg, March 18, 1986).

Research results and their discussion.

In the experiment, all females survived, were active and consumed food and water well. The extraction of embryos was carried out on the 13th and 19th days of embryogenesis operatively under thiopental anesthesia of females. Already during the operative removal of embryos, attention was drawn to the fact that in the group of isolated introduction of cadmium chloride, a decrease in the number of embryos and their asymmetric location in the horns of the female uterus was observed (fig.).

Such a situation indicates a negative influence of the studied factors on the morphofunctional state of the ovaries of the female. As is known, cadmium compounds have a high level of gonadotoxicity and cause a violation of their functional state. In our experiment, such a violation led to an asymmetric arrangement of embryos with a significant advantage of the number of embryos in one horn of the uterus in 34.3% of females of the group of isolated influence to cadmium chloride.

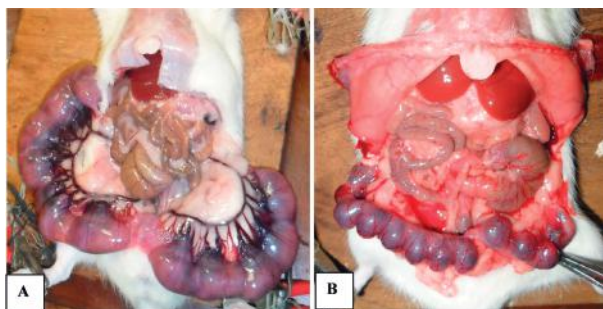


Figure – Asymmetric arrangement of embryos in the uterine horns of a female rat of the control group (A) and the group of isolated cadmium chloride injection (B).

In the groups of combined influence, the asymmetry of the location of the embryos was determined only in 4.2% of females, and in the control group this violation was not determined at all.

To achieve this goal, we calculated quantitative indicators of the number of embryos in groups at two stages of embryonic development and all types of embryonic mortality, which are standard indicators of embryotoxicity. Calculation and analysis of the obtained data of embryotoxicity indicators of the studied compounds showed the following. On the 13th day of embryonic development in the control group, the average number of embryos was 13.45 ± 0.26 , and in the group isolated injection to cadmium chloride, a significant decrease in the number of embryos was observed, namely – 9.3 ± 0.54 . In the groups of combined injection of cadmium chloride with metal succinates, the studied indicators demonstrate the modifying effect of zinc and copper succinates on the embryotoxic effect of cadmium chloride, namely, in both groups, the number increased and amounted to 11.27 ± 0.63 at this term of development. Such a difference of the presented indicator had reliability ($p = 0.05$) in comparison with the group of isolated influence of cadmium chloride.

The next point of the experimental study was the 19th day of pregnancy, that is, the end of rat embryogenesis. In the group of isolated exposure to cadmium, the same tendency to decrease the number of embryos was determined – 9.18 ± 0.74 against the control indicators – 13.78 ± 0.35 , which in our opinion is logical, because exposure to the cadmium solution is continued. In groups of combined injection of cadmium chloride, the results of calculations were unexpected at this time. The lowest indicator of the number of embryos was in the group of combination with zinc succinate and was 7.76 ± 0.25 , despite the high indicator on the 13th day in comparison with the group of isolated exposure to cadmium chloride. In the group of combined exposure to cadmium chloride and copper succinate, this indicator on the 19th day had no significant difference with the control and was equal to 10.50 ± 0.73 embryos, which indicates the modifying effect of copper succinate on the toxicity of cadmium chloride when combined in the experiment on pregnant female rats.

The calculation of embryonic mortality rates on the 13th day of embryogenesis showed the following: the total embryonic mortality in the control group was 0.03 ± 0.01 , while in the group of isolated cadmium chloride administration it increased ($p=0.001$) to 0.25 ± 0.03 . In groups of combined administration, the rate of total embryonic mortality decreased by more than half com-

pared to the group of isolated exposure to cadmium chloride. With the combination of cadmium and zinc succinate, the total embryonic mortality on the 13th day was 0.10 ± 0.03 , and in the combination with copper succinate – 0.14 ± 0.02 , which was significantly less than the isolated injection group. The study indicators on the 19th day kept the same downward trend compared to the isolated injection of cadmium chloride.

The rate of total embryonic mortality is proportional and actually consists of indicators of pre-implantation and post-implantation embryonic mortality. Pre-implantation embryonic mortality is also present in the control and is 0.03 ± 0.01 , but cadmium influence increased this indicator to 0.09 ± 0.02 . In the combined injection groups, this indicator decreased on average to 0.07 ± 0.04 , which indicates the modifying effect of copper and zinc succinates on the embryotoxicity of cadmium chloride. The rates of post-implantation embryonic mortality were, respectively: 0.01 ± 0.01 in the control and 0.17 ± 0.03 with isolated injection to cadmium, and in combination with zinc and copper to $0.07-0.09$, which also indicates a decrease in cadmium toxicity.

Thus, according to all criteria of embryotoxicity, the isolated introduction of cadmium has a pronounced embryotoxic effect, which is expressed in a decrease in the number of embryos in the litter and an increase in all types of embryonic mortality. In the groups of combined injection to cadmium chloride with metal succinates, a compensatory effect on indicators of cadmium embryotoxicity was determined. The obtained data prove that the combined injection of zinc and copper succinates have a modifying effect on the embryotoxicity of cadmium chloride in an experiment on rats, and the succinates themselves can be considered as potential bioantagonists of cadmium chloride in the body of experimental animals with combined intragastric administration.

Conclusions

1. Isolated chronic intragastric administration of cadmium chloride at a dose of 2.0 mg/kg has a pronounced embryotoxic effect on indicators of the number of embryos, total embryonic mortality, pre-implantation and post-implantation embryonic mortality on the 13th and 19th days of embryogenesis in an experiment on rats.

2. The combined injection of zinc or copper succinates with cadmium chloride has a modifying effect on the embryotoxicity of cadmium chloride according to the main indicators of embryotoxicity, and the succinates themselves can be considered as potential bioantagonists of cadmium during chronic exposure in an experiment on rats.

Prospects for further research.

In our opinion, the detection of morphological changes in the organs of the intestinal-gastric tract of embryos of experimental animals after influence to the studied factors is promising.

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ПОШУК НОВИХ БІОАНТАГОНІСТІВ ЕМБРІОТОКСИЧНОСТІ ХЛОРИДУ КАДМІЮ У ХРОНІЧНОМУ ЕКСПЕРИМЕНТІ НА ЩУРАХ

Шаторна В. Ф., Кононова І. І., Гарець В. І., Нефьодова О. О., Ломига Л. Л.

Резюме. Малодослідженими є аспекти хронічного впливу солей кадмію на перебіг ембріогенезу як при ізольованому впливі так і з мікроелементами, що мають біоантагоністичну властивість щодо токсичності важких металів. Таким чином, актуальним напрямом морфологічних експериментальних досліджень є пошук нових можливих біоантагоністів токсичності кадмію серед мікроелементів, здатних впливати на диселементози, підтримуючи гомеостаз організму, у тому числі під час вагітності. Метою експерименту було визначення ступеня ембріотоксичності хлориду кадмію у дозі 2,0 мг/кг при ізольованому внутрішньошлунковому введенні та проведення пошуку можливих біоантагоністів серед сукцинатів цинку та міді при комбінованому введенні з хлоридом кадмію у хронічному експерименті. Дослідження проведено на 32 вагітних самках щурів лінії Wistar, які були розподілені на 4 експериментальні групи: перша – контроль; друга – ізольованого введення розчину кадмію хлориду у дозі 2,0 мг/кг; третя – комбінованого введення розчину кадмію хлориду у дозі 2,0 мг/кг + сукцинат цинку у дозі 5 мг/кг; четверта – комбінованого введення розчину кадмію хлориду у дозі 2,0 мг/кг + сукцинат міді 0,1 мг/кг. Можливу негативну дію речовин, що вивчаються на ембріональний розвиток визначали по здатності підвищувати рівень всіх видів ембріональної смертності. Аналіз отриманих результатів продемонстрував, що ізольоване хронічне введення хлориду кадмію в дозі 2,0 мг/кг має виражений ембріотоксичний вплив на показники кількості ембріонів, загальної ембріональної смертності, доімплантаційної та постімплантаційної ембріональних смертностей у щурів. Комбіноване введення сукцинатів цинку або міді з хлоридом кадмію має модифікуючий вплив на ембріотоксичність хлориду кадмію за основними показниками ембріотоксичності, а самі сукцинати можуть розглядатися як потенційні біоантагоністи кадмію при хронічному впливі на експеримент.

Ключові слова: щури, ембріотоксичність, кадмій, цинк, мідь.

SEARCH OF NEW BIOANTAGONISTS OF EMBRYOTOXICITY OF CADMIUM CHLORIDE IN A CHRONIC EXPERIMENT ON RATS

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Abstract. Aspects of the chronic effect of cadmium salts on the course of embryogenesis, both as an isolated effect and with microelements that have bioantagonistic properties in relation to the toxicity of heavy metals, are poorly studied. Thus, the actual direction of morphological experimental studies is the search for new possible bioantagonists of cadmium toxicity among trace elements that can affect dyselementoses, maintaining body homeostasis, including during pregnancy. The purpose of the experiment was to determine the degree of embryotoxicity of cadmium chloride at a dose of 2.0 mg/kg with isolated intragastric administration and to search for possible bioantagonists among zinc and copper succinates when combined with cadmium chloride in a chronic experiment. The study was carried out on 32 pregnant female Wistar rats, which were divided into 4 experimental groups: the first – control; the second – isolated administration of a solution of cadmium chloride at a dose of 2.0 mg/kg; the third – combined administration of a solution of cadmium chloride at a dose of 2.0 mg/kg + zinc

succinate at a dose of 5 mg/kg; the fourth – combined administration of a solution of cadmium chloride at a dose of 2.0 mg/kg + copper succinate 0.1 mg/kg. The possible negative effect of the studied substances on embryonic development was determined by the ability to increase the level of all types of embryonic mortality. The analysis of the obtained results showed that the isolated chronic administration of cadmium chloride at a dose of 2.0 mg/kg has a pronounced embryotoxic effect on the number of embryos, total embryonic mortality, pre-implantation and post-implantation embryonic mortality in rats. The combined administration of zinc or copper succinates with cadmium chloride has a modifying effect on the embryotoxicity of cadmium chloride in terms of the main indicators of embryotoxicity, and the succinates themselves can be considered as potential bioantagonists of cadmium during chronic exposure in the experiment.

Key words: rats, embryotoxicity, cadmium, zinc, copper.

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Conflict of interest:

The authors declare no conflict of interest.

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