DOI 10.29254/2077-4214-2023-2-169-397-401
UDC 611.12–053.13:546.48'131:546.461.4:616–091.8–092.9

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CHANGES IN EMBRYOTOXICITY INDICATORS OF CADMIUM CHLORIDE IN A CHRONIC EXPERIMENT ON RATS

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Studying the influence of cadmium on embryogenesis remains relevant in our time, since man-made load in industrial regions can have negative consequences for people, especially women during pregnancy, because of causing pathological changes in offspring, as it is able to overcome the placental barrier.

The article presents the results of a study of the effect of cadmium chloride at a dose of 2 mg/kg on rats' embryogenesis in a chronic experiment, as well as a comparative analysis with previous results of studies of the influence of cadmium chloride at a dose of 1 mg/kg. During the consideration of the results, there was a significant decrease in the number of living embryos per 1 female in the cadmium chloride group compared to the control group, as well as a significant increase in the total, preimplantation and postimplantation embryonic mortalities. These indicators have also been compared with the corresponding results of previous studies of the influence of cadmium chloride at a dose of 1 mg/kg for rats' embryogenesis. Comparison of results revealed a significant increase in general and preimplantation mortality with the introduction of cadmium chloride at a dose of 2 mg/kg, especially on the 20th day of the experiment, while the indicator of postimplantation mortality is almost indistinguishable from the results of previous studies.

Thus, the results obtained suggest that the chronic effect of cadmium chloride at a dose of 2 mg/kg has an apparent embryotoxic effect, and compared to relative indicators of general, preimplantation and postimplantation mortalities with similar indicators in previous experiments its toxicity in the early stages of embryogenesis.

Key words: *embryotoxicity, cadmium chloride, rats, embryogenesis.*

The connection of the publication with planned research works.

The experimental study was performed within the framework of the research work of the Department of Medical Biology, Pharmacognosy, Botany and Histology of DSMU "Biological bases of morphogenesis of organs and animals under the influence of trace elements and ultramicroelements in the experiment" (№ state registration 0118U00635).

Introduction.

Cadmium (Cd) is a heavy metal that has a toxic effect on many eukaryotic organisms because it does not have a clear biological role, and all its real effects are toxic. From the environment into the human body, it gets in different ways: air, food, aquatic, as well as at high expositions of cadmium, transplacental, with its subsequent accumulation in the placenta [1]. When entered into the human body, CD is stored in hepatocytes in complexes with protein metallothionein (CdMT). Subsequently, the hepatic CdMT is released into blood circulation, filtered with renal glomeruli and reabsorbed with proximal tubules. After the process, which requires several stages, the Cd is again stored as CdMT, but is capable of damaging, the severity of which is proportional to the amount of load, which can increase for many years after ending exposure due to the constant release of cadmium from the liver. Epidemiological data indicate that cadmium induces the development of hypertension, liver inflammation, kidney degeneration and Alzheimer's disease, is a carcinogen, especially in lungs and kidneys, and enhances the renal effects of diabetes [2]. Separately pay attention to research on the influence of cadmium on the reproductive system. It is established that cadmium has a negative effect on fertility indicators in men and women. In addition, this effect is manifested during the embryogenesis of offspring and postnatal development [3].

Increasing the level of cadmium in the placenta correlates with a decrease in the size and weight of the baby at birth [4, 5, 6]. The effect of cadmium on the mother's body disturbs the preimplantation development of the embryo in vivo, causes its death, fragmentation or blocks further development, disrupting epigenetic modification and causing damage to DNA, as well as partially inhibiting the expression of genes associated with DNA reparation [7]. The study of toxic effects of cadmium chloride in rats experiments showed a significant increase in embryonic mortality rates, as well as disturbing of histo – and organogenesis of offspring [8, 9, 10, 11, 12]. But previous studies were performed with the effects of cadmium chloride at a dose of 1.0 mg/kg, that is, twice less than the one we chose in our experiments.

Thus, the relevance of studies on the influence of heavy metals on ontogeny is undeniable, and the use of experimental models allows obtaining important material for extrapolation on human embryogenesis of developmental disorders during chronic exposure.

The aim of the study.

Determine the level of embryotoxic effects of cadmium chloride at a dose of 2mg/kg with isolated intragastric administration in a chronic experiment on pregnant female rats. Compare the level of exposure to cadmium chloride at a dose of 2mg/kg with embryotoxicity levels with 1 mg/kg.

Object and research methods.

The research was conducted on females of rats Wistar (nurser "Next 2000", Kyiv), which was kept in vivarium of the Dnipro State Medical University, with compliance with all the necessary conditions for keeping animals. To obtain females with a dated pregnancy, their set cycle was examined by a microscopy of vaginal smears. For the experiment, 32 females were selected with a permanent rhythm of the cycle and paired with

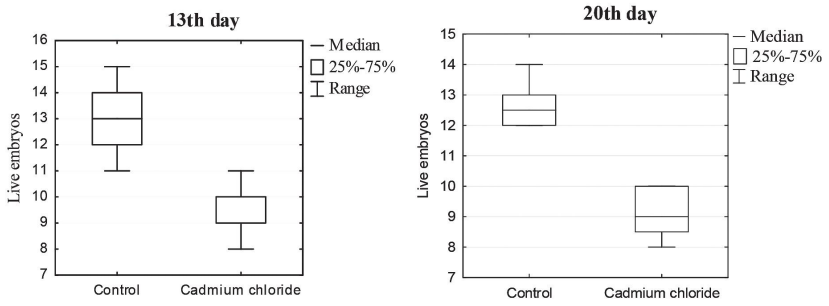

Figure 1 – The average number of living embryos per 1 female.

intact males according to the 2:1 scheme. The determination of the first day of pregnancy of females was indicated if the sperm in the vaginal smears of females.

All pregnant female rats were divided into 2 main groups: the first group – control; the second group – an administration of chloride cadmium solution at a dose of 2.0 mg/kg, which, in turn, were divided into 2 subgroups according to the term of withdrawal from the experiment: the 13th and 20th day of the experiment.

During the withdrawal from the experiment under thiopental anesthesia, embryos were excluded; the number of yellow bodies of pregnancy in the ovaries of females and resorptions in each horn of the uterus were calculated.

To determine the embryotoxicity of cadmium chloride used an average number of embryos per female, as well as standard indicators of embryonic development, which were calculated by formulas:

1. Total embryonic mortality (TEM), units

$$\mathrm{TEM} = \frac{\mathrm{C}-\mathrm{A}}{\mathrm{C}},$$

where A is the number of living embryos,
C is the number of yellow bodies of pregnancy.

2. Preimplantation mortality (PreIM), units

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

where A is the number of living embryos,
B is the number of dead (resorbed) embryos,
C is the number of yellow bodies of pregnancy.

3. Postimplantation mortality (PostIM), units

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

where A is the number of live embryos,
B is the number of dead (resorbed) embryos.

Research was conducted in accordance with the principles of the Helsinki Declaration, adopted by the World Medical Association General Assembly (2000), the Council of Europe Convention on Human Rights and Biomedicine (1997), relevant provisions of WHO, International Medical Ethics Council, International Code of Medical Ethics (1983), "General Ethical Principles of Animal Experiments", approved by the First National Congress in Bioethics (Kyiv, 2001) in accordance with the provisions of the "European Convention for the Protection of Vertebrates used in experiments and other educational purposes" (Strasbourg, 18 March 1986).

The statistical analysis of the obtained data was carried out using the STATISTICA program and AtteStat from Microsoft Excel. Differences were considered statistically significant at $p < 0.05$ (5% significance). Since not all experimentation indicators had a normal distribution, we used a median (Me) to describe the average values with 25% and 75% quartiles and confidence intervals (CI), and to comparison between groups – Mann–Whitney U test.

Research results and their discussion.

The obtained results demonstrate a significant increase in all indicators of embryonic mortality in the cadmium chloride group compared to the control group. Thus, the average numbers of living embryos per female in the cadmium chloride group on the 13th day Me was 10 (9; 10) units, while in the control group Me was 13 (12; 14) units (p=0.001). On the 20th day, the numbers of living embryos Me was 9 (8.5; 10), and in the control group Me was 12.5 (12; 13) (p=0.0001) **(fig. 1)**.

In this case, the number of yellow bodies in the group of exposure to cadmium chloride is not significantly different from the control group: on the 13th day their number Me was 13 units (12; 13.5) in the group of exposure to cadmium chloride and Me was 13 units (12.5; 14) in the control group (p=0,5053); on the 20th day – 13 units (12; 13.5) in the group of exposure to cadmium chloride and 13 units (13; 13.5) in the control group (p=0.2786).

The number of resorbed embryos was counted with calculating preimplantation and postimplantation mortalities. A small amount of resorptions was observed in the control group. The frequency of resorption in the cadmium chloride exposure to the 13th day was 8.74%, which is 9 times higher than this indicator in the control group (0.93%), at the 20th day the frequency of resorption was 7.77% compared to 1.87% in the control group, i.e. it increased by 4 times **(fig. 2)**. Thus, the results prove a high level of embryotoxicity of chronic administration

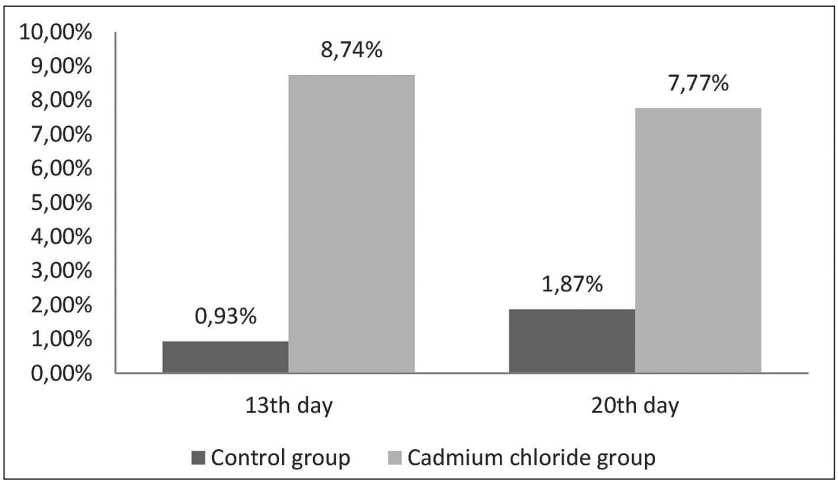

Figure 2 – Frequency of resorptions in the group exposed to cadmium chloride and the control group on the 13th and 20th days of the experiment.

of cadmium chloride at the specified dose and method of administration in experiments on rats.

When comparing preimplantation and postimplantation mortalities for the 13th and 20th days, it was noted that in the control group they are very low and varied in the range of 0.00 to 0.07, which complicated the calculation of the median, whereas in the group the effects of cadmium chloride, they were essential and reliable from the control group **(table)**. In addition, the preimplantation mortality rate exceeded the indicator of postimplantation mortality: on the 13th day – by 1.7 times, and on the 20th – by 2.3 times.

Table – Medians of total embryonic mortality (TEM), preimplantation (PreIM) and postimplantation embryonic mortality (PostIM)

	13th day				
	Control group	CI	Cadmium chloride group	CI	p-value
TEM	Me 0 (0; 0,07)	0; 0,08	Me 0,24 (0,19; 0,29)	0,17; 0,38	0,0001
PreIM	Me 0 (0; 0,03)	0; 0,08	Me 0,17 (0,15; 0,20)	0,07; 0,23	0,0003
PostIM	Me 0 (0; 0,00)	0; 0,08	Me 0,10 (0,05; 0,16)	0,00; 0,20	0,0148
	20th day				
TEM	Me 0,07 (0; 0,08)	0; 0,08	Me 0,31 (0,26; 0,33)	0,17; 0,36	0,0001
PreIM	Me 0 (0; 0,07)	0; 0,08	Me 0,23 (0,16; 0,27)	0,08; 0,29	0,0001
PostIM	Me 0 (0; 0,04)	0; 0,08	Me 0,10 (0,09; 0,11)	0,00; 0,20	0,0030

The calculation of these relative indicators also allows us to compare the results obtained during the experiment with the results of previous studies, the dose of cadmium chloride in which was 1 mg/kg (i.e., twice as low). Analyzing the indicators of total embryonic mortality, preimplantation and postimplantation embryonic mortalities at different doses of exposure, it is possible to note the preservation or deviation from the main trends of toxic effects of cadmium chloride on embryonic development of rats. Thus, the total embryonic mortality in the exposure group of cadmium chloride at a dose of 2 mg/kg has increased significantly compared to total embryonic mortality in previous studies with cadmium exposure 1mg/kg. In addition, it draws attention to the increase in preimplantation mortality in the cadmium chloride group, especially on the 20th day. In our experiment, this indicator was 0.23, while in previous studies it ranged from 0.09 to 0.1. Accordingly, the total embryonic mortality rate at the 20th day was 0.31 compared to 0.20-0.22 previous studies **(fig. 3)**. It should be noted that the rate of postimplantation embryonic mortality remained at the same level as in previous experiments and was 0.10 both on the 13th and 20th days of the experiment.

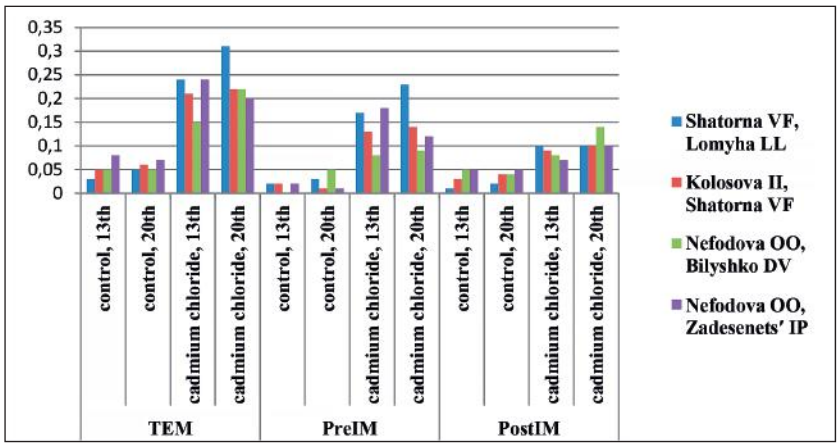

Figure 3 – Comparison of the average values of the TEM, PreIM, PostIM in our experiment with previous studies.

Thus, based on the results obtained and previous studies, it can be argued that chronic administration of cadmium chloride significantly affects the embryogenesis in rats, reduces the number of living embryos per 1 female and increases the risk of preimplantation and postimplantation mortality.

Conclusions.

1. Chronic administration of cadmium chloride at a dose of 2.0 mg/kg during embryogenesis has a significant influence on the indicators of the number of living embryos per female, total embryonic mortality, preimplantation and postimplantation embryonic mortalities for the 13th and 20th day of the rat's embryogenesis.

2. Analysis of the results obtained in comparison with the experiments of the previous years allows to indicate the preservation of the general tendency of toxic effects of cadmium chloride on embryonic development during the chronic experiment on rats with increasing levels of preimplantation and, accordingly, general embryonic mortality.

Prospects for further research.

In order to determine the negative influence of cadmium chloride on organogenesis, in our opinion, the study of embryos organs to identify morphological changes and further histological examination to determine possible developmental disorders.

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ЗМІНИ ПОКАЗНИКІВ ЕМБРІОТОКСИЧНОСТІ ХЛОРИДУ КАДМІЮ В ХРОНІЧНОМУ ЕКСПЕРИМЕНТІ НА ЩУРАХ

Шаторна В. Ф., Ломига Л. Л.

Резюме. Дослідження ембріогенезу на фоні хронічного впливу кадмієм залишається актуальним і, водночас, малодослідженим питанням. Відомо, що кадмій здатний долати плацентарний бар'єр та впливати на ріст і розвиток ембріона, викликати патології окремих органів та їх систем, обумовлювати когнітивні дисфункції у нащадків.

Метою дослідження було визначення ступеню ембріотоксичності хлориду кадмію у дозі 2мг/кг при внутрішньошлунковому введенні на вагітних самицях щурів протягом 13-ти (завершення органогенезу) та 20-ти (завершення ембріогенезу) діб, а також порівняння відносних показників загальної ембріональної смертності, доімплантаційної та постімплантаційної смертностей під впливом хлориду кадмію у дозі 2мг/кг з цими ж показниками при введені 1 мг/кг у попередніх дослідженнях.

Для визначення впливу хлориду кадмію на показники ембріонального розвитку щурів було відібрано 32 самиці, які були поділені на 2 групи: основна і контрольна. Основній групі вводили внутрішньошлунково хлорид кадмію у дозі 2 мг/кг починаючи з 1 дня вагітності, контрольна група також внутрішньошлунково отримувала воду. Кожна група в подальшому була розподілена на 2 підгрупи (самиці обирались випадковим чином) за строками виведення з експерименту (13-та та 20-та доба).

Аналіз отриманих результатів засвідчує суттєвий вплив хлориду кадмію на всі показники ембріогенезу, а саме: зменшення середньої кількості живих ембріонів на 1 самицю у групі впливу хлоридом кадмію у порівнянні з контрольною, а також зростання показників загальної ембріональної смертності, доімплантаційної та постімплантаційної ембріональної смертності в основній групі. Крім того, при співставленні відносних показників із результатами досліджень щодо впливу хлориду кадмію у дозі 1 мг/кг, звертало на себе увагу суттєве зростання доімплантаційної ембріональної смертності, особливо на 20-ту добу, і відповідно, загальної ембріональної смертності під впливом хлориду кадмію у дозі 2 мг/кг в порівнянні із дослідженнями впливу 1 мг/кг. Водночас, показник постімплантаційної ембріональної смертності зберігся на тому ж рівні, що і в попередніх дослідженнях.

Ключові слова: ембріотоксичність, кадмій хлорид, показники ембріогенезу.

CHANGES IN EMBRYOTOXICITY INDICATORS OF CADMIUM CHLORIDE IN A CHRONIC EXPERIMENT ON RATS

Shatorna V. F., Lomyha L. L.

Abstract. The study of embryogenesis with the influence of chronic exposure to cadmium remains a relevant and, at the same time, an understudied issue. It is known that cadmium is able to cross the placental barrier and affect the growth and development of the embryo, cause pathologies of individual organs and their systems, and cause cognitive dysfunction in the offspring.

The aim of the study was to determine the level of embryotoxicity of cadmium chloride at a dose of 2 mg/kg which was administered intragastrically to pregnant female rats during the 13th (end of organogenesis) and 20th (end of embryogenesis) days, and to compare the relative rates of total embryonic mortality, preimplantation and postimplantation mortalities under the influence of cadmium chloride at a dose of 2 mg/kg with the same indicators when 1 mg/kg was administered in previous studies.

To determine the effect of cadmium chloride on the indicators of embryonic development of rats, 32 females were selected and divided into 2 groups: the croup of cadmium chloride and control group. The cadmium chloride group was injected intragastrically with cadmium chloride solution at a dose of 2 mg/kg starting from day 1 of pregnancy; the control group also received water intragastrically. Each group was further divided into 2 subgroups (females were chosen randomly) according to the time of withdrawal from the experiment (13th and 20th day).

The analysis of the obtained results proves the significant influence of cadmium chloride on all indicators of embryogenesis, namely: a decrease in the average number of live embryos per 1 female in the group exposed to cadmium chloride compared to the control group, and an increase in the indicators of total embryonic mortality (TEM), preimplantation (PreIM) and of postimplantation (PostIS) embryonic mortalities in the cadmium chloride group. In addition, when comparing the relative indicators with the results of studies on the influence of cadmium chloride at

a dose of 1 mg/kg, attention was drawn to a significant increase in preimplantation embryonic mortality, especially on the 20th day, and, accordingly, in total embryonic mortality under the influence of cadmium chloride at a dose 2 mg/kg compared to 1 mg/kg exposure studies. At the same time, the rate of postimplantation embryonic mortality remained at the same level as in previous studies.

Key words: embryotoxicity, cadmium chloride, indicators of embryogenesis.

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Conflict of interest:
The authors declare no conflict of interest.

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A – Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article.

Received 20.11.2022
Accepted 02.05.2023