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## THE EFFECT OF CADMIUM SALTS ON THE DEVELOPMENT OF VESSELS AND ATRIOVENTRICULAR VALVES OF RAT HEART UNDER THE CONDITIONS OF ZINC CITRATE CORRECTION

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**Annotation.** *The article presents the results of an experimental study of the effect of cadmium salts on the development of blood vessels and atrioventricular valves of the rat heart during isolated administration and under the condition of correction of zinc citrate. The internal diameters of the vessels, the thickness of the atrioventricular valves, as well as the accumulation of cadmium and zinc in the heart of embryos of the rat of the 20th day of development were studied.*

**Key words:** *cadmium, zinc citrate, cardiogenesis, cardiac vessels, atrioventricular valves.*

The increasing pollution of industrialized countries requires the study of the effects of environmental factors on human health. Heavy metals are one of the most common and dangerous environmental pollutants. The latter, when they enter the human body, lead to acute and delayed complications, change the balance of trace elements, the course of diseases, pregnancy, and cause different malformations [1,2,3].

Together with lead, mercury and arsenic, cadmium belongs to a group of heavy metals that pose a serious health risk. Cadmium is one of the few toxic metals that has no known physiological functions in the body. It is toxic at very low levels, has acute and chronic effects on human health. Cadmium accumulates in the human body throughout life and has a long biological half-life (17-30 years) [4]. Investigating the morphological changes that occur under the influence of cadmium in both postnatal and prenatal ontogenesis is an important task for scientists. Due to scientific and technological progress, compounds of heavy metals with different physical and chemical properties (nanometals) have appeared. Their effects on the human body have differences compared to ionic forms [8,9,10]. The search for antagonists that reduce the toxic effect of cadmium is a priority in scientific researches [5,6].

**The aim of the study.** To investigate the effect of cadmium salts on the development of blood vessels and atrioventricular valves and the accumulation in the heart of rat embryos during isolated administration and in combination with zinc citrate.

**Materials and methods.** To simulate chronic toxicity during cadmium exposure throughout pregnancy, female Wistar rats were administered cadmium citrate or cadmium chloride on a daily basis per os via the probe in isolation (at a dose of 1.0 mg/kg) or in combination with zinc citrate. The selected dose of cadmium-containing compounds

corresponds to 1/100 LD50 for cadmium [7].

In the experimental model, solutions of cadmium and zinc citrate obtained by the aquanotechnology method were used [10].

Solutions of citrate forms of metals (cadmium and zinc) were obtained according to the agreement on scientific cooperation at the Scientific Research Institute of Nanobiotechnology and Resource Conservation of Ukraine (Kyiv).

Given the pronounced antagonistic properties of zinc-containing compounds with respect to cadmium *in vivo*, it was decided to use the zinc citrate form as a potential biological antagonist against cadmium intoxication [11,12,13].

In accordance with the requirements of embryonic experiments, pregnant rats were provided with a complete nutritional diet, water *ad libitum* and careful care. Intra-gastric injection of metal solutions was performed from the first day of pregnancy daily at the same time of day (from 9 to 11 hours).

Female rats with a dated pregnancy were divided into 5 groups:

1. Control (animals received 0.5 ml of 0.9% NaCl solution). Number of females - n = 8; number of embryos - n = 71
2. Cadmium chloride (dose 1.0 mg/kg). Number of female rats - n = 8; number of embryos - n = 62
3. Cadmium citrate (dose 1.0 mg/kg). Number of female rats - n = 8; number of embryos - n = 68
4. Cadmium chloride and zinc citrate (received doses of 1.0 mg/kg and 1.5 mg/kg, respectively). Number of female rats - n = 8; number of embryos - n = 72
5. Cadmium citrate and zinc citrate (received doses of 1.0 mg/kg and 1.5 mg/kg, respectively). Number of female rats - n = 8; number of embryos - n = 69

For embryonic studies, female rats with a dated term of pregnancy were obtained. On the 20th day of pregnancy, female rats were. The embryos were removed from the uterus, tested for a "live-dead", weighed, photographed and fixed in 10% formalin solution for further histological examination. After fixation of 20-day-old embryos, the hearts of embryo rats were separated from body. From isolated hearts, sections of 4-6 microns thick (hematoxylin-eosin staining) were made using standard histological methods for further morphometric studies.

Part of the 20-day-old embryos of all the experimental groups were frozen to measure cadmium and zinc accumulation in the thoracic organ complexes using the atomic emission method with electric arc atomization. The study was conducted at the State Enterprise "Ukrainian Research Institute of Transport Medicine" of the Ministry of Health of Ukraine (Odessa) according to the agreement on scientific and technical cooperation (2018).

ZEISS Axiocam ERc 5s light microscopy camera with P95-C 1/2 "0.5x adapter was used to obtain digital images with subsequent structure size calculations.

The size of the heart structures was determined using the ZEN 2.0 software, which is software for ZEISS's Primo Star series of light microscopes. We used software tools to measure the linear dimensions of structures.

On histological sections of the heart of the rat embryos of the 20th day of development

in all groups we measured:

1) average width of the atrioventricular valve (AVV) flaps in the middle third ( $\mu\text{m}$ ),  $M \pm m$ ;

2) average internal diameters of subepicardial vessels and myocardial vessels of compact myocardium in the middle part of both ventricles on identical sections of embryonic heart;

3) average internal diameter of the coronary arteries in the proximal part ( $\mu\text{m}$ ),  $M \pm m$ ;

Statistical processing and analysis of results were performed according to conventional methods using the licensed statistical programs Statistica v.6.1 and Microsoft Excel. The reliability of the statistical surveys was performed using the Student's t-test. A level of  $p < 0.05$  was considered to be statistically significant.

Animal studies were conducted in accordance with the "General Ethical Principles for Animal Experiments" (Kyiv, 2001), which are consistent with the European Convention for the Protection of Experimental Animals (Strasbourg, 1985).

**Results and discussion.** All the female rats in the experiment survived. Defects of embryonic development were absent. Isolated exposure to cadmium chloride leads to a decrease in the internal diameter of the vessels of the myocardium against the background of thickening of the walls of the vessels and pronounced perivascular edema. Subepicardial vessels were dilated (see Table 1).

In the ventricles of the heart of embryos of the cadmium citrate influence group, the tortuosity of the subepicardial vessels was determined. Also in this group we observed a large number of subepicardial vessels, among which there were single vessels with increased internal diameter and with increased blood filling compared with the control group and a large number of vessels of small diameter.

To find out the compensatory potential of zinc citrate in relation to cadmium salts, we compared the groups of combined exposure with the respective groups of isolated exposure and control. The results obtained for the cadmium chloride + zinc citrate group were compared with the control and isolated administration of cadmium chloride groups.

In the cadmium chloride group, there was a significant increase in the average internal diameter of the subepicardial vessels by 1.54 and 1.83 times in the left and right ventricles, respectively, compared to the control group ( $p < 0.001$ ). In the cadmium citrate group, there was a significant decrease in the average internal diameter of the subepicardial vessels by 1.43 ( $p < 0.001$ ) and 1.33 times ( $p < 0.01$ ), respectively, in the left and right ventricles compared to the control group.

In the cadmium chloride group, there was a significant decrease in the mean diameter of the myocardial vessels by 1.4 times ( $p < 0.001$ ) and 16% ( $p < 0.05$ ) in the left and right ventricles, respectively, compared to the control group. In the cadmium citrate group, the mean diameter of the myocardial vessels was 22% less than that of the control group in the left ventricle ( $p < 0.001$ ). The mean internal diameter of the right ventricular myocardial vessels was significantly greater by almost 1.5 times that of the same group ( $p < 0.001$ ). Also, myocardial vessels of the right ventricle of the cadmium citrate group were 22% larger than the control group ( $p < 0.01$ ) and 1.42 times larger compared to the

cadmium chloride group ( $p < 0.001$ ).

Table 1

**The average inner diameter of the vessels of the rat embryos heart on the 20th day of embryonic development, ( $\mu\text{m}$ ),  $M \pm m$**

Control group	Left ventricle	Right ventricle
Subepicardial vessels	47.31 $\pm$ 3,17	48.73 $\pm$ 2.61
Myocardial vessels	38.83 $\pm$ 2,19	38.69 $\pm$ 2.06
Cadmium chloride	Left ventricle	Right ventricle
Subepicardial vessels	72.84 $\pm$ 2.89 ***	89.22 $\pm$ 3.49 ***
Myocardial vessels	27.83 $\pm$ 1.71 ***	33.32 $\pm$ 1.66 *
Cadmium citrate	Left ventricle	Right ventricle
Subepicardial vessels	33.06 $\pm$ 2.06***,###	36.51 $\pm$ 2.84 **, ###
Myocardial vessels	31.83 $\pm$ 2.07 *	47.28 $\pm$ 1.9 **, ###
Cadmium chloride + zinc citrate	Left ventricle	Right ventricle
Subepicardial vessels	59.09 $\pm$ 2.58 **, ###	68.9 $\pm$ 2.85 ***, ###
Myocardial vessels	33.9 $\pm$ 1.1 *, ##	37.68 $\pm$ 1.37 #
Cadmium citrate + zinc citrate	Left ventricle	Right ventricle
Subepicardial vessels	42.74 $\pm$ 1.53 @@@	43.9 $\pm$ 1.9 @
Myocardial vessels	36.71 $\pm$ 1.34 @	40.31 $\pm$ 1.37 @@

Notes: \* -  $p < 0.05$ ; \*\* -  $p < 0.01$ ; \*\*\* -  $p < 0.001$ ; in relation to control. # -  $p < 0.05$ ; ## -  $p < 0.01$ ; ### -  $p < 0.001$  in relation to the cadmium chloride group. @ -  $p < 0.05$ ; @@ -  $p < 0.01$ ; @@@ -  $p < 0.001$ ; in relation to the cadmium citrate group.

In the CdCl<sub>2</sub> + Zn citrate group, a 23.3% reduction in the diameter of subepicardial vessels was observed compared to the cadmium chloride group ( $p < 0.001$ ). In the control group, the inner diameter of the subepicardial vessels was 24.9% less than in the CdCl<sub>2</sub> + Zn citrate group. A similar result was obtained in the CdCl<sub>2</sub> + Zn citrate group for the inner diameter of the subepicardial vessels of the right ventricle, namely: the latter was 29.5% less than in the cadmium chloride group ( $p < 0.001$ ), and 41.4% greater than in the control group ( $p < 0.001$ ). In the Cd citrate + Zn citrate group, an increase in the internal diameter of the subepicardial vessels was obtained by 29.3% ( $p < 0.001$ ) and 20.2% ( $p < 0.05$ ) for the left and right ventricles respectively compared to the cadmium citrate group.

Comparing the internal diameters of the myocardial vessels of the cadmium chloride + zinc citrate group with the isolated cadmium chloride administration group, the latter increased by 21.8% ( $p < 0.01$ ) and 13.1% ( $p < 0.05$ ) for the left and the right ventricle, respectively. Zinc citrate in the combination with cadmium citrate showed an increase in the internal diameter of myocardial vessels by 15.3% ( $p < 0.05$ ) for the left ventricle and a

decrease by 17.3% ( $p < 0.01$ ) for the right ventricle compared with group of cadmium citrate.

In the cadmium chloride group, wall thickening and perivascular coronary artery edema were registered. In the cadmium citrate group, the primitive media of the coronary vessels was also significantly distinguished in the histological examination compared to the control group.

A statistical comparison of the inner diameters of the coronary vessels of the rat embryos of the 20th day of development in the groups of combined and isolated administration is given in Table 2.

Table 2

**The average internal diameter of the coronary arteries of rat embryos on the 20th day of embryonic development, ( $\mu\text{m}$ ),  $M \pm m$**

Group	Right	Left
Control	47.62 $\pm$ 2.07	45.56 $\pm$ 2.01
Cadmium chloride	55.56 $\pm$ 2.3 *	56.45 $\pm$ 2.25 ***
Cadmium citrate	46.82 $\pm$ 1.86 ##	51.14 $\pm$ 1.8 *
Cadmium chloride + zinc citrate	49.73 $\pm$ 1.47 #	49.8 $\pm$ 1.76 #
Cadmium citrate + zinc citrate	48.49 $\pm$ 1.46	45.12 $\pm$ 1.65 @

Notes: \* -  $p < 0.05$ ; \*\* -  $p < 0.01$ ; \*\*\* -  $p < 0.001$ ; in relation to control. # -  $p < 0.05$ ; ## -  $p < 0.01$ ; ### -  $p < 0.001$  in relation to the cadmium chloride group. @ -  $p < 0.05$ ; @@ -  $p < 0.01$ ; @@@ -  $p < 0.001$ ; in relation to the cadmium citrate group.

Analysis of the results showed a significant increase in the internal diameter of the coronary arteries in the cadmium chloride group by 16.7% ( $p < 0.05$ ) and by 23.9% ( $p < 0.001$ ) for the left and right arteries, respectively, compared with the control group.

In the Cd group of citrate + Zn citrate, a significant reduction of the internal diameter of the right coronary artery by 13.3% ( $p < 0.05$ ) was obtained compared to the cadmium citrate group. With the combined administration of cadmium chloride with zinc citrate, we observed a decrease in the diameter of the coronary vessels by 11.7% ( $p < 0.05$ ) and 13.4% ( $p < 0.05$ ) for the left and right coronary arteries, respectively, compared with the isolated group introduction of CdCl<sub>2</sub>.

The results of the obtained measurements of the middle part of the valves of the atrioventricular (PSC) valves and their statistical analysis are shown below (Table 3).

Analyzing the data obtained and comparing them with isolated exposure groups, we received reliable differences. In the combined groups of cadmium and zinc introduction, positive dynamics was obtained in relation to the parameter of the average thickness of the cusps of atrioventricular valves. Isolated administration of cadmium chloride led to a significant decrease in this parameter, and in the cadmium citrate group the opposite result was obtained - the average thickness of the leaflets of the atrioventricular valves increased reliably. In the CdCl<sub>2</sub> + Zn citrate group, this indicator was 36.4% less than in the control group ( $p < 0.001$ ) and almost twice greater the same as that of the cadmium chloride group ( $p < 0.001$ ) for the mitral valve and 25% less than in the control group ( $p < 0.05$ ) and 44.5% greater than the cadmium chloride group ( $p < 0.001$ ) for the tricuspid valve. The obtained



data indicate a positive effect of zinc citrate on the index of thickness of the flaps of atrioventricular valves on the background of intoxication with cadmium chloride.

Table 3

**The average thickness of the middle part of the inner flaps of the mitral and tricuspid valves of the heart of rat embryos on the 20th day of embryonic development in the experiment, (µm), M ± m**

Group	Mitral valve	Tricuspid valve
Control	52.58 ± 3.01	43.84 ± 2.82
Cadmium chloride	19.18 ± 1.46 ***	24.26 ± 2.04 ***
Cadmium citrate	69.87 ± 4.28 **,###	59.72 ± 3.46 ***,###
Cadmium chloride + zinc citrate	38.55 ± 1.91 ***,###	35.06 ± 1.93 *,###
Cadmium citrate + zinc citrate	59.51 ± 2.96 @	51.2 ± 2.53@

Notes: \* -  $p < 0.05$ ; \*\* -  $p < 0.01$ ; \*\*\* -  $p < 0.001$ ; in relation to control. # -  $p < 0.05$ ; ## -  $p < 0.01$ ; ### -  $p < 0.001$  in relation to the cadmium chloride group. @ -  $p < 0.05$ ; @@ -  $p < 0.01$ ; @@@ -  $p < 0.001$ ; in relation to the cadmium citrate group.

In the Cd citrate + Zn citrate group, the thickness of the AVV flaps was non-reliably greater (13.2% for the mitral valve ( $p=0.1$ ) and 16.8% for the right PNC ( $p=0.053$ )) than in the control group, and reliably less (17.4% for the mitral valve ( $p<0.05$ ) and 16.6% for the right AVV ( $p<0.05$ )) than in the cadmium citrate alone group. This fact also indicates the positive effect of zinc citrate on the thickness of the AVV cusps on the background of cadmium citrate intoxication.

Studying the accumulation of cadmium and zinc in the hearts of embryos by the method of atomic emission with electric arc atomization revealed changes in the accumulation of the trace elements under study (see Table 4).

Table 4

**Accumulation of cadmium and zinc in rat embryos hearts at the 20th day of embryogenesis in the experiment (µg/g)**

Group	Zinc	Cadmium
Control	6.04±0.27	0.00046±0.000024
Cadmium chloride	14.36±0.43***	0.00096±0.000047***
Cadmium citrate	4.62±0.35 **,###	0.,0052±0.00033 ***,###
Cadmium chloride + zinc citrate	9.21±0.63 ***,###	0.00049±0.000035 ###
Cadmium citrate + zinc citrate	7.05±0.25 **,###,@@@	0.00162±0.000039 **,@@@

Notes: \* -  $p < 0.05$ ; \*\* -  $p < 0.01$ ; \*\*\* -  $p < 0.001$ ; in relation to control. # -  $p < 0.05$ ; ## -  $p < 0.01$ ; ### -  $p < 0.001$  in relation to the cadmium chloride group. @ -  $p < 0.05$ ; @@ -  $p < 0.01$ ; @@@ -  $p < 0.001$ ; in relation to the cadmium citrate group.

In the cadmium chloride group, the average cadmium accumulation was  $0.00096\pm 0.000047$  µg/g, ie, twice higher than in the control group ( $p < 0.001$ ). The

introduction of cadmium chloride increased the average zinc content to  $14.36 \pm 0.43 \mu\text{g/g}$  ( $p < 0.001$ ) in the heart embryos of rats of the 20th day of development. Isolated administration of cadmium citrate led to an increase in cadmium accumulation in the rat embryo heart to  $0.0052 \pm 0.00033 \mu\text{g/g}$ , which was 11.3 times higher than in the control values ( $p < 0.001$ ).

In the combined exposure group of cadmium chloride + zinc citrate, the content of cadmium in the hearts of embryos of the 20th day of development was  $0.00049 \pm 0.000035 \mu\text{g/g}$ , that is, there was no statistically significant difference with the control ( $0.00046 \pm 0.000047 \mu\text{g/g}$ ). These results suggest a modifying effect of zinc citrate on the ability of cadmium chloride to accumulate in embryonic tissues and to overcome the placental barrier. The level of zinc in the same group exceeded the control values by 1.5 times and amounted to  $9.21 \pm 0.63 \mu\text{g/g}$  ( $p < 0.001$ ). In the group of cadmium citrate combination with zinc citrate, the level of cadmium accumulation was  $0.00162 \pm 0.000039 \mu\text{g/g}$ , which is 3.2 times less than the isolated cadmium citrate administration group ( $p < 0.001$ ). It is interesting to note that in the cadmium and zinc combined administration groups, we obtained the above cadmium accumulation level without significantly increasing the zinc accumulation level observed in the cadmium chloride isolated administration group. With high cadmium accumulation, we observed a slight increase in zinc compared to the control group (16.7% ( $p < 0.01$ )) and a significantly lower (2.04 times ( $p < 0.001$ )) zinc accumulation level compared to of the cadmium chloride group and 1.53 times higher than in the cadmium citrate group ( $p < 0.001$ ).

Thus, it should be noted that the simultaneous use of cadmium citrate and zinc citrate leads to a decrease in cadmium accumulation and increased zinc accumulation, however, when comparing the cadmium chloride groups and cadmium citrate and cadmium citrate + zinc citrate, the zinc level is much higher in the group of the isolated administration of cadmium, despite the fact that no additional zinc was administered to the female rats during pregnancy. It also makes sense that zinc exhibits high antagonistic activity against cadmium, which is not inconsistent with literature data and has also been confirmed in our experiment [11, 13].

The interaction of heavy metal citrate forms exhibiting antagonistic properties requires further investigation. In our opinion, the mechanism of accumulation of citrate forms of cadmium and zinc differs from their ionic forms.

**Conclusions.** Summarizing all mentioned above, we can assume that, despite the use of identical doses of different cadmium salts in the experimental groups, as compounds with toxic properties, we obtained different results for the citrate and ionic forms of cadmium, indicating the excellent mechanism of toxic action of these forms of cadmium. Zinc citrate reduced the cardiotoxic properties of cadmium salts: the thickness of the AVV cusps, the internal diameter of the myocardial and subepicardial vessels, the coronary arteries approached the values of the latter of the control group. However, the combined administration of zinc and cadmium citrates had a different effect on the accumulation of cadmium and zinc in the heart of rat embryos. In view of the above, we believe that the interaction of heavy metal citrate forms in vivo needs further investigation. Zinc citrate can be regarded as an antagonist for cadmium compounds. However, its toxicological profile needs in-depth study.

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