

SKELETAL AND EXTERNAL TERATOGENIC EFFECTS OF CADMIUM DURING GESTATION IN RATS

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Abstract - The teratogenicity of cadmium exposure was studied in virgin NIMRI rats aged 10-13 weeks. Pregnant animals were fed cadmium sulfate equivalent to doses of 1, 4 and 7 mg/kg/day of cadmium by oral gavage on the day 7 of gestation. On the day 18 of gestation, fetuses were delivered and examined for external and skeletal malformations. Total percent of malformed fetuses in rats treated by different doses of cadmium were significantly higher than of control group. In this study the length of arm, big diameter of chest, big diameter of pelvis, length of femur, length of tibia, length between crown to sacrum, large diameter of skull and length between crown to bottom were examined. The appearance of total skeletal malformation in treated groups started by dose of 1 mg/kg/day of cadmium ($p < 0.05$) excluded by length of arm, big diameter of chest and big diameter of pelvis. Growth retardation was also observed significantly in all doses of cadmium treatment. Regression analysis showed a significant relationship between rate of total malformations and doses of cadmium ($r^2 = 0.97$); and between growth retardation and doses of cadmium ($r^2 = 0.99$). The most toxicity of cadmium on length of skeletons appeared in tibia and the lowest one was in arm. Of width skeletons, the highest toxic effect of cadmium was on big diameter of pelvis and the lowest was on big diameter of chest. In total toxicity of cadmium was observed most frequently on the lower limbs. In the external malformation category, only cleft palate and open eyelid were observed in live fetuses.

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INTRODUCTION

Cadmium(Cd) is an essential trace metal that progressively accumulates in the body. Cd appears in the workplaces in solder, a neutron absorbent in the nuclear industry, alkaline storage batteries, as amalgam in dentistry, a stabilizer for polyvinyl chloride etc. Cd is considered to have no biological function and is highly toxic. After absorption, Cd is transported in blood cells and albumin. Cd exposure produce renal dysfunction, emphysema and osteomalacia. It has been shown that Cd is a teratogenic and carcinogenic agent (1). Previous studies with various animal species have shown that

exposure to Cd during pregnancy could result in fetal death, growth retardation and a variety of fetal malformations or developmental anomalies in the offspring. Although these experiments showed teratogenicity of Cd in animal species but its effect on skeletal system remains unknown (3 - 13). In this regard we were interested to design this study to investigate the potency of Cd in various doses to disturb development of skeletal system in embryos during organogenesis.

MATERIALS AND METHODS

Cadmium sulfate (CdSO_4), Alizarin red, glycerin and potassium hydroxide were purchased from Merck Co., Germany. Alcohol 96% was purchased from Ararat Co., Iran. Normal saline was purchased from Toli - darou pharmaceutical Co., Iran. The study was conducted with 40 virgin - NMRI female rats aged 10-13 weeks and weighing of 195-203 g with free access to food and water. Rats were allowed more than one week for acclimatization prior to use in the experiments. One female rat was housed with one male overnight, and successful mating was confirmed by vaginal smear. The day of positive vaginal smear observation was considered as day zero of pregnancy. Mated females were divided into four main groups including control (Cd-0), Cd at the dose of 1 mg/kg/day (Cd-1), Cd at the dose of 4 mg/kg/day (Cd-4), and Cd at the dose of 7 mg/kg/day (Cd-7). Cadmium sulfate was dissolved in sterile normal saline and the animals received 1, 4 and 7 mg/kg/day of Cd by gavage syringe on day 7 of pregnancy equivalent to time of organogenesis with maximum teratogenic effect (14). The control group rats received only normal saline. The

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pregnant rats were delivered on day 18 of pregnancy, then sacrificed by cervical dislocation and their reproductive status were examined. The litters then were examined for external malformations and growth retardation using stereomicroscope. Growth retardation was determined as decrease in embryo weight. Then fetuses were sacrificed and dehydrated in 96% ethyl alcohol. The skin of fetuses was removed and then cleared in 1% KOH. The samples were stained with Alizarin red according to Dawson's technique (15). Fetal skeletons were assessed for the maturity of ossification and checked for malformations like fusion, gaps or defects and deformity with a stereoscope (magnification 6x). Skeletal systems were studied for deduction in length and width of bones completely. Each litter was considered as an experimental unit for statistical analysis to account experimental and control groups for skeletal malformation and growth retardation.

Chi-square and Fisher Exact tests were used for analysis of non-parametric variables. Student t-test was used to analyze parametric changes.

RESULTS

Total skeletal malformation: This was tested in all of treated groups. The range of changes varied between 11.70% (Cd-1) to 53.17% (CD-7) which were significantly higher than that of control group ($p < 0.05$). There were not any significant differences in length of arm, big diameter of pelvis, big diameter of chest, among (Cd-1)-treated and control groups (Table 1). These data also show that the highest teratogenic effect of Cd appeared as deduction in length of tibia and big diameter of pelvis. Although the lower limbs were more affected by Cd treatment ($p < 0.05$), but changes in length and width of skeletons were similar (Table 1).

External malformation

Among different variables examined, only the appearance of cleft palate and open eyelid in Cd treated groups were significantly different in comparison with those of controls respectively at $p < 0.001$ and $p < 0.008$.

Table 1. Effects of various doses of cadmium on skeletal system in rat fetuses

Parameters	control (Cd 0 mg/kg)	Cd (1 mg/kg)	Cd (4 mg/kg)	Cd (7 mg/kg)
Length bones				
Length of arm (mm)	8.50±0.07	8.30±0.04	7.90±0.21*	3.02±0.11*
Length of femur (mm)	7.5±0.04	6.80±0.04*	5.56±0.02*	3.68±0.08*
length of tibia (mm)	6.48±0.01	4.92±0.01*	3.22±0.03*	2.04±0.02*
length between crown to sacrum (mm)	43.00±0.09	40.00±0.09*	35.68±0.06*	20.48±0.07*
Length between crown to bottom (mm)	46.84±0.06	45.04±0.04	41.60±0.05*	26.8±0.06*
Total malformation in length skeletons (%)	3.10	10.27*	24.10*	56.30*
Wide bones				
Big diameter of pelvis(mm)	8.16±0.04	5.52±0.03*	3.90±0.02*	3.78±0.04*
Big diameter of skull(mm)	9.88±0.05	8.86±0.05*	8.12±0.04*	5.68±0.07*
Big diameter of chest(mm)	8.14±0.03	7.84±0.05	7.48±0.03*	4.00±0.04*
Total malformation in wide skeletons (%)	3.10	14.43*	27.00*	48.61*
Total skeletal malformations (%)	3.15±0.05	11.70±0.04*	25.12±0.06*	53.17±0.07*

All data are Mean ± SEM; All measurements were done by cholis vernier, 0.02.

* Difference between treated and control group is significant at $p < 0.05$.

Table 2. Reproductive status with different doses of cadmium

Parameters	Control 0 mg/kg	Cd 1 mg/kg	Cd 4 mg/kg	Cd 7 mg/kg
Number of fetuses/ number of dams	138/10	122/10	123/9	108/10
Number of pregnant/ number of mated dams	10/10	10/10	9/10	10/10
Mean fetal weight (g)*T	5.12	4.5*	3.58**	2.18***
Mean weight of dams(g)	197.10	195.70	202.10	203.00
Mean weight of males (g)	355	359	393	400
Dead fetuses(%)	%9.4	%7.4	%7.3	%8.3

* - Difference between treated and control group is significant at $p < 0.05$.

** - Difference between treated and control group is significant at $p < 0.02$.

*** - Difference between treated and control group is significant at $p < 0.008$.

*T - Growth retardation determined as decrease in animal weight.

Reproductive status

Rate of growth retardation in groups of Cd-1, Cd-4 and Cd-7 were higher than that of controls ($p < 0.05$, $p < 0.02$ and $p < 0.008$ respectively). Table 2. The rate of mortality varied between 7.3% to 8.3%. This variable was not significant when compared to control group ($p > 0.05$). Correlation between different doses of Cd and growth retardation or total skeletal malformations are shown in figures 1 and 2. There is a linear relationship between dose and growth retardation and also between dose and total skeletal malformations ($r^2 = 0.99$ and $r^2 = 0.97$ respectively).

Discussion

Cadmium was first associated with bone disease after environmental exposure in Japan, resulting in identification of "Itai-Itai" disease (16), also after occupational exposure among Cd workers in France (17) and Sweden (18). In these chronic exposure situations, bone disease was concomitant with kidney damage. Patients were treated with large doses of vitamin-D with varying degrees of success to overcome the lack of vitamin-D activation by the damaged kidney.

Cd can directly induce bone loss, without kidney dysfunction. Bone loss was demonstrated by decreased bone mineral densities in mice (19, 20), dogs (21) and decreased bone strength in rats (22). Decreased bone loss may be due to a decreased bone formation as measured by a decreased number of active bone formation sites in beagles (23) or by increased bone resorption as measured by ^{45}Ca release from the prelabeled skeleton (19 - 26). It was also reported that toxic effects of Cd on bone has a threshold level (27). As shown in table 1 and figures 1, and 2, results of our investigation somehow support the above mentioned reports. Our data (figs. 1 and 2) seem to emphasize on the dose dependency of Cd toxicity that is also supported by another report (28). It was shown (6) that the fusion ribs by Cd using CdCl_2 and gavage method can be seen in a dose range of 3-10 mg/kg/day; this was not confirmed in our investigation; none of the groups treated by 1,4 and 7 mg/kg/day showed such defects

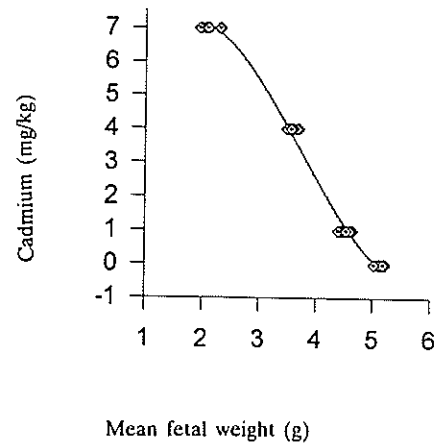


Fig. 1. Correlation between different cadmium doses and fetus growth retardation. Dams were treated by single oral administration of cadmium by gavage on day 7 of gestation period. $n = 8$ for each dose ($r^2 = 0.99$).

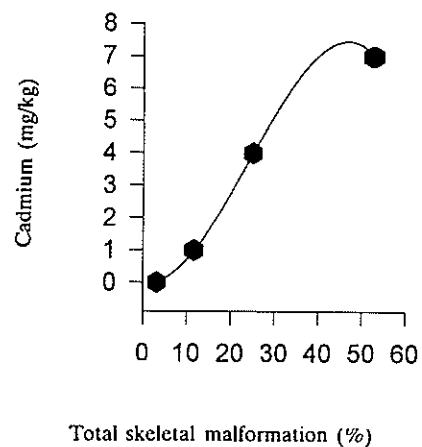


Fig. 2. Correlation between different cadmium doses and total percentage of skeletal malformed fetuses. Dams were treated by single oral administration of cadmium by gavage on day 7 of gestation period. Each point is mean percentage of 7 data ($r^2 = 0.97$).

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(Table 2). The minimal teratogenic dose was determined as 6.1 mg/kg/day (by oral gavage) while in our experiment it was found to be 1 mg/kg/day. The dose effect relationship for Cd-induced teratogenicity suggests that there must be a threshold level for Cd toxic effects. Based on our knowledge there is concern about the threshold level which remains to be clearly defined. In another experiment it was shown (9) that greater rates of exencephaly occur in fetuses of pregnant rats treated by 2 mg/kg/day, while none of the treated animals in our study showed this malformation (Table 2).

Several possible explanations for these differences can be discussed. Among them we would like to emphasize on difference between types of Cd salts used in each experiment. The solubility of different Cd salts and their diffusion into systemic circulation, thereby from placenta into fetuses could explain these differences. However we have not decided to study the mechanism of Cd toxic effects but it is postulated that maternal synthesis of metallothionein with binding to Cd may prevent the embryotoxic effects during sensitive stages of development. This hypothesis could be the subject for future experiments. In summary, these data suggest that in dams¹ Cd exposure during organogenesis increases bone defects in litters, supporting the hypothesis of particular teratogenic effects of Cd on skeletal system. More caution concerning the teratogenic potential of Cd during human pregnancy is proposed.

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