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Selenium Protection against Cadmium Toxicity in Hamster Embryos

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Abstract

The present studies were carried out in order to assess the protective effects of selenium against cadmium toxicity in pregnant hamsters. On the 8th day of gestation the females were dosed subcutaneously either with cadmium or with cadmium and selenium in the following doses (mg per kilogram of body weight): 0, 1 Cd, 1 Cd + 1 Se, 2 Cd, 2 Cd + 2 Se. In groups treated with cadmium decreased litter size, increased postimplantation losses, reduced foetal weight, elevated percentage of resorptions and increased incidence of malformations were noted. Injection of Cd and Se resulted in significantly lower embryo- and foetotoxic indices. Selenium also reduced Cd concentrations in maternal tissues but not in the placenta nor in the foetuses.

Keywords: cadmium, selenium, hamster, embryotoxicity

Introduction

Cadmium is a naturally occurring element present in soil and water. Additionally, it is released to the atmosphere from a wide range of antropogenic sources. Recently there has been some concern that cadmium levels in soil are increasing due to the disposal of domestic sewage sludge on agricultural areas as well as wide use of phosphate fertilizers contaminated with this toxic metal. As a mobile element Cd is taken up by plants and animals and bioaccumulated. Especially high bioconcentration of this heavy metal has been found in aqueous organisms such as molluscs, crustaceans and fish. Increased levels of cadmium lead to greater human exposure from the food chain. It should be mentioned that smoking is also an important source of cadmium exposure.

However, the reproductive effects of cadmium have not been reported in humans, though many studies on animals have revealed its developmental and teratogenic effects [1, 2, 3]. Cadmium administered parenterally to pregnant laboratory animals produced a variety of adverse reproductive outcomes. Decreased litter size, resorptions, foetal death, growth retardation and congenital malformations in offsprings of exposed animals have been the most frequently reported [4, 5, 6, 7]. Cd has also been observed to cause oppressive acute pathological changes in animal gonads, especially in males. Inflammation of the testes followed by necrosis and atrophy in male rats was reported by several authors [8, 9, 10].

Studies from the last decade evidenced that cadmium toxic effects may be mediated by other elements, e.g. zinc, cobalt, calcium or selenium [11, 12, 13]. Our previous study on hamsters demonstrated that selenium can completely protect the male reproductive system against cadmium induced damage [14]. The purpose of our recent studies was to find out if the selenium protects the hamster embryos against cadmium developmental toxicity. The previous literature data as well as our own experience pointed out that the hamster is one of the most sensitive models for screen environmental teratogens even after single treatment in the morning on the eighth day of gestation. This period (24-hr) corresponds to the period of very rapid differentiation and major organogenesis in this animal.

Materials and Methods

Chemicals

Cadmium chloride, $CdCl_2 \cdot 2_{1/2} H_2O$ (POCh, Gliwice). Sodium selenite, Na_2SeO_3 anhydrous (Fluka Switzerland).

Animals

The experiment was carried out according to governmental regulations (Ustawa o ochronie zwierząt Dz. U. Nr 111 z 21.08.1997) and internationally acknowledged principles for the use of animals for experimental and other scientific purposes (European Convention for the Protection of Vertebrae Animals Used for Experimental and Scientific Purposes, Strasbourg, 1986).

The mature nulliparous female golden hamsters, 10-16 week old, derived from our own colony (National Veterinary Research Institute, Pulawy) were used in this study. They were housed in a light controlled room (light: dark cycle, 14:10 hr) at 20 ± 2°C and relative humidity 50 ± 10%. A commercial pelleted diet (Murigran) and water were provided ad libitum.

The animals were mated overnight and the following day was designated day one of gestation. The primiparous females (international recommendations) were randomly assigned to five groups of 20 animals each and housed one per cage.

Treatment

On day eighth of gestation the females were weighed and dosed subcutaneously (s.c.) with either cadmium alone or with cadmium and selenium (10 min interval, different plays of injection):
group I - Control *(aqua pro injectione)* group II - 1 mg Cd/kg body weight group III - 1 mg Cd + 1 mg Se/kg body weight group IV - 2 mg Cd/kg body weight group V - 2 mg Cd + 2 mg Se/ kg body weight The aqueous solutions of cadmium and selenium were given at a volume of 0.5 ml/100 g body weight. Doses were estimated on the basis of the results of our previous studies [7, 20].

Maternal and Foetal Examination

All the animals were observed for death and other signs of toxicity during the whole experiment. On the 15th day of gestation (1 day before parturition) the animals were weighed and euthanized by CO_2 inhalation. The uterus with ovaries was removed, weighed and the numbers of corpora lutea, implantations, normal and dead foetuses as well as the number of resorption sites were recorded. The foetuses were sexed, weighed, measured (crown-rump length) and examined for gross malformation. Approximately one-third of the foetuses were fixed

Table 1. Effect of cadmium and cadmium with selenium on pregnant hamsters.

Group	I	II	III	IV	V
Dose mg/kg	0 control	1 Cd	1 Cd +1 Se	2 Cd	2 Cd +2 Se
Pregnant females, No.	21	20	20	20	18
Body weight gains (g)					
Total weight gain (1–15)	24.4 ± 4.0	21.2 ± 6.7	21.7 ± 5.8	20.0 ± 8.5*	22.7 ± 6.4
– Gravid uterine weight	28.6 ± 4.3	21.0 ± 7.1*	25.4 ± 4.5*	20.7 ± 8.0*	23.1 ± 7.2*
Net weight gain (1–15)	–4.6 ± 5.3	0.1 ± 6.7*	–3.7 ± 4.6	–0.7 ± 4.0*	–0.3 ± 5.5*
Maternal organs weight/100 g of body weight (g)					
Liver	5.54 ± 0.54	5.14 ± 0.39*	5.83 ± 0.38	5.43 ± 0.37	5.85 ± 0.25*
Kidneys	0.98 ± 0.06	0.95 ± 0.11	1.00 ± 0.09	0.97 ± 0.11	1.00 ± 0.10
Spleen	0.32 ± 0.16	0.26 ± 0.10	0.34 ± 0.07	0.28 ± 0.25	0.30 ± 0.10
Embryotoxic indices/ litter					
Corpora lutea	10.9 ± 2.4	9.6 ± 1.7	9.1 ± 1.5	0.7 ± 1.5	9.8 ± 2.2
Implantations	9.8 ± 1.7	8.5 ± 1.6*	8.6 ± 1.6	9.6 ± 1.9	8.9 ± 2.0
Postimplant. losses,	0.6 ± 0.93	1.5 ± 1.99*	0.4 ± 0.5	3.1 ± 2.84*	1.2 ± 1.95
Foetotoxic indices					
Litter size	9.2 ± 1.9	7.0 ± 2.7	8.2 ± 1.7	6.5 ± 3.1*	7.8 ± 2.8
Foetal weight, g	1.97 ± 0.19	1.80 ± 0.20*	1.86 ± 0.20	1.60 ± 0.30*	1.78 ± 0.23*
Crown-rump length, mm	26.9 ± 1.5	26.8 ± 1.3	27.4 ± 1.6	24.8 ± 1.8*	26.9 ± 1.3
Placental weight, g	0.34 ± 0.06	0.28 ± 0.07*	0.33 ± 0.06	0.30 ± 0.09	0.29 ± 0.03*
Malformed, No. (%)	0	11 (7.4)**	0	25 (19.2)**	14 (10.0)**
Runts, No. (%)	11 (5.7)	14 (9.4)	13 (9.8)	38 (29.2)**	25 (17.8)**

The values are presented as the mean ± SD;
Significantly different from control, $p < 0.05$: * – Student's t-test; ** – χ^2 test.

in Bouin's solution and then examined for visceral alteration by microdissection according to Wilson's technique [15]. The remaining two-thirds of foetuses were placed in 95% ethanol, cleared with KOH and stained with Alizaryn Red S. After processing the foetuses were examined for skeletal abnormalities.

Additionally cadmium concentration in maternal and foetal tissues was measured. The procedure consisted of digestion of biological samples at 450˚C, dissolution in hydrochloric acid solutions, chelation of cadmium with ammonium pyrrolidynodithiocarbamate (APDC), extraction of the chelate into methyl isobutyl ketone and determination of the complex by atomic absorption spectrometry at 228.8 nm.

The results obtained were analyzed using a computer-based statistical package (SYSTAT for Windows: Statistics, version 5, 1992). To determine the significance of treatment effects the normally distributed data (body and evaluated organs weight, length of the foetuses as well as the concentration of cadmium in tissues) were analyzed using Student's t-test. The implantation losses, stunted and malformed foetuses were analyzed using $\%^2$ test. The level of significance for all comparisons was set at $p < 0.05$.

Results

Maternal Toxicity

There were no clinical signs of cadmium toxicity in animals during the whole experiment, however, an adverse effect on body weight gain was noted. Total weight gain and gravid uterine weight were lower in all (Cd and Cd + Se) treated groups than in control (Table 1). This effect was dose dependent and correlated with significantly decreased litter size and increased postimplantation losses. There were no statistical differences in kidneys and spleen weight between cadmium-exposed and control group.

Embryo and Foetal Toxicity

The foetal weight was reduced in the three groups (1 mg Cd, 2 mg Cd and 2 mg Cd + 2 mg Se). Additionally,

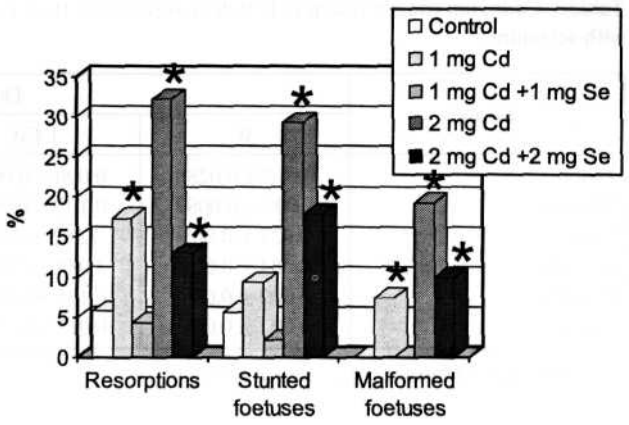

Fig. 1. Embryotoxic effects of cadmium or cadmium with selenium administered on day 8 of gestation in hamsters. * - significantly different from control ($p < 0.05$).

significantly more incidences of stunted foetuses (whose weight was less than the mean of the base control minus 2-fold standard deviation) were found in groups treated with a higher dose of cadmium alone and with selenium (Figure 1).

The mean percentage of resorptions was markedly elevated in both groups treated with 1 or 2 mg Cd (32.1% and 17.3%, respectively) and in the group exposed to 2 mg Cd + 2 mg Se (13%), compared to control (5.8%). In the same groups significant increase of malformation incidences was noted (Figure 1). All major malformations detected in foetuses, either by external, visceral or skeletal examination are presented in Table 2. There was no evidence of malformation in the control foetuses. It should be noted that there was a direct relationship between Se dose and the incidences of foetal abnormalities and resorptions. Simultaneous injection of Cd + Se resulted in at least two times lower incidence of these embryotoxic indices. The teratological findings observed in hamster foetuses after exposure to cadmium were dose-related.

Cadmium Distribution

Evaluation of cadmium distribution in hamsters revealed that this element was accumulated mainly in the

Table 2. Effect of cadmium and cadmium with selenium on the incidence of anomalies in hamsters.

Dose mg/kg	Incidence*		Type of anomalies (number of affected foetuses)
	Litters	Foetuses	
1 Cd	5/20	11/148	Amelia (4), Ectrodactylia (3), Hemimelia (2), Fused ribs (1), Cleft lip (1), Cleft Palate (1), Encephalocele (1)
1 Cd + 1 Se	0/16	0/132	No gross malformations were found
2 Cd	10/17	25/130	Cleft lip (13), Cleft palate (13), Fused ribs (12), Fused sternal element (12), Microphthalmia (6), Short tail (6), Exophthalmia (6), Umbilical hernia (5), Hypognathia (3), Encephalocele (3), Exencephaly (3), Amelia (2)
2 Cd + 2 Se	8/18	14/140	Fused ribs (13), Amelia (5), Hemimelia (4), Ectrodactylia (4), Short tail (4),

* Number of affected/number of examined.

Table 3. Cadmium concentration in female hamsters and their foetuses (μg/g, mean ± SD) following exposure to cadmium and cadmium with selenium.

Tissue	Dose (mg/kg), s.c. on day 8 of gestation				
	0	1 Cd	1 Cd + 1 Se	2 Cd	2 Cd + 2 Se
Blood	0.012 ± 0.0288	0.090 ± 0.060*	0.041 ± 0.038	0.062 ± 0.056*	0.078 ± 0.036*
Muscles	0.005 ± 0.0087	0.102 ± 0.083*	0.055 ± 0.029*	0.306 ± 0.251*	0.216 ± 0.154*
Liver	0.027 ± 0.005	12.82 ± 3.22*	4.697 ± 1.914*	20.10 ± 4.73*	8.624 ± 4.153*
Kidneys	0.084 ± 0.003	3.984 ± 2.003*	3.064 ± 0.990*	9.020 ± 3.618*	6.089 ± 1.846*
Placenta	0.003 ± 0.004	0.125 ± 0.050*	0.175 ± 0.079*	0.377 ± 0.476*	0.953 ± 0.330*
Foetus	0.002 ± 0.003	0.015 ± 0.007*	0.042 ± 0.017*	0.012 ± 0.013*	0.059 ± 0.570*

* – significantly different from control ($p < 0.05$).

liver and kidneys. Mean level of Cd in these organs in hamsters injected with cadmium were several dozen to several hundred times higher than in the control group. The general pattern of Cd distribution in tissues was the same in all cadmium treated groups i.e. liver > kidneys > placenta > muscles > blood > foetus. Simultaneous injection of Cd and Se resulted in a sharp decrease of Cd level in the liver, kidneys and muscles but neither in the placentas nor in the foetuses (Table 3).

Discussion

In the present study we have confirmed the protective activity of selenium against cadmium's toxic effects. Cadmium injected to pregnant hamsters at a dose of 1 or 2 mg/kg b.w. exerted strong teratogenic and embryotoxic influence on foetuses but not in rats, as was found in our previous studies [7]. On the contrary, in the group injected with Cd (1 mg/kg) and Se (1 mg/kg) simultaneously we have observed neither teratogenic nor embryotoxic effects. In the above group selenium completely blocked the toxic properties of cadmium.

We have demonstrated a similar protective action of selenium in a study on male hamsters. In that experiment Se entirely saved the male reproductive system against cadmium-induced damage [14].

The mechanism of protection by Se treatment against acute cadmium toxicity is not quite recognized. However, it has been known that Se and Cd form a non-toxic high molecular weight complex in blood. In the next step Cd is distributed to various organs, especially to liver, kidneys and testes in which it exists as a cadmium-metallothionein form. It was also found that Se promotes Cd excretion [16, 17].

The data from the cadmium concentration study in our experiment are surprising to some extent. In general a significantly lower level of cadmium was found in the groups treated with Cd + Se compared to groups receiving only Cd. This pattern of Cd concentration was similar in all samples of analyzed tissues except for placentas and embryos. On the contrary in placentas and embryos from groups II and IV (1 mg Cd and 2 mg Cd) cadmium concentrations were much lower than in respective groups III and V injected simultaneously with selenium. This observed reverse pattern of Cd concentration remains unclear; however, it could be due to the more stable complex of cadmium-metallothionein, which could not so readily cross the placental barrier. On the other hand, even such high Cd concentrations (placenta and embryo) observed in group III receiving 1 mg of Cd + 1 mg Se did not induce any embryotoxic effects.

In the group of hamsters injected with higher doses of Cd (2 mg) simultaneously with Se (2 mg) we did not observe such spectacular protection effectiveness. Though the embryotoxic and teratogenic indices in this group were about 50% lower than in group IV treated with 2 mg of Cd alone. Moreover, one should remember that selenium at higher doses could be extremely toxic and it has proven to be a developmental toxicant in animals [18, 19]. In our previous study in the group of hamsters injected with selenium at the dose of 2 mg/kg b.w. on day eighth of gestation we have observed a significantly higher incidence of stunted as well as malformed foetuses [20].

The results obtained from this and previous studies indicate that selenium used at a low enough dose could be a very effective protection against cadmium-induced developmental toxicity. However, it is necessary to remember that species differences in susceptibility, route and time of exposure are of great importance in extrapolation of the results to humans.

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