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Influence of cadmium salts on gestation and foetuses in rabbits

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ABSTRACT

Fifteen female rabbits of the Chinchilla bastard breed of reproductive age (7 months) were used in the study. The animals were naturally mated with 5 male rabbits at 3-day intervals. On day 10 of mating, pregnancy was established by abdominal palpation and ultrasonography in all 15 female rabbits. The rabbits were then divided into two experimental groups and one control group of 5 animals each. In group 1 spontaneous abortion occurred on day 20-22 of gestation. In group 2 abortion occurred on day 17-18 of gestation in three rabbits, whereas in two animals in which abortion did not occur, only a small part of necrotic placenta was found on section in the uterus. There were no teratogenic alterations of the aborted foetuses, whereas the placentae showed very pronounced changes in the form of necroses with abundant fibrin deposits and little structure preserved. In the control group of animals, pregnancies resulted in the delivery of healthy neonates, while the placental tissue showed normal structure. There were no macroscopic uterine lesions. In group 1 and 2 pathohistologic examination revealed decidual cell destruction and massive fibrin deposition in the decidual area.

Key words: rabbit, cadmium chloride, pregnancy, placenta, pathohistologic lesions

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Introduction

Cadmium (Cd) is widespread in the environment and is classified as a first-group carcinogen. In addition, it is unique among metals for its toxicity in low doses, long half-life, low elimination rate and significant soft tissue residues (RAJAN et al., 2001). Therefore, cadmium poses a significant risk for human and animal health. Studies have shown that in animals, cadmium binds harmlessly to metallothionein (MT), inducing its formation even at a low level of exposure. Chronic toxicity is believed to occur when renal concentration of cadmium reaches or exceeds the MT binding capacity (MIN et al., 2001). Studies of the effect of cadmium on reproductive organs and foetuses have been stimulated by scientific, health, as well as economic interests.

Studies in humans and animals have shown a very low proportion of cadmium to cross the placenta, being instead accumulated in the placental tissue. According to LEVIN and MILLER (1983), MILLER et al. (1983), MILLER et al. (1988), MOBERG et al. (1992), GOYER (1995), CARMICHAEL et al. (1982), and LAGERKVIST et al. (1992), the low concentration of cadmium in human foetuses indicates that placenta is not an absolute barrier for cadmium passage. Recent studies of placental cadmium and progesterone concentrations in female cigarette smokers have provided additional evidence that maternal cigarette smoking reduces the placental level of progesterone and confirms the association between cigarette smoking and placental cadmium concentration (PIASEK et al., 2001). The same group of authors investigated the acute effect of cadmium on ovarian steroidogenesis in different reproductive states. A subcutaneously administered dose of 3 or 5 mg Cd/kg body mass did not cause general toxic effect, oestrous cycle interruption, or foetal viability modifications in female rats. Histology revealed moderate thecal congestion in the ovaries of pregnant rats. Besides the liver, highest cadmium concentrations were observed in the foetal segment of the placenta (PIASEK and LASKEY, 1994). CHIQUOINE (1965) reports on placental necrosis and foetal death in rats on day 6 and 17 of gestation, respectively, following single subcutaneous administration of 3 mg cadmium chloride ($CdCl_2$) per kilogram body mass, but without any foetal lesions. PARIZEK (1964 and 1965) reports on 100% foetal lethality and severe placental necrosis in pregnant rats administered 20 to 40 mmol $CdCl_2$/kg

body mass subcutaneously on day 17 and 21 of gestation. CHIQUOINE (1965) and FERM et al. (1969) describe similar observations in mice, emphasizing foetal death despite low cadmium accumulation in the placenta, its concentration being at the borderline of placental permeability. SAMARAWICKRAMA and WEBB (1979) performed histologic analysis of the placentae of rats exposed to cadmium chloride and found uterine vasculature stenosis, haemorrhages, and placental thromboplastic necroses. In the study by PADMANABHAN (1986), pregnant mice were administered an aqueous solution of cadmium chloride subcutaneously in a dose of 4 or 6 mg/kg body mass. In the treated groups of animals, foetal malformations were observed in 60% to 75% of all aborted foetuses. The malformations included mandibular and maxillary pulmonary hypoplasia, diaphragmal hernia, and kidney hypoplasia. DALTON et al. (1996) gave cadmium to normal and transgenic mice in a dose of 7.5 or 10.0 mmol/kg body mass. Testicular histology showed no changes in male mice exposed to the lower dose of cadmium. However, testicular necrosis developed in both transgenic and normal groups of animals administered 10.0 mmol cadmium *per* kg body mass. In female animals, impediment of the foetal uterine implantation was observed in both dosage groups. The authors conclude that specific mRNA had no protective action against the effect of cadmium in mice. LAU et al. (1998) investigated the role of placental metallothioneine in the maternal to foetal transfer of cadmium in genetically modified mice. Their results suggest that placental metallothioneine reduces the maternal cadmium transfer to the foetus, although low cadmium doses resulted in the high level of cadmium accumulated in the liver and kidneys in all experimental groups of mice. In spite of this, however, a small amount of cadmium was found to have deposited in the placenta. Therefore, the authors state that the role of placental metallothioneine as a barrier for cadmium remains inconclusive. LEVIN and MILLER (1980) report on only 12% mortality in rabbit foetuses following direct uterine administration of cadmium chloride. The authors conclude that foetal 'resistance' to direct uterine administration of cadmium chloride indicates that foetal mortality was not exclusively caused by the direct effect of cadmium, but was also due to some extra-foetal effects.

Our studies were focused on a macroscopic and histologic follow-up of changes in the placentae and foetuses of rabbits exposed to multiple peroral administration of cadmium chloride.

Materials and methods

Fifteen female rabbits of the Chinchilla bastard breed of reproductive age (7 months), weight 3200-3800 g were used in the study. The animals were caged individually, fed commercial rabbit chow manufactured by Pliva Inc., and had access to water *ad libitum*. All rabbits were allowed to mate in groups of five at 3-day intervals. Female rabbits were placed in cages with male rabbits for mating. On day 10 after mating, female rabbits were examined for pregnancy by abdominal palpation and ultrasonography. Pregnancy was confirmed in all 15 female rabbits. Experimental group 1 and 2 rabbits were given 5 and 10 mg $CdCl_2$/kg body mass, respectively, starting from day 10 of gestation, on a daily basis for the first 15 days, and thereafter every other day. In the control group (group 3) animals, all conditions were the same as in the experimental groups, but they were not treated with cadmium chloride. Cadmium chloride was dissolved in 2 ml distilled water and applied onto the root of the animal tongue by use of a suitable catheter. Following abortions in experimental group 1 and 2, the animals were sacrificed by jugular vein exsanguination, being previously anesthetized with 25 mg/kg ketamine chloride. On section, each individual animal was thoroughly examined, including the placenta. Placental and uterine samples for pathohistologic examination were fixed in 10% buffered paraformaldehyde, submitted to serial alcohol dehydration, and paraplast embedded. Histologic sections were stained with hemalaun eosin.

Results

In group 1 (5 mg $CdCl_2$/kg body mass), abortions occurred between day 20 and day 22 of gestation. The aborted foetuses showed no teratogenic alterations. However, they were light-pink in colour. Macroscopic changes of the placenta included thin fibrin deposits on its surface and small dark-brown necrotic areas.

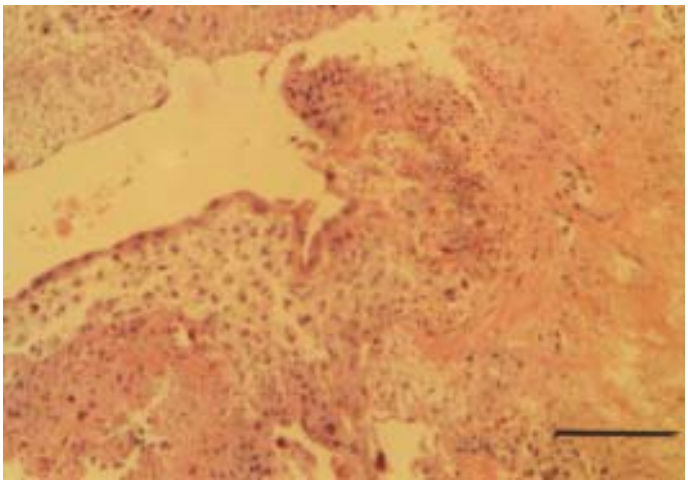

Fig. 1. Placenta of rabbits treated with 5 mg $CdCl_2$/kg body mass. Abundant fibrin deposits with largely extended necrotic areas. Only rare sites with preserved placental structure are seen. H&E; ×200; scale bar = 100 μm

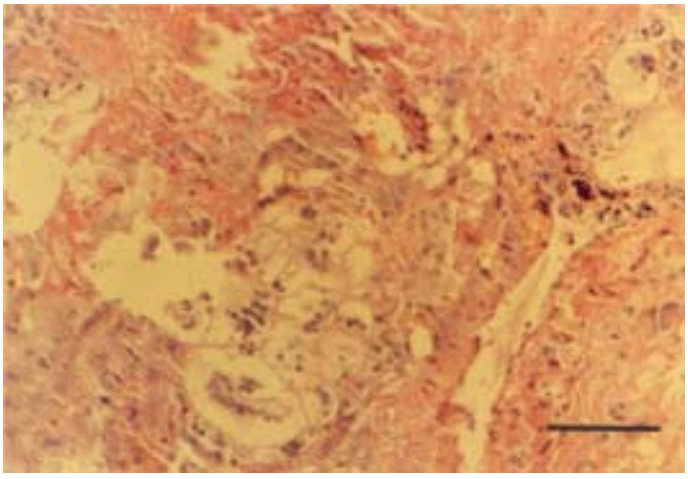

Fig. 2. Placenta of rabbits treated with 10 mg $CdCl_2$/kg body mass. Severe lesions of the placental tissue, with areas of total necrosis. H&E; ×200; scale bar = 100 μm

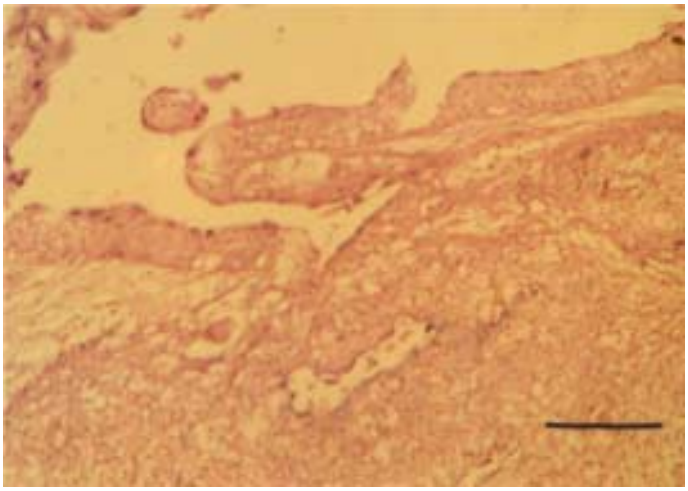

Fig. 3. Placenta of control group rabbits. Normal structure of the placental tissue. H&E; ×200; scale bar = 100 µm

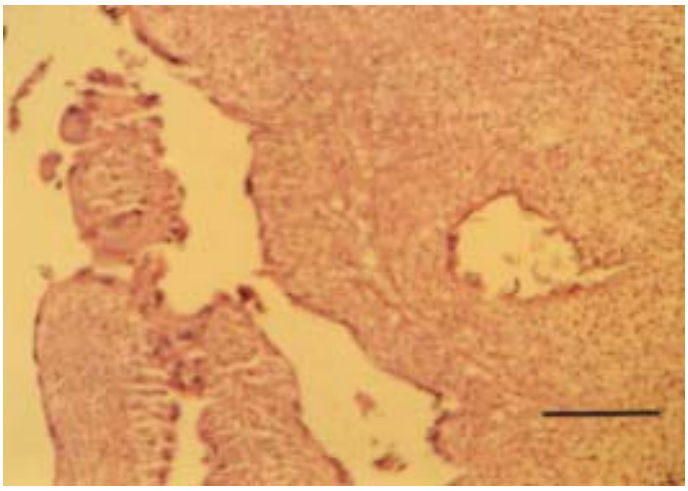

Fig. 4. Uterus of rabbits treated with 5 mg $CdCl_2$/kg body mass. Occasional fibrin deposits in decidual tissue; distinct margin between decidual cells has disappeared. H&E; ×200; scale bar = 100 µm

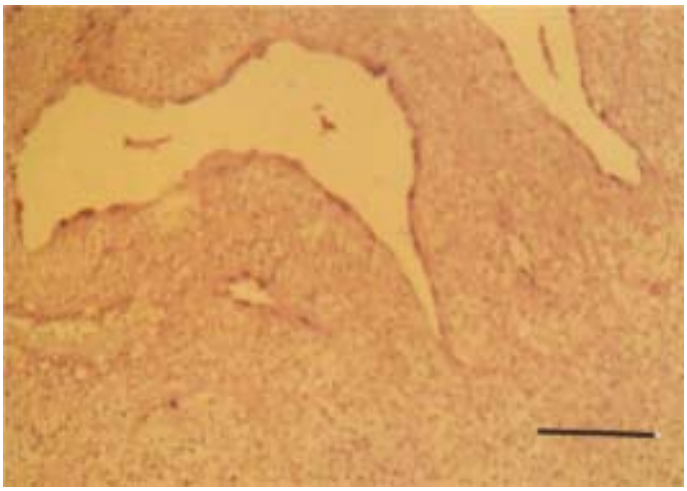

Fig. 5. Uterus of rabbits treated with 10 mg $CdCl_2$/kg body mass. Abundant fibrin deposits in decidual area, with large areas of decidual cell necrosis. H&E; ×200; scale bar = 100 μm

Pathohistologic slides of group 1 rabbit placentae showed abundant fibrin deposition and an extended necrotic area. There were only rare spots of structurally preserved placenta (Fig. 1).

In experimental group 2 (10 mg $CdCl_2$/kg body mass), abortion occurred between days 17 and 18 of gestation. The aborted foetuses were dark-brown to black in colour. Abortion did not occur in two rabbits. However, section revealed a small portion of the placenta and occasional foetuses in a stage of resorption. Macroscopically, thick fibrin deposits and placental tissue necroses were clearly seen. Pathohistologic slides showed severe lesions of the placental tissue, with areas of total necrosis (Fig. 2).

In the control group (group 3) animals, the course of pregnancy terminated with uneventful delivery. The placenta showed normal placental tissue structure both macroscopically and histologically (Fig. 3).

In group 1 and 2 animals, no macroscopic changes of the uterus were observed. Pathohistologic slides of group 1 animals showed occasional initial fibrin accumulation in decidual tissue. Decidual cells appeared to

coalesce, with indistinct margins between them, as if disappearing (Fig. 4). In group 2 animals, pathohistologic slides showed abundant fibrin deposits and large areas of decidual cell necrosis (Fig. 5).

Discussion

The concentration and properties of cadmium compounds, and the route of their entering the body are the major factors that determine the effects of cadmium in a particular animal species. It is somewhat difficult to arrive at any definite conclusion on the basis of literature data on the effect of cadmium on various species, because the studies reported differed significantly in some of the above mentioned factors. Simple recomputation of the cadmium doses used in various studies yields them to mostly range between 0.5 and 10.0 mg $CdCl_2$/kg animal body mass. Also, the fact that only a minor portion of cadmium ingested with feed or water is being resorbed should be taken in consideration. According to GRUDEN (1982) and RUDE (1982), an average of 6% cadmium is absorbed in the duodenum and jejunum. SREBOČAN et al. (1986) added up to 0.222 mg $CdCl_2$ *per* kg cockerel feed, which is significantly below the daily doses of cadmium reported in the quoted literature. However, they found hydropic degeneration of testes in all experimental groups of birds, and also atrophy in the birds that died. DOBRANIĆ et al. (2001) report on considerable testicular reduction, along with rough surface and a light-yellow colour of testes instead of a smooth surface and pink colour in both experimental groups of rabbits after peroral administration of 5 or 10 mg $CdCl_2$/kg body mass. After 30-day cadmium exposure, pathohistology showed significant reduction of spermatogenesis, or even a complete lack of formed sperm, in both groups of experimental animals.

Considering the doses and effects of cadmium on reproductive organs and placenta mentioned in the introduction, we decided to use a multiple daily dose of 5 or 10 mg $CdCl_2$/kg body mass, expecting to induce considerable placental and foetal alterations in rabbits. Rabbits were used for their economic importance. Although paid due attention by only a small number of rabbit breeders, macroscopic and pathohistologic examinations of rabbit placenta provide a simple and inexpensive method for the study

of cadmium teratogenicity in these animals, which was the reason for our choice of these analyses in the present study.

In our experiments, rabbits treated with 5 or 10 $CdCl_2$/kg body mass showed major placental alterations that led to abortion and embryonal death. Macroscopic and microscopic examinations of the placental tissue revealed abundant fibrin deposits and severe placental necrosis. CHIQUOINE (1965) describes placental necrosis and foetal death after the administration of cadmium, while PARIZEK (1964 and 1965) relates 100% foetal lethality to placental necrosis following cadmium administration. These results are consistent with our findings. However, those authors used rats and a different route of cadmium administration in their studies. SAMARAWICKRAMA and WEBB (1979) think that cadmium chloride leads to vascular stenosis, thus causing placental necrosis and haemorrhage. BARTUM et al. (1974) investigated uteroplacental blood exchange as a possible mechanism of foetal death, ascribing it to reduced placental and uterine circulation. In their study of cadmium foetal toxicity, LEVIN and MILLER (1980) found a reduced uteroplacental circulation. When cadmium chloride was applied directly into the uterus of rabbits, foetal death rate was only 12%. Thus, the authors believe that foetal death was caused by some extra-foetal effects other than direct cadmium chloride action.

In our study, histopathologic changes of the uterus were observed in group 1 rabbits administered 5 mg $CdCl_2$/kg body mass. The changes involved decidual tissue, where initial fibrin deposits were occasionally observed, while the clear margin between decidual cells gradually disappeared. In group 2 animals given 10 mg $CdCl_2$/kg body weight, uterine changes manifested as abundant fibrin accumulation in the decidual area, with large areas of decidual cell necrosis with more pronounced necrotic lesions of the placental tissue. In spite of the low intestinal cadmium resorption, the repeated daily administration of 5 or 10 mg $CdCl_2$ was expected to induce uterine and placental circulatory impairments in some 10 days, with a consequential toxic effect on the foetus. However, our results suggest that the real toxic effect of cadmium on the foetus is predominantly of an extra-foetal nature, as confirmed by LEVIN and MILLER (1980).

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DOBRANIĆ, T., L. ŠTILINOVIĆ, M. CERGOLJ, A. TOMAŠKOVIĆ, D. MARKOVIĆ: Učinak peroralno davanog kadmijeva klorida na gravidnost i plodove u kunica. Vet. arhiv 71, 203-214, 2001.

SAŽETAK

Cilj ovog istraživanja bio je dokazati toksičnost različitih koncentracija $CdCl_2$ na gravidnost i plod. U istraživanju smo imali 15 spolno zrelih kunica pasmine »činčila-bastard« u dobi 7 mjeseci. Kunice su bile prirodno parene s pet mužjaka u razmaku od tri dana. Kod svih 15 kunica 10. dana od parenja palpacijom i ultrazvukom trbuha utvrđena je gravidnost. Nakon toga kunice su bile podijeljene u dvije pokusne i jednu kontrolnu skupinu. U svakoj skupini bilo je po 5 kunica. Kunice u prvoj pokusnoj skupini pobacile su između 20. i 22. dana gravidnosti. U drugoj pokusnoj skupini došlo je do pobačaja kod tri kunice nakon 17. i 18. dana gravidnosti, dok je kod dvije kunice koje nisu pobacile prilikom razudbe u maternici nađen samo malen dio nekrotične posteljice. Na pobačenim plodovima nisu zapažene teratogene promjene, dok su promjene na posteljici bile izražene u obliku nekroza s obilnim nakupljanjem fibrina i malo očuvane strukture. Kod kontrolne skupine gravidnost je završena porođajem zdrave mladunčadi, a posteljično je tkivo imalo uobičajenu strukturu. Makroskopski na maternici nisu bile uočene promjene. U patohistološkom preparatu u prvoj i drugoj pokusnoj skupini bilo je vidljivo propadanje decidualnih stanica, te obilno taloženje fibrina u decidualnoj zoni.

Ključne riječi: kunić, kadmij-klorid, gravidnost, posteljica, patohistološke promjene