Bull. Environ. Contam. Toxicol. (2002) 69:829–834

DOI: 10.1007/s00128-002-0134-2

Bulletin of Environmental Contamination and Toxicology

Cadmium Accumulation in Fetus and Placenta of Bank Voles (*Clethrionomys glareolus*, Schreber 1780)

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Received: 15 March 2002/Accepted: 15 October 2002

Cadmium was found to have unique biological role (Yu et al. 1993). However it is well known for its toxicity with regard to the reproduction and development of young animals. Cadmium is easily absorbed and remains a relatively long time within the tissues. Its general occurrence in the environment is a significant threat to young organisms, during both prenatal and postnatal development. Placenta is supposed to be an effective barrier to toxic elements (Whelton et al. 1993; Lau et al. 1998). However, other research has proven that cadmium is able to cross through the placenta during early foetal development, when the embryo is most vulnerable, inducing teratogenic effects in rodents (Domingo 1994) and causing the death of the foetus (Levin et al. 1987). Another harmful influence of cadmium on pregnant females and their embryos is connected with its antagonistic effect on absorption and distribution of nutrition in the organism (Sowa 1985; Fiala et al. 1998).

While several studies have examined heavy metal accumulation in laboratory-raised animals (Sowa 1985; Sawicka-Kapusta et al. 1994) few have considered animals captured from the natural environment. Using wild animals avoids the negative influences of long-term captive breeding on laboratory animals. It also increases possibility that former generations were exposed to pollutants in the natural environment. They might develop a defence mechanism. The aim of our study was to assess the influence of a dose of cadmium given with their food to parents and its level in the foetus and placenta as well as its effect on concentrations of essential metals (copper, zinc and iron) in these samples.

MATERIALS AND METHODS

The bank vole (*Clethrionomys glareolus*, Schreber, 1780), is a wild rodent species, common in forest ecosystems all over Europe. As it is relatively easy to breed, the bank vole has become a model animal for numerous laboratory studies of physiology and pollution (Zakrzewska 1988; Sawicka-Kapusta et al. 1990; Sawicka-Kapusta 1995).

For these experiments adult bank voles were exposed to cadmium for two generations. Pairs of voles mated with special consideration to their relationship, were kept in separate cages, in an animal room with a regulated temperature (20 -

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23°C) and 12 h light/dark regime. Animals were fed with high quality food (produced by SPECIAL DIET SERVICE, Po Box 705, Witham, Essex CM8 3AB, UK, Rat & Mouse No. 1 Maintenance Diet) containing all essential minerals, vitamins, proteins and additionally contaminated with 35 or 7 ppm of Cd or without cadmium (control). Those cadmium doses were found in a heavily polluted environment (Kucharski et al. 1994). The voles were given tap water to drink.
Pregnant females from the 2nd generation of studied animals were killed by anaesthesia, some in the earlier (about 14th day) and others in the later (about 19th day) stage of pregnancy. We used 23 younger embryos of 5 females and 59 older embryos of 11 females in the group received 35 ppm of Cd; 37 younger embryos of 7 females and 47 older embryos of 9 females in the group received 7 ppm of Cd; and 33 younger embryos of 6 females and 43 older embryos of 9 females in the control group. Embryos and placentas were dissected and oven-dried to constant weight.

Dry samples were digested in 4:1 HNO_3 : $HClO_4$ mixture. Cadmium was determined by means of atomic absorption spectrophotometer (GF-AAS, Perkin-Elmer ZL 5100), zinc, copper and iron by IL 251 flame spectrophotometer. Standard Reference Material BCR 397 (Brussels) (human hair) was used as the certified reference material. Our detection limit was 0,01 ppm. Two-way Anova on logarithmic data was used to determine differences in concentrations of cadmium. This was followed by post-hoc Tukey HSD in the case of statistically significant differences (Sachs 1984). A Kruskal-Wallis nonparametric test was used to determine differences among experimental groups in concentrations of physiological metals (Sachs 1984).

RESULTS AND DISCUSSION

Placenta is supposed to be an effective barrier against the transfer of toxic elements. A dosage of less than 1% cadmium given in water to mice mothers during pregnancy was accumulated in the bodies of offspring (Whelton et al. 1993). According to Lau et al. (1998), 0.1 - 0.3% of cadmium given to the mothers was detected in the new-borns.

On the other hand, cadmium transfer across the placenta has been proven by several studies (Bell 1984). Cadmium accumulations were two times higher both in the hair of human mothers occupationally exposed to this metal and in their babies exposed during pregnancy (Huel et al. 1983).

In our study the cadmium concentrations were significantly higher in placentas compared to embryos in all studied groups (Table 1).

Placentas of bank voles exposed to 7 or 35 ppm Cd in their food accumulated significantly more cadmium ($p<0.05$) than placentas from the control group.
Also in females exposed to cadmium a transfer of that metal through the placenta still existed.

The foetuses of females fed with "cadmium-food" had significantly higher levels of this element ($p<0.05$) than the ones of the control animals.

Table 1. Average cadmium concentrations (ppm d.w.) in the 14th and 19th days foetus and placenta of bank voles.

Metal		Control	7 ppm Cd	35 ppm Cd
Cadmium	Foetus	$0.05^{1,*}$ SE = 0.014 N = 15	$0.13^{1,**}$ SE = 0.067 N = 16	$0.21^{1,**}$ SE = 0.094 N = 16
	Placenta	$0.66^{2,*}$ SE = 0.310 N = 15	$2.34^{2,**}$ SE = 0.598 N = 16	$5.91^{2,**}$ SE = 1.248 N = 16

*,** – different numbers of stars denote significant differences between experimental groups ($p<0.05$)
1, 2 – different numbers denote significant differences within foetus and placenta in the same experimental group ($p<0.05$)

We noticed an increase in cadmium accumulations in placentas in the group exposed to 7 ppm cadmium in their food. In the case of the group receiving 35 ppm cadmium, the concentration of that metal, despite the pregnancy period, maintained a higher level than in other groups. Cadmium levels in placentas and embryos from the control group did not change with their development. In contrast, embryos of the bank voles receiving cadmium in their food had a statistically significant increase in the levels of that metal during the length of pregnancy (Table 2).
Our research showed a significantly higher level of cadmium in placentas than in embryos despite the pregnancy period in all cases studied, except embryos and placentas from earlier pregnancy in the control group (Table 2). Cadmium's ability to cross the placenta barrier is probably connected with the concentration of cadmium in the mother's diet, as well as the presence and concentration of optimal dietary elements in the food. Sawicka-Kapusta (1995) noticed significantly higher cadmium levels in tissue of foetuses fed with 100 ppm Cd compared to the control group. In this case poor-quality food increased cadmium toxicity. This work also suggested that the placenta is an effective barrier to cadmium transport when the cadmium concentration in food is less than 50 ppm.

Any disturbance in the function of the placenta by changing its metabolism or morphology might cause embryotoxic and teratogenic effects on developing organisms (Soukupova and Dostal 1991). Cadmium can change some physiological parameters such as an enzymatic activity in placenta, so cadmium may influence foetal development even though its level in the embryo is relatively low. Taking part in early cellular changes, cadmium might cause the death of the foetus by dysfunction of uterus and placenta (Boadi et al. 1992).

Table 2. Concentrations of heavy metals (ppm d.w.) in the foetus and placenta of bank voles. Mean ± SE.

Metal	Samples	Control	7 ppm Cd	35 ppm Cd
Cd	Embryos from earlier pregnancy	$0.04^{*} \pm 0.023$ N = 6	$0.06^{a,1,*} \pm 0.039$ N = 7	$0.02^{a,1,*} \pm 0.003$ N = 5
	Placenta of younger embryos	$0.23^{*} \pm 0.080$	$0.77^{a,2,*} \pm 0.213$	$2.44^{2,**} \pm 0.301$
	Foetus from later pregnancy	$0.06^{1,*} \pm 0.019$ N = 9	$0.25^{b,1,**} \pm 0.115$ N = 9	$0.27^{b,1,**} \pm 0.127$ N = 11
	Placenta of older foetus	$0.92^{2,*} \pm 0.485$	$3.72^{b,2,**} \pm 0.85$	$7.07^{2,**} \pm 1.528$
Zn	Embryos from earlier pregnancy	121 ± 4.5	107 ± 3.1	119 ± 8.7
	Placenta of younger embryos	$109^{a} \pm 8.8$	$104^{a} \pm 6.5$	$129^{a} \pm 6.5$
	Foetus from later pregnancy	$121^{1} \pm 3.4$	$111^{1} \pm 4.7$	$106^{1} \pm 6.4$
	Placenta of older foetus	$99^{b,2} \pm 5.2$	$89^{b,2} \pm 6.6$	$90^{b,2} \pm 4.6$
Fe	Embryos from earlier pregnancy	428 ± 36.2	397 ± 12.3	377 ± 19.2
	Placenta of younger embryos	543 ± 38.4	688 ± 54.1	315 ± 19.1
	Foetus from later pregnancy	$365^{1} \pm 15.6$	$386^{1} \pm 17.3$	$375^{1} \pm 12.1$
	Placenta of older foetus	$668^{2} \pm 43.9$	$693^{2} \pm 38.6$	$663^{2} \pm 54.6$
Cu	Embryos from earlier pregnancy	$12.0^{1} \pm 1.57$	$10.4^{1} \pm 0.41$	$10.4^{1} \pm 0.53$
	Placenta of younger embryos	$26.2^{2} \pm 5.02$	$16.0^{2} \pm 0.94$	$18.2^{2} \pm 3.29$
	Foetus from later pregnancy	$9.4^{1} \pm 0.88$	$9.9^{1} \pm 0.55$	$7.9^{1} \pm 0.57$
	Placenta of older foetus	$22.2^{2} \pm 2.98$	$15.1^{2} \pm 0.70$	$20.3^{2} \pm 2.74$

$^{a,\,b}$ – different letters denote significant differences within foetus or placenta in different pregnancy period (p<0.05)

$^{1,\,2}$ – different numbers denote significant differences within foetus and placenta in the same pregnancy period (p<0.05)

$^{*,\,**}$ – different numbers of stars denote significant differences between experimental groups (p<0.05)

Cadmium given to the mother with food may influence the development of the embryo due to a decrease in the amount of basic physiological metals (Iyengar and Rapp 2001).

In our study, zinc concentrations reached between 106 and 121 ppm in the foetus and between 89 and 129 in the placenta. The level of zinc in the placenta decreased during pregnancy development in all studied groups (Table 2).
The average concentrations of iron varied from 365 to 428 ppm in foetuses and between 315 and 693 ppm in the placenta (Table 2).
The average concentrations of copper were about 9.4 ppm in foetuses and between 15.1 and 26.2 ppm in the placenta (Table 2).
Our study showed significantly higher concentrations of zinc and iron in the foetuses than in the placentas from the same group on 19^{th} day of pregnancy. Additionally, we noticed higher ($p<0.05$) copper levels in placentas compared to embryos during the whole period of pregnancy (Table 2).

Our study did not show statistically significant changes in the amount of nutrition (copper, zinc and iron) transferred to the foetus from mothers exposed to cadmium in their food. This was probably caused by the fact that such an effect was noticed only in some organs. Sowa (1985) noticed a decrease in levels of zinc in the liver of embryos while copper concentrations were lower in the liver, brain, kidney and digestive canal of the embryos. Except for this, bank voles in this study were supplemented with food containing all needed nutrition and vitamins, what might protect against the negative effects of cadmium on physiological elements' metabolism. Due to the realistic lack of such a diet in the natural environment, we would suspect the stronger effect of cadmium on these metals in bank voles living in the wild.

Acknowledgments. The work was financed by the Polish State Committee for Scientific Research, Grant No. 6 P04G 016 15.

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