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Cadmium, zinc and iron interactions in the tissues of bank vole *Clethrionomys glareolus* after exposure to low and high doses of cadmium chloride

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Abstract

In present study, bank voles *Clethrionomys glareolus* were peritoneally injected with different doses of cadmium, 0, 1.5, 3.0 mg Cd/kg body mass. Animals were sacrificed on the 21st day after cadmium exposure and the liver and kidney were obtained for cadmium, zinc and iron analysis using atomic absorption spectrometry. Results showed that cadmium had accumulated in the tissues according to dosage and sex. Cadmium affected the survival and body masses of dosed females. Cadmium decreased the iron concentrations in the liver of voles, whereas zinc concentrations increased in both the kidney and liver.

Introduction

Studies on cadmium toxicity have been performed ever since this metal was recognized as being potentially toxic to humans and animals, and which is often present in high concentrations in the environment (Nordberg 1972; Friberg *et al.* 1974, 1986; Luckey & Venugopol 1977; Stoeppler & Piscator 1988). It is known that cadmium is well absorbed by organisms, with doses up to 8% built up during a lifetime (Amdur *et al.* 1991; Świergosz-Kowalewska 2001). The metal accumulates in different tissues to a different extent, but the main storage and action sites are the liver and kidney. The absorbed cadmium causes direct or indirect physiological and histopathological changes in the tissues (Chmielnicka & Cherian 1986; Ballantyne *et al.* 1995; Floriańczyk 1995; Świergosz *et al.* 1998; Waalkes 2000). Despite numerous studies on its toxicity, some aspects are not fully recognized yet, for example genotoxicity. One of the important characteristics of cadmium toxicity is its interaction with physiologically essential elements such as zinc, iron or copper (Bonner *et al.* 1980; Peraza *et al.* 1998). The interactions with other elements may have a synergistic or antagonistic character. Depending on this, a decrease or increase in concentrations of essential metals occurs in the tissues of exposed animals, which can generate various metabolic alterations. Recent studies demonstrate that a decrease in iron levels in tissues cause not only anemia, but also lipid peroxidation (Casalino *et al.* 1997). Such data were obtained by Włostowski *et al.* (2003) in the experiment on bank voles, where elevated lipid peroxidation was found in the liver or kidney of the animals exposed to cadmium. If this is the case, the doses of cadmium resulting in the reduction of tissue iron concentration should be extensively studied.

In our previous field studies, the interactions between cadmium and zinc or iron were also tested. In most cases, the positive correlations between cadmium and zinc in the liver and/or in the kidney were observed (Świergosz-Kowalewska *et al.* 2005; Świergosz-Kowalewska *et al.* 2006).

Because cadmium was not the only contaminant in the studied sites, we conjectured that the high concentrations of zinc in the tissues were due to high zinc contamination rather than the effects of the interaction with cadmium.

The present study was performed in order to clarify our findings and those of other researchers, concerning the interactions between nutritionally essential metals. Our objective was to assess cadmium accumulation and retention in the tissues of bank voles exposed to different cadmium doses, and to study the interactions between cadmium and two physiologically important metals; namely: zinc and iron. For this purpose, the injection route of exposure was chosen in our study, when a dose of cadmium, as well as other conditions, are fully controlled. Even if it is not an environmentally realistic exposure, it allowed us to estimate precisely the cadmium toxicity. The experiment was performed on bank voles, *Clethrionomys glareolus*, which is a wild animal, commonly used for biomonitoring and ecotoxicological studies.

Material and methods

Animal treatment

The experiment was performed on 25 individuals, which were bred for five generations in a breeding laboratory after collecting specimens in the field. The animals were 4 months old at the start of the experiment. They were kept individually in standard cages, in an animal room at 21 °C and 12/12 h light/dark regime. The animals were assigned to three experimental groups according to sex and body weight. Animals from each experimental group were injected peritoneally with a single dose of cadmium: 0.0 (control), 1.5 and 3.0 mg/kg body mass. Cadmium was injected as a solution of cadmium chloride ($CdCl_2$) in deionized water. The animals from the control group were injected with physiological salt. The animals were given commercial food (Wytwórnia Pasz, A. Morawski) and water *ad libitum*.

Measurements during the experiment

The body mass of each vole was recorded on the 1st and 21st days of the experiment.

All animals were sacrificed on the 21st day of the experiment and the liver and kidneys were sampled for Cd, Fe, and Zn analyses.

Metal analysis

The kidney and liver samples for metal analyses were dried at 60 °C and then wet-digested in a 4:1 mixture of nitric and perchloric acid (super-pure grade, Merck, Germany). After digestion, the samples were transferred to 10-ml volumetric flasks. Metal concentrations were determined using atomic absorption spectrometry (Perkin-Elmer, AAnalyst 800). Certified reference material (BCR No. 185; bovine liver) was then used to check the analytical precision for all elements. The obtained results were 0.305 ± 0.025 μg Cd/l, 141.69 ± 0.0 μg Zn/l, 206.64 ± 12.4 μg Fe/l, while the certified values were 0.289 ± 0.025 μg Cd/l, 142 ± 3 μg Zn/l, 214 ± 5 μg Fe/l. Heavy metal concentrations in tissues are expressed in milligrams per kilogram dry weight (mg/kg DW) throughout the article.

Statistical analysis

The mean and standard errors ($\pm$SE) for each dataset were calculated. The data were not distributed normally, and logarithmic transformation was used (Zar 1999) for further analysis. Multiple regressions were performed to determine the relationship between the dosage and cadmium accumulation in the tissues, differences in metal accumulation between sexes and the effects of cadmium on zinc or iron concentrations in the liver and kidneys of bank voles (Zar 1999).

Results and discussion

Bank voles survival and biomass change

Although mammals are relatively resistant to cadmium, its toxicity may be manifested in a decrease in the survival rates of exposed animals (Eisler 1985). In the present study, the high mortality of bank voles (44%), was observed in the experimental group exposed to high doses of cadmium, with a 3 mg/kg body weight. Females were more sensitive than males as the ratio of dead individuals was 3:1, respectively. Survival in the other groups was 100%.

Table 1. Body mass of bank voles (g) on the 1st and 21st day of the experiment.

Dose (mg Cd/kg body mass)	Body mass on the 1st day (g)			Body mass on the 21st day (g)		
	Male	Female	Average for both sexes	Male	Female	Average for both sexes
0.0	21.8	20.6	21.1	23.1	22.0	22.5
1.5	20.4	19.9	20.1	21.9	18.6	20.1
3.0	20.6	22.2	21.2	23.5	19.7	22.0

Other sub-lethal effects of cadmium include tissue damage, anemia, growth retardation and body mass changes. In our study, a body mass decrease was observed in some individuals. The mean body masses of females, males, and both sexes grouped together are shown in Table 1. It was found that only the body mass of females treated with 1.5 and 3.0 mg Cd/kg body mass, decreased during the 21 days after the cadmium injection, whereas the body mass of males increased in most cases. The body mass of all animals from the control group also increased. The data showed that the metabolic alterations resulting in body mass decrease was stronger in females than in males. Suzuki *et al.* (1983) also observed a decrease in the body mass of Wistar rat females although cadmium was injected in doses of 3 mg Cd/ kg body weight, 4 times a week for up 6 weeks. In a similar study performed by Karmakar *et al.* (2000) on balb/c mice, the body mass of males did not change after injection of 2.5 mg Cd/kg on alternate days for 21 days.

Tissue accumulation of cadmium

Cadmium absorption and accumulation in the tissues depends on many factors, chief among them being the dose, route of administration, interaction with other substances, and rate of elimination from the body. Cadmium accumulates mainly in the liver and kidney, and has a long half-life in an organism. In the present study, the accumulation of cadmium, in both tissues, was significantly dose dependent (liver: $p = 0.0096$, kidney: $p = 0.0121$) (Figures 1 and 2). Average Cd concentrations in the liver and kidney of bank voles were similar, ranging from 0.2 to 28.5 and 0.7–29.5 mg Cd/kg dry weight, respectively (Table 2). As was highlighted earlier, the cadmium distribution in an organism depends also on the method of metal administration (Amdur *et al.* 1991). Jonah and Bhattacharyya (1989) showed in the experiment on mice, that

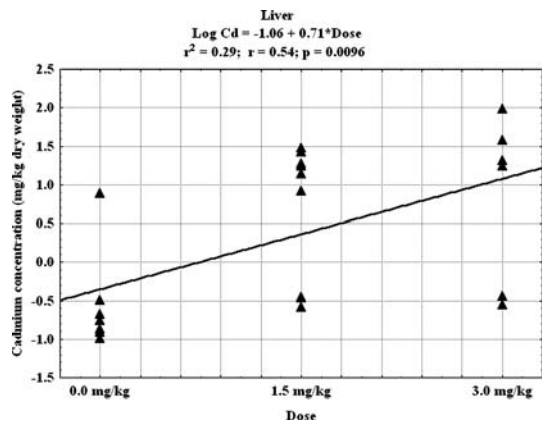

Figure 1. The relationship between the Cd dose (mg/kg body mass) and concentrations of cadmium (mg/kg dry weight) in the liver of bank voles. The regression line for y and x (solid line) is shown.

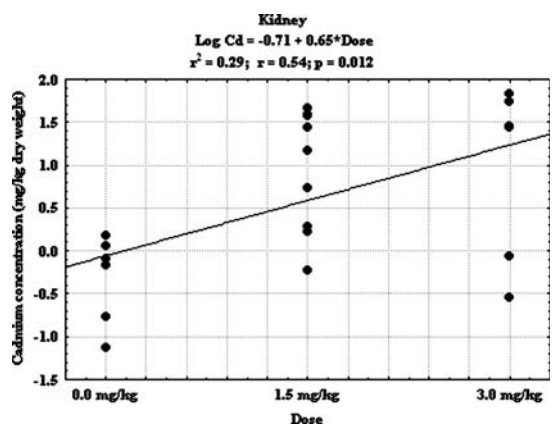

Figure 2. The relationship between the Cd dose (mg/kg body mass) and concentrations of cadmium (mg/kg dry weight) in the kidney of bank voles. The regression line for y and x (solid line) is shown.

during the first few days after exposure, the ratio between liver and kidney cadmium concentration was 13:1 when injected, and 3:1 when administered orally. Injected cadmium binds to albumin, plasma

protein and is transported first to the liver, which is the main detoxification organ. Because the post-exposure time was long in our experiment, (21 days), we did not expect such a big difference in cadmium accumulation between the studied tissues. Average cadmium concentrations in both tissues of animals from the high dose group were the same, suggesting that accumulation and elimination of metal occurs with the same intensity. The only difference in average cadmium levels between the studied tissues was obtained for animals treated with low dose of cadmium, 1.5 mg Cd/kg body mass. The average cadmium concentration in the kidney was 1.5 times higher than the amount in the liver of voles injected with this dose. It shows that cadmium may be intensively accumulated in this organ and still eliminated via the urine from the organism. In addition, reasonably high amounts of cadmium were still present in the liver and kidney of bank voles on the t 21st day after exposure, up to 29.4 mg/kg, suggesting that this metal has to be bound to a storage protein such as metallothionein and other low molecular proteins. This hypothesis is supported by results obtained by Rie *et al.* (2001), in an experiment on a painted turtle injected with cadmium chloride and by other authors discussing the role of metallothionein in metal detoxification (Chan & Cherian 1992; Roesijadi 1996).

The present data showed the differences between females and males in cadmium accumulation in the liver and kidney (Figures 3 and 4). Cadmium levels in both tissues were significantly dose dependent for females. The slow elimination of cadmium from the body by females resulted in a high mortality rate and body mass decrease.

Zinc and iron levels in the tissues

Zinc and iron are nutritionally essential elements present in all tissues, incorporated in many enzymes and proteins (Eisler 1993; Ballantyne *et al.* 1995). The body levels of both elements are modulated by homeostatic mechanisms. However, tissue concentrations may change after exposure to very high (toxic effect) or very low (deficiency) doses of zinc. About 20–30% of ingested zinc is absorbed. Liver is an organ which remains, after the prostate, the most enriched with zinc in rodents. In the present study, average concentrations of zinc in the livers of bank voles were

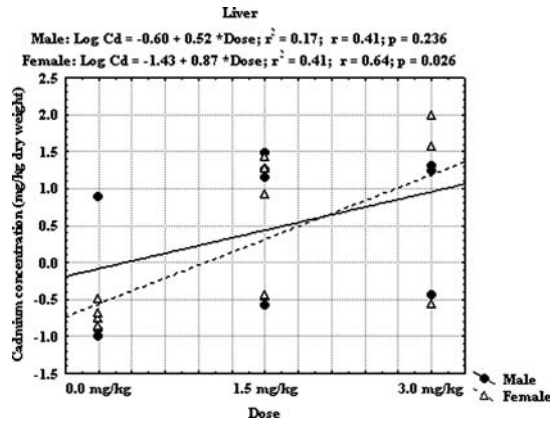

Figure 3. The relationship between the Cd dose (mg/kg body mass) and concentrations of cadmium (mg/kg dry weight) in the liver of male and female bank voles. The regression lines for male (solid line) and female (dashed line) are shown.

slightly lower than in the kidneys, ranging from 103 to 121 mg Zn/kg dry weight (Table 2). The kidney tissues contained up to 147 mg Zn/kg dry weight.

Iron absorption from intestine is lower than zinc, from about 2% to 15%. The highest amounts of iron are found in the liver and pancreas (Amdur *et al.* 1991). Our study showed that mean concentrations of iron in the livers of bank voles ranged from 869 to 2267 mg Fe/kg dry weight (Table 2). Lower levels of iron were found in the kidneys, up to 670 mg Fe/kg dry weight.

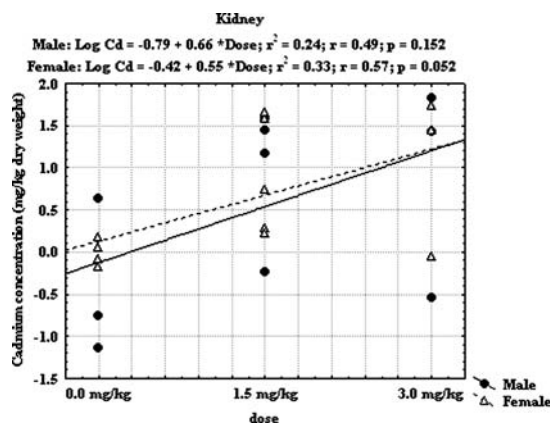

Figure 4. The relationship between the Cd dose (mg/kg body mass) and concentrations of cadmium (mg/kg dry weight) in the kidney of male and female bank voles. The regression lines for male (solid line) and female (dashed line) are shown.

Table 2. Average metal concentrations (mg/kg dry weight) in bank vole tissues on the 21st day of the experiment.

Metal	Tissue	Dose (mg Cd/kg)	*N*	*X*	SE	Range
Cd	Liver	0.0	6	0.2	0.03	0.1–0.3
		1.5	9	12.9	3.7	0.3–30.1
		3.0	6	28.5	14.5	0.3–95.4
	Kidney	0.0	6	0.7	0.2	0.1–1.5
		1.5	9	19.2	6.0	0.6–45.6
		3.0	6	29.4	11.1	0.3–66.7
Zn	Liver	0.0	6	103	3	90–108
		1.5	9	121	6	102–142
		3.0	6	121	10	92–156
	Kidney	0.0	6	131	6	106–146
		1.5	9	144	7	103–171
		3.0	6	147	22	45–194
Fe	Liver	0.0	6	2267	324	1104–3476
		1.5	9	1531	252	588–2719
		3.0	6	869	77	650–1127
	Kidney	0.0	6	535	50	376–686
		1.5	9	670	29	518–768
		3.0	6	449	93	75–737

Effects of cadmium on essential metals

Cadmium and zinc belong to the same group of IIB metals and have very similar chemical properties (Ballantyne *et al.* 1995; Peraza *et al.* 1998). Interactions between them occur as early as in an intestine during absorption, but more intensive interactions take place during accumulation in the tissues. Cadmium is absorbed in the duodenum and early jejunum, whereas zinc in the jejunum and ileum. Absorbed cadmium can replace zinc in enzymes, which leads to metabolic disturbances and, as a consequence, to various diseases. When a diet is poor in zinc, the toxicity from cadmium can be induced even at lower intake levels (Sato & Nagai 1989). Zinc application lowers the cadmium toxicity (Eisler 1997). Both metals are strong inducers of metallothionein and compete for the binding site in it. The model for coupled metallothionein induction by cadmium was described in detail by Roesijadi (1996).

The mechanisms of the interactions are discussed extensively, showing competitive and non-competitive relationships. In some papers, the authors showed a decrease in zinc levels in tissues after cadmium exposure (Suzuki *et al.* 1983; Peraza *et al.* 1998). In the case of data presented by Suzuki et al. (1983), the first reaction to cadmium exposure was a zinc increase followed by a decrease in the liver. As was mentioned before, the results obtained in our earlier studies on bank voles and shrews collected from cadmium and zinc contaminated sites, showed a clear increase in zinc levels, sometimes in both liver and kidney (Świergosz-Kowalewska *et al.* 2005, 2006). This positive relationship between cadmium and zinc in the tissues of wild animals was supported in our present studies on the bank vole. In this experiment, all animal groups were fed with the same standard food, which contained the same amount of zinc, approximately 140 mg/kg. The exposure to zinc was the same, but retention of zinc due to cadmium exposure was higher for cadmium dosed animals. The positive relationship between these metals was found in both studied tissues, albeit stronger in the liver than in the kidney (liver: $p < 0.01$; kidney: $p = 0.035$) (Figures 5 and 6). Indirect induction of metallothionein production by cadmium through zinc replacement and release from ligands, follows an increase of binding sites not only for cadmium but also for zinc (Roesijadi 1996). This process can take place because cadmium has a higher affinity to ligands than zinc (Brzóska & Moniuszko-Jakoniuk 2001). Thus, stimulation of the metallothionein synthesis explains the high accumulation of zinc in the tissues of cadmium-dosed bank voles.

It seems that stronger competition occurs between cadmium and iron than between cadmium and zinc, during metals intake and accumulation in tissues (Schümann *et al.* 1996). Absorption of

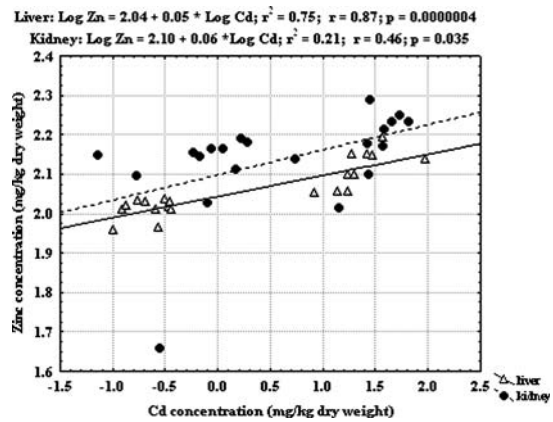

Figure 5. The relationship between the Cd and Zn concentrations (mg/kg dry weight) in the liver and kidney's of bank voles. The regression lines for the liver (solid line) and kidney (dashed line) are shown.

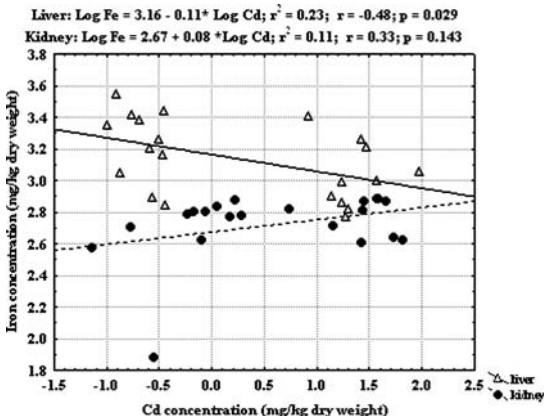

Figure 6. The relationship between the Cd and Fe concentrations (mg/kg dry weight) in the liver and kidney's of bank voles. The regression lines for liver (solid line) and kidney (dashed line) are shown.

iron takes place in the same section of the intestine as cadmium (Hamilton & Valberg 1974; Suzuki *et al*. 1983). They compete for the same proteins, ferritin and transferrin. In physiological conditions, both proteins are responsible for iron intake and transfer within the body. Cadmium, through binding to ferritin in the intestinal mucosa, lowers iron intake and results in decreased iron levels in tissues, especially in the liver. Transferrin, which is a donor of iron for hemoglobin synthesis, can bind different ions, including cadmium. In the present study, the negative cadmium effect on iron tissue concentration was observed in the liver of bank voles ($p = 0.029$) (Figure 6). Cadmium could not lower the intestinal absorption because the animals were exposed to cadmium by injection. The only explanation for the observed iron decrease is that cadmium replaced iron in iron binding molecules and it was excreted from the organism. Kidney iron was not affected by cadmium. As we know from other studies, the indirect effect of cadmium toxicity is to lower concentrations of iron especially in the liver, which results in anemia and induction of lipid peroxidation (Blazka & Shaikh 1992).

We can conclude that cadmium accumulated in the tissues of bank voles in a dose-depended manner. The animals exposed to cadmium also demonstrated differences between sexes. Males were less sensitive to cadmium toxicity than females, which displayed high mortality and a decrease in body mass. Zinc concentrations in the both the liver and kidney of animals, were positively correlated with tissue cadmium content. Depletion of iron levels was characteristic for the liver of bank voles. The study confirmed that even low doses of cadmium can affect the nutritional status of an organism.

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