BioMetals **17:** 1–14, 2004.

Low iron diet and parenteral cadmium exposure in pregnant rats: the effects on trace elements and fetal viability

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Received 5 September 2002; accepted 21 November 2002; Published online: June 2003

Key words: cadmium, fetus, iron deficiency, liver, zinc

Abstract

The effects of latent iron deficiency combined with parenteral subchronic or acute cadmium exposure during pregnancy on maternal and fetal tissue distribution of cadmium, iron and zinc, and on fetal viability were evaluated. Timed-pregnant Sprague-Dawley rats were fed on semisynthetic test diets with either high iron (240 mg kg) or low iron (10 mg kg), and concomitantly exposed to 0, 3 or 5 mg cadmium (as anhydrous $CdCl_2$) per kilogram body weight. Animals were exposed to cadmium from gestation day 1 through 19 by subcutaneously implanted mini pumps (Subchronic exposure) or on gestation day 15 by a single subcutaneous injection (Acute exposure). All rats were killed on gestation day 19. Blood samples, selected organs and fetuses were removed and prepared for element analyses by atomic absorption spectrometry. Low iron diet caused decreases in maternal body weight, maternal and fetal liver weights, placental weights and tissue iron concentrations. By cadmium exposure, both subchronic and acute, tissue cadmium concentrations were increased and the increase was dose-related, maternal liver and kidney zinc concentrations were increased, and fetal zinc concentration was decreased. Cadmium concentration in maternal liver was additionally increased by low iron diet. Acute cadmium exposure caused lower maternal body and organ weights, high fetal mortality, and decreased fetal weights of survivors. In conclusion, parenteral cadmium exposure during pregnancy causes perturbations in essential elements in maternal and fetal compartments. Acute cadmium exposure in the last trimester of gestation poses a risk for fetal viability especially when combined with low iron in maternal diet.

Introduction

Cadmium is a toxic metal with ability to accumulate into body during lifetime. Aside from occupational exposure, general population is chronically exposed to low level of cadmium from food and drinking water. Another important sources are parenteral exposures to cadmium inhaled from the main stream in active cigarette smokers, and from the environmental tobacco smoke in both smokers and non-smokers (Blanuša *et al.* 1991; Järup *et al.* 1998; Bhattacharyya *et al.* 2000; Nordberg *et al.* 2000; Piasek *et al.* 2001).

Iron is essential for life and toxic in excess. Iron deficiency, latent and overt, continues to be one of the most prevalent single-nutrient deficiencies affecting approximately 20% of the human populations in both developed and developing countries (Schümann *et al.* 1998; Monsen 1999; Beard 2000, 2001). In long term, iron status is fundamentally dependent on iron absorption and genes encoding proteins that are necessary for iron metabolism (DiDonato & Sarker 2000). Regulatory mechanisms are developed for efficient transport of iron into cells. A divalent metal transporter (DMT1), the first mammalian transmembrane iron transporter, a key factor in the uptake of

dietary iron, has been recently discovered (Andrews *et al.* 1999). Most recent findings indicate that DMT1 is a nonspecific metal transporter, which can also mediate the uptake of cadmium and probably other toxic metals into human absorptive enterocytes (Tallkvist *et al.* 2001; Park *et al.* 2002).

Intake of dietary iron depends on biological bioavailability and physiological demands and loss. Women are population groups with higher iron demands during their reproductive years and increased blood loses caused by menstruation and/or pregnancy. In small infants, iron demand is increased by rapid growth, which increases the intestinal iron transfer. In both groups iron deficiency is favored by a diet which contains insufficient or low available iron. The major liabilities of iron deficiency include defects in psychomotor development in infants, impaired educational performance in school children, increased perinatal morbidity, depressed immunobiological response, and impaired work ability (*e.g.*, Cook 1999; Beard 2000, 2001).

Iron and zinc are most bioavailabe from many of the same foods and their absorption is inhibited by many of the same dietary substances, such as phytate, certain dietary fibers and calcium. Deficiencies of iron and zinc are common, they can occur simultaneously, and even mild-to-moderate deficiencies impair neuropsychologic function (Sandstead 2000).

Rat has been widely accepted as a model for studying iron deficiency because it mirrors iron metabolism in man in most respects. In experimental animals, iron deficiency has been induced by means of a semisynthetic diet and a scrupulous elimination of all iron containing materials from the feed and/or by bleeding (Hamilton & Valberg 1974; Valberg *et al.* 1976).

The concentration of iron in the diet is known to have impact on the absorption of cadmium and its disposition in organs and tissues. Population groups with reduced body stores of iron, zinc and/or calcium absorb more cadmium than those with replete stores of iron and other essential elements. As a result, these populations seem to be more susceptible to adverse health effects of cadmium than those on more nutritious diets and with similar cadmium intakes (Berglund *et al.* 1994; Järup *et al.* 1998; Reeves & Chaney 2001). Animal experiments have shown that under iron deficiency and concomitant oral cadmium exposure, amount of duodenal mucosal cadmium, transfer of cadmium from the intestinal mucosa to the body, and the proportion of absorbed cadmium deposited in the liver and kidneys are increased (Hamilton & Valberg 1974; Ragan 1977; Flanagan *et al.* 1978; Valberg *et al.* 1976; Kollmer & Berg 1989; Blanuša *et al.* 1993; Tandon *et al.* 1993, 1994; Reeves & Chaney 2001; Park *et al.* 2002).

Women generally show higher concentrations of cadmium in the blood, urine and kidney than men, especially cigarette smokers or those habitually eating a diet rich in cadmium (shellfish, high-fiber meals). Higher risk of cadmium toxicity can be expected during their reproductive years due to 'physiological' deficiency of iron and/or other essential elements. This can be explained by higher absorption rate of cadmium due to lower-to-empty iron body stores during reproductive age (Flanagan *et al.* 1978; Berglund *et al.* 1994; Järup *et al.* 1998; Akesson *et al.* 2002). The findings of increased cadmium body burden in women mostly acquired during reproductive age when they are prone to essential element (calcium, iron, zinc) deficiencies contributed to elucidate the etiopathogenetic mechanisms of high incidence of osteomalacia in Japanese women with the history of multiple pregnancies, so called Itai-itai disease (Tschuchiya 1978; Bhattacharyya *et al.* 2000).

It has been proved by both human and animal studied that very little cadmium crosses the placenta to the fetus as it is actually accumulated in the placental tissue. Cadmium in the placenta may interact with essential elements and placental capacity to transfer iron and other nutrients may be inhibited, which can lead to fetal and neonatal iron deficiency and anemia after birth (Carmichael *et al.* 1982; Goyer 1995; Piasek *et al.* 1996).

Mineral supplementation has protective effect against cadmium accumulation and toxicity in rats fed inorganic cadmium salt (Groten *et al.* 1991, 1992; Matek Sarić *et al.* 2002). Groten *et al.* (1991) found that the protective effect of combined mineral supplementation was mainly due to the presence of the ferrous ion (Fe^{2+}). When compared to the other essential elements, calcium/phosphorus and zinc, Fe^{2+} protected most effectively against intestinal absorption of cadmium from cadmium-metallothionein, decreasing cadmium uptake by 80% (Groten *et al.* 1992).

On the other hand, intestinal absorption of various metals, such as cadmium, zinc, cobalt, manganese and copper, can impair intestinal iron absorption and increase iron deficiency in both man and animals. Oral cadmium exposure (such as by contaminated foodstuffs or industrial wastes) may reduce availability of dietary iron for absorption. Investigations on experimental animals have shown that iron absorption

is strongly inhibited by cadmium, and oral cadmium causes anemia even under sufficient iron in the diet (Hamilton & Valberg 1974; Sansi & Pond 1974; Valberg & Hamilton 1976; Petering *et al.* 1979; Radi & Pond 1979; Sugawara *et al.* 1984, 1988; Schäfer & Forth 1984; Schäfer & Elsenhans 1985; Schäfer *et al.* 1990; Sugawara & Sugawara 1991; Oishi *et al.* 2000). Iron and cadmium most probably compete at the binding sites of the iron intestinal transfer system (Schäfer & Forth 1984;. Bhattacharyya *et al.* 2000; Tallkvist *et al.* 2001; Park *et al.* 2002). It was recently found in growing rats that cadmium inhibits iron absorption only at low to normal levels of dietary iron, and at high levels of cadmium intake, iron and cadmium are largely absorbed by other, non-competitive mechanisms (Crowe & Morgan 1997).

If concentrations of daily cadmium intake continue to rise, population groups with low iron reserves, first of all women of reproductive age and growing children, will be at higher risk for toxic cadmium effects than those with replete iron stores. There are generally insufficient data on the nutritional iron deficiency and concomitant cadmium exposure by either oral or parenteral route of exposure (*e.g.*, through cigarette smoke) during perinatal period that involves expectant mother and both unborn and newborn baby. The aim of this investigation was to evaluate in animal models the effects of combined latent nutritional iron deficiency and parenteral cadmium exposure during pregnancy on cadmium, iron and zinc distribution in maternal and fetal organs as well as on fetal viability. We compared these biomarkers after continuous (subchronic) *versus* single (acute) cadmium exposure to the same cadmium doses in pregnancy.

Materials and methods

Animals

Adult cycling Sprague-Dawley female rats (Charles River Breeding Laboratories, Raleigh, NC), 60 day-old, and weighing 200–300 g at arrival were used. Animals were housed two per cage in clear plastic cages (20 × 25 × 47 cm) with laboratory grade heat-treated pine shavings as bedding and maintained under controlled temperature (20 °C), humidity (40–60%), and light (12:12 h light:dark cycle; lights out at 6:00 p.m. eastern time). The rats were allowed free access to the research diets and tap water.

All required principles of laboratory animal care and use according to the institutional research guidelines on the protection of animal welfare were followed.

Diets

Semisynthetic pelleted research diets Teklad (TD, Madison, WI, USA) were used. Feed contained either 240 mg iron (Fe) kg (TD 93273) (*High Fe diet* group) or 10 mg Fe kg (TD 80396) (*Low Fe diet* group). At the beginning of the investigation, animals were weighed, ranked, and assigned to one of the two dietary groups.

Estrous cycles synchronization

After two-weeks on different feeding regime, to obtain timed-pregnant rats, the animals were synchronized in the estrous cycles by subcutaneous (s.c.) injection of luteinizing hormone-releasing factor (LHRH) agonist (DES-Gly^{10}, D-Ala^{6} Pro N HET^{9}, Sigma Chemical Co., No. l-4513). A single s.c. 80 μg dose of LHRH in 0.1 ml per rat was administered according to the method described earlier (Cukierski *et al.* 1991). Approximately 110 h after injection, the females were let to mate over night. Next morning sperm positive (pregnant) females were detected by vaginal smear cytology. Day of sperm positive finding was assigned as gestation day 1.

Cadmium exposure

Part of the animals fed on either high or low iron diet (as described above) was concomitantly exposed to cadmium during pregnancy. Anhydrous cadmium chloride ($CdCl_2$, Fisher Scientific Co., NJ 07410, USA, No. C10) dissolved in 0.9% NaCl (physiological saline) solution (USP, sterile diluent, Abbott Laboratories, North Chicago, IL 60064, USA, No. NDC 0074-4888-50) was used. Doses of cadmium (Cd) were: 0 (control, administered physiological saline), 3 mg Cd kg body wt or 5 mg Cd kg body wt. Cadmium solutions in either dose were administered parenterally (subchronically or acutely) during pregnancy.

Experimental design

Two experiments were performed with the same feeding regime and different exposure duration to one of the three cadmium doses during pregnancy.

Subchronic cadmium exposure. In the first experiment, pregnant rats fed on either high or low iron diet were exposed to cadmium from gestation day

1 through 19. Cadmium was administered by surgically sc. implanted osmotic mini pumps (MP 2ML4 ALZET® , ALZA Co., Palo Alto, CA, USA) in the intrascapular region of each rat under short-term light anesthesia (with intraperitoneally administered 50 mg kg body wt of sodium pentobarbital). Nominal performance of used pumps (at 37 °C) were: reservoir volume 2.0 ml, maximal exposure duration 28 days, constant pumping rate of the compound 2.5 μg h. Producer's instructions for filling the pumps were strictly followed to obtain the exposure to the total dose of 0 (control), 3 or 5 mg Cd kg body wt over 19 days of pregnancy.

Acute cadmium exposure. In the second experiment pregnant rats fed on either high iron or low iron diets were administered 0, 3 or 5 mg Cd kg body wt cadmium on gestation day 15 by a single sc. injection.

In both experiments a sample size was at least six animals in each group. Body weights were recorded on experiment day 0 (beginning of the feeding regime with different iron content), on experiment day 15 (gestation day 1), and last day of each experiment, that is, on gestation day 19. On the termination of each experiment, rats were anesthetized in CO_2 (Piasek & Laskey 1994), exsanguinated by cardiac puncture, and blood and selected organs were removed. Following tissues were taken for trace element analyses: blood (3–5 ml), liver (three parts; one from each lobe, in total 1–2 g), right kidney, ovaries (composite samples of all left ovaries from each group), placentas (composite samples of 4–6 maternal and fetal placental portions from both uterine horns to get minimum 0.5 g tissue) and whole fetuses (two samples per mother rat, one from each end of the uterine horns). In the first experiment (*Subchronic cadmium exposure*) elements were also analyzed in the fetal liver (composite samples from 4–6 fetuses taken from both uterine horns), and adrenal glands were removed and their fresh weights were recorded as an indicator of potential surgically-induced stress.

Trace element analysis

Fresh (wet) weights of whole organs were recorded. Tissue samples (except blood) were blotted on a filter paper, homogenized in 1–2 ml deionized water and dried at 105 °C. Samples were then dry ashed at 450 °C in a muffle furnace (Blanuša & Breški 1981) in quartz glass crucibles and dissolved in 2% nitric acid. Concentrations of cadmium in maternal liver and iron and zinc in all tissues were analyzed by flame atomic absorption spectrometry (F-AAS) (Varian, AA375, Australia). Cadmium in all other samples was analyzed by electrothermal atomic absorption spectrometry (ET-AAS) (Varian SpectrAA 300A, Australia) with a graphite tube atomizer (GTA 96). Deuterium background correction was applied in all measurements. The limit of detection by F-AAS was 0.012 μg g wet wt for cadmium 0.15 μg g wet wt for iron and 0.04 μg g wet wt for zinc, and by ET-AAS it was 0.08 ng g wet wt for cadmium. Tissue cadmium values in the control groups were close to respective detection limit, they could not be detected with accuracy, and therefore these findings have not been shown in the paper.

Required recommendations on tissue collection, sample preparation and quality assurance for applied analytical techniques for metal analyses in biological samples were strictly followed (Piasek & Laskey 1994; Piasek *et al.* 2001; Matek Sarić *et al.* 2002). The accuracy and precision of element analysis were tested by means of certified standard reference material (SRM). Bovine Liver SRM 1577b (from National Institute of Standards and Technology, USA) was prepared and analyzed by the same procedure as the samples from the experiments. For cadmium, iron and zinc in Bovine Liver SRM analyzed values were 0.55, 184 and 127 μg g (certified values are 0.50, 184 and 127), respectively.

Statistical analysis

Data were analyzed using both one-way and two-way analyses of the variance (ANOVA) in the general linear model procedure (PROC GLM) by The Statistical Analysis System (SAS, Cary, NC, USA). Within each dietary group (High Fe group and Low Fe group), differences were analyzed among 0, 3 and 5 mg Cd kg exposed groups, and for tissue cadmium concentrations only between 3 and 5 mg Cd kg exposed groups. Where the data appeared to have a linear trend, *post hoc* PROC GLM was used to test for linearity. Two main treatment effects were evaluated for each biomarker: main effect of iron (Fe) and main effect of cadmium (Cd). Potential Fe $\times$ Cd interactions were also determined. In all analyzes the least significant difference method was used to separate means at the level of significance $P < 0.05$.

Results

General effects

At autopsy on gestation day 19 (end of each experiment), in the rats fed on low iron diet were noticed pale livers, ovaries, placentas and fetuses comparing to the same tissues in rats fed on high iron diet and irrespective of cadmium exposure.

In the first experiment (*Subchronic cadmium exposure*), during the course of the experiment in three rats (all fed on low iron diet and exposed to 0, 3 and 5 mg Cd kg, respectively) osmotic pumps were partly extruded. The pumps were surgically re-fixed and skin re-sutured. After that, in one animal (on low iron, 0 mg Cd kg) the pump remained still partially exposed, and one rat (on low iron, 3 mg Cd kg) eventually died.

In the second experiment (*Acute cadmium exposure*), by acute cadmium exposure to 5 mg Cd kg on gestation day 15, at autopsy four days later, almost all placentas were destroyed and fetuses died in both dietary groups. Therefore element analyses and statistical evaluations of biomarkers in placental and fetal tissues at acute dose of 5 mg Cd kg were hardly possible (as indicated in the tables by lower number of samples or no samples at all).

Body and fresh organ weights

Subchronic cadmium exposure. There was no effect of cadmium exposure during 19 days of pregnancy on maternal body weights (Table 1, upper part). Mean body weights in the rats fed on low iron diet were significantly lower than in rats fed on high iron diet (indicated by main iron effect on whole body weight marked with 'a'). There was no effect of subchronic cadmium exposure on fresh organ weights. By low iron diet fresh weights of the maternal and fetal liver were decreased, and weight of maternal portion of the placenta was increased.

An interesting observation should be mentioned. In one mother rat (on high iron diet, exposed to 3 mg Cd kg) kidney, adrenal, ovary and uterine horn on the left side were missing. Consequently, wet weights of these organs on the right side were approximately two-fold higher (kidney 2.9 g, ovary 0.123 g, adrenal gland 0.105 g) than in other rats with 'normal' paired organs (kidney ∼1.3 g, ovary 0.06–0.07 g, adrenal gland 0.08 g). This 'side findings' had no relevance to the experimental results as the data were excluded from statistical analysis.

Acute cadmium exposure. Due to acute cadmium exposure, reduced maternal body weights and maternal liver weights, increased right kidney weights, and decreased ovarian and placental (fetal portion) weights were found (Table 1, lower part). The effect of cadmium on decreased ovarian weight was linear. Weights of remaining live fetuses at 3 mg Cd kg dose were significantly lower than in controls. Low iron diet was again (as in the first experiment) associated with significantly reduced maternal liver weight.

Tissue cadmium concentrations

Subchronic cadmium exposure. By continuous 19-day exposure during pregnancy, concentrations of cadmium were increased in all analyzed tissues. Most of the increases were significantly dose-related (Table 2, upper part, marked with asterisks). Low iron diet caused additional cadmium increase in the maternal liver (Table 2, main iron effect marked with 'a').

Acute cadmium exposure.
With exception of maternal kidney, single administrations of the same cadmium doses were associated with higher cadmium concentrations in measured tissue compartments than with continuous (subchronic) exposure to the same cadmium doses (Table 2). There was about a 10-fold increase in tissue cadmium in maternal portions of the placent as and five to 10-fold increase in fetal portions of the placent as in rats exposed to 3 mg Cd kg dose. At this cadmium dose, this increase in cadmium concentrations in the fetuses was about 30 times. After acute exposure, there was again main effect of iron in the diet on increase in cadmium concentration in maternal liver, as it was observed after continuous 19-day cadmium exposure (Table 2, lower part, marked with 'a').

Tissue iron concentrations

In both experiments, low iron diet caused decreased iron concentrations in all analyzed tissues of both maternal and fetal origin (Table 3, main iron effect marked with 'a').

Subchronic cadmium exposure. Continuous cadmium exposure during 19 days of pregnancy caused changes in fetal iron with increased values of iron concentration in fetal body and liver at both cadmium doses (3 and 5 mg kg).

Table 1. Average maternal and fetal body and organ weights on gestation day 19 in rats fed on diet with 240 mg iron (Fe)/kg feed (*High Fe diet*) or 10 mg Fe/kg feed (*Low Fe diet*) and exposed to cadmium (Cd) (3 or 5 mg Cd /kg body wt) either by sc. implanted osmotic mini pumps from gestation day 1–19 (*Subchronic Cd exposure*) or by a single sc. injection on gestation day 15 (*Acute Cd exposure*)

Group	N	Average weights (g)								
		Mother rats					Placenta		Festuses	
		Whole body	Liver	Right kidney	Adrenals	Ovaries	Maternal portion	Fetal portion	Weight per live fetus	Fetal liver
					Subchronic Cd exposure					
High Fe diet										
Control	16	381±11.4	19.3±0.622	1.28±0.026	0.079±0.003	0.137±0.004	0.112±0.006	0.172±0.007	1.36±0.029	0.114±0.004
Cd 3 mg/kg	8	398±6.46	19.9±0.269	(7) 1.32±0.037	(7) 0.079±0.001	(7) 0.139±0.006	0.123±0.005	0.187±0.009	1.37±0.034	0.119±0.005
Cd 5 mg/kg	7	402±10.6*	20.2±0.690	1.35±0.060	0.085±0.006	0.139±0.004	0.109±0.003	0.173±0.010	1.31±0.021	0.113±0.003
Low Fe diet										
Control	14	370±5.51	16.5±0.547	1.19±0.054	(13) 0.083±0.003	0.126±0.004	0.130±0.008	0.187±0.006	1.26±0.032	0.088±0.004
Cd 3 mg/kg	7	385±12.6	18.4±0.589	1.30±0.051	0.080±0.005	0.133±0.011	0.131±0.008	0.190±0.008	1.31±0.051	0.090±0.005
Cd 5 mg/kg	8	378±11.5	17.3±0.509	1.27±0.055	0.087±0.006	0.137±0.005	0.132±0.009	0.208±0.011	1.35±0.034	0.101±0.008
MAIN EFFECT		a	a				a			a
					Acute Cd exposure					
High Fe diet										
Control	6	419±15.5	21.5±0.854	1.41±0.027	n.d.	0.152±0.010	0.117±0.020	0.188±0.013	1.50±0.178	n.d.
Cd 3 mg/kg	8	406±11.7	21.4±0.901	1.44±0.061*	n.d.	0.129±0.007	(6) 0.102±0.011	(6) 0.153±0.017	(6) 1.40±0.081	n.d.
Cd 5 mg/kg	9	375±8.53*	19.1±1.34	1.48±0.052*	n.d.	0.121±0.008*	(1) 0.003	(1) 0.169	-§	n.d.
Low Fe diet										
Control	7	410±11.3	19.5±0.468	1.38±0.054	n.d.	0.141±0.006	0.138±0.010	0.205±0.009	1.51±0.153	n.d.
Cd 3 mg/kg	7	382±10.9*	18.8±1.08	1.46±0.068	n.d.	0.131±0.012	(3) 0.114±0.026	(3) 0.176±0.025	(4) 1.14±0.344	n.d.
Cd 5 mg/kg	8	369±9.67*	16.9±0.906	1.45±0.048*	n.d	0.110±0.006*	-§	-§	-§	n.d.
MAIN EFFECT		b	a,b	b		b,c		b	b	

Results are presented as means ± S.E.M. (N in brackets if different than shown in second column); n.d. – not determined.
§No data due to high fetal mortality at Cd 5 mg/kg dose. Significant differences (at $P < 0.05$ by ANOVA): *different from respective control value; [a]main effect of Fe; [b]main effect of Cd.

Table 2. Tissue cadmium concentrations on gestation day 19 in rats fed on diet with 240 mg iron (Fe)/kg feed (*High Fe diet*) or 10 mg Fe/kg feed (*Low Fe diet*) and exposed to cadmium (Cd) (3 or 5 mg Cd /kg body wt) either by sc. implanted osmotic mini pumps from gestation day 1-19 (*Subchronic Cd exposure*) or by a single sc. injection on gestation day 15 (*Acute Cd exposure*)

Group	N	Tissue cadnium concentrations (μg/g wet wt)							
		Mother rat				Placenta		Fetus	
		Blood	Liver	Kidney	Ovary[#]	Maternal portion	Fetal portion	Whole body	Fetal liver
					Subchronic Cd exposure				
High Fe diet									
Cd 3 mg/kg	8	0.159±0.060	12.2±0.685	39.2±3.93	1.01	0.321±0.043	0.704±0.132	0.001±0.0001	(6) 0.011±0.002
Cd 5 mg/kg	7	0.174±0.010	36.6±2.35*	85.0±2.70*	1.87	1.19 ±0.145*	2.74 ±0.302*	0.005±0.002	(7) 0.015±0.001
Low Fe diet									
Cd 3 mg/kg	7	0.145±0.069	16.0±3.89	47.5±5.12	0.663	0.381±0.055	0.829±0.133	0.001±0.0001	(6) 0.013±0.006
Cd 5 mg/kg	8	0.151±0.017	43.4±3.98*	80.0±7.40*	1.54	1.18 ±0.169*	±0.509*	0.007±0.002*	(7) 0.021±0.004
MAIN EFFECT			a,b	b		b	b	b	
					Acute Cd exposure				
High Fe diet									
Cd 3 mg/kg	8	(7) 0.174±0.022	33.2±0.781	19.5±0.933	2.60	3.54 ±0.115	7.18 ±0.746	0.034±0.009	n.d.
Cd 5 mg/kg	9	(8) 0.454±0.145	50.3±3.26	36.1±3.73*	2.29	-[§]	-[§]	(2) 0.059±0.023	n.d.
Low Fe diet									
Cd 3 mg/kg	7	0.183±0.025	38.8±2.29	19.4±1.52	3.86	3.21 ±0.016	5.31 ±0.350	(4) 0.028±0.002	n.d.
Cd 5 mg/kg	8	0.307±0.022	67.9±6.47*	40.0±3.48*	4.35	-[§]	-[§]	-[§]	n.d.
MAIN EFFECT		b	a,b	b					

Results are presented as means ± S.E.M. (N in brackets if different than shown in second column); n.d. – not determined.
[#]Pooled samples of left ovaries from each group;
[§]No data due to high fetal mortality at Cd 5 mg/kg dose.
Significant differences (at $P < 0.05$ by ANOVA): *difference between values at 3 and 5 mg Cd/kg dose; [a]main effect of Fe; [b]main effect of Cd.

Table 3. Tissue iron concentrations on gestation day 19 in rats fed on diet with 240 mg iron (Fe)/kg feed (*High Fe diet*) or 10 mg Fe/kg feed (*Low Fe diet*) and exposed to cadmium (Cd) (3 or 5 mg Cd/kg body wt) either by sc. implanted osmotic mini pumps from gestation day 1–19 (*Subchronic Cd exposure*) or by a single sc. injection on gestation day 15 (*Acute Cd exposure*)

Group	N	Tissue cadnium concentrations (μg/g wet wt)							
		Mother rat				Placenta		Fetus	
		Blood	Liver	Kidney	Ovary[#]	Maternal portion	Fetal portion	Whole body	Fetal liver
					Subchronic Cd exposure				
High Fe diet									
Control	16	629±29.7	191±12.4	89.1±3.95	59.5	86.7±5.01	(15) 121±3.56	50.8±1.93	164±11.5
Cd 3 mg/kg	8	616±39.1	197±13.9	92.3±9.76	n.d.	87.7±5.00	129±6.72	60.5±5.45*	203±6.67*
Cd 5 mg/kg	7	542±38.4	152±13.0	74.7±3.87*	88.8	79.1±3.38	126±7.25	66.5±4.13*	195±8.97*
Low Fe diet									
Control	14	475±15.2	53.3± 3.20	39.4±1.43	51.9	(13) 51.6±2.61	(13) 63.0±3.53	16.6±0.999	(13) 73.0±2.40
Cd 3 mg/kg	7	407±22.5	51.7± 8.03	34.7±1.65	54.6	57.0±4.19	63.5±3.61	19.0±4.28	(7) 80.1±2.09
Cd 5 mg/kg	8	432±14.8	51.5± 3.64	40.5±3.80	53.7	48.1±4.33	58.8±7.75	24.3±1.92	(7) 77.4±5.72
MAIN EFFECT		a	a	a		a	a	a,b	a,b
					Acute Cd exposure				
High Fe diet									
Control	6	600±33.0	162±12.9	97.7±2.45	59.0	71.2±4.12	101 ±6.33	65.8±3.15	n.d.
Cd 3 mg/kg	8	551± 29.1	155± 6.54	92.4±6.93	79.0	(5) 62.0±6.93	(5) 86.6±9.17	(6) 46.9±2.86*	n.d.
Cd 5 mg/kg	9	635±120	188±12.7	79.1±4.00*	90.0	-[§]	-[§]	(2) 33.0±2.00*	n.d.
Low Fe diet									
Control	7	464±20.0	40.6± 4.76	42.4±2.93	32.0	65.6±20.1	57.4±6.20	22.8±1.98	n.d.
Cd 3 mg/kg	7	452±19.6	48.4± 3.60	41.7±2.61	37.0	(2) 40.5± 6.50	(2) 44.5±6.50	(4) 26.1±4.88	n.d.
Cd 5 mg/kg	8	392±36.4	60.3± 7.67	41.1±4.14	49.0	-[§]	-[§]	-[§]	n.d.
MAIN EFFECT		a	a,b	a			a	a,b,c	

Results are presented as means ± S.E.M. (N in brackets if different than shown in second column); n.d. – not determined [#]Pooled samples of left ovaries from each group; [§]No data due to high fetal mortality at Cd 5 mg/kg dose. Significant differences (at $P<0.05$ by ANOVA): *different from respective control value; [a]main effect of Fe; [b]main effect of Cd.

Acute cadmium exposure.
Single cadmium administration increased iron concentration in the maternal liver (Table 3, lower part, marked with 'b'). In fetal body, iron concentration was significantly decreased and a significant Fe × Cd interaction was determined (Table 3, marked with 'c').

Tissue zinc concentrations

By both subchronic and acute cadmium exposures, concentrations of zinc in maternal liver and kidney were significantly increased (Table 4). Concentrations of zinc were decreased in fetal portion of the placenta, whole fetus and fetal liver after subchronic, and in whole fetus after acute maternal cadmium exposure.

Low iron diet in the first experiment (*Subchronic cadmium exposure*) was associated with significant increases in zinc concentration in both maternal and fetal liver. It caused decreases in zinc concentration in fetal portion of the placenta and whole fetus (Table 4, upper part).

Discussion

Exposure to cadmium via food and drinking water presents an important route in general population (Blanuša *et al.* 1991; Järup *et al.* 1998; Akesson *et al.* 2002). Gastrointestinal absorption of ingested cadmium in adults is rather low (about 1–6%). However, as it has been shown in humans, about 50% of the cadmium inhaled via cigarette smoke could be absorbed (Järup *et al.* 1998; Jin *et al.* 1998). Parenteral route via main-stream cigarette smoke and/or environmental, second-hand tobacco smoke present a route of increasing concern in general population, especially in its most vulnerable high at risk groups – women in reproductive age and growing children – for a variety of cadmium health effects. The majority of literature data on cadmium toxicokinetics and interaction of cadmium and iron (and other essential elements) so far have been obtained under condition of oral (enteral) route of cadmium exposure. Such data on parenteral route of cadmium exposure are lacking, and in a view of above mentioned facts, their relevance is undoubtful.

We found in our earlier investigation that parenteral exposure to the same cadmium doses (3 and 5 mg kg as a single s.c. injection) caused target organ cadmium accumulation with concomitant decreases in tissue iron and perturbations in ovarian steroidogenesis that were related to the reproductive stage (Piasek & Laskey 1994). Present investigation was aimed to give more insight in toxicokinetics and effects of cadmium in both maternal and fetal tissue compartments after parenteral route comparing biomarkers of subchronic and acute exposures. We had on mind that during pregnancy uptake and retention of metals (both essential and toxic) are increased due to specific physiological state with higher nutritional demands. Parenteral cadmium administration enabled us to maintain exact exposure levels under strictly controlled conditions during particular periods of gestation. By this route of exposure we avoided duodenal cadmium absorption and its interaction on intestinal level (competition with trace elements in the process of mucosal uptake), which has been mostly studied so far. We evaluated post-absorptive interactions of cadmium, iron and zinc in blood and other tissue compartments.

It has been shown that different routes and doses of cadmium intake influence the intestinal distribution of cadmium, metallothionein and trace metals differently. Elsenhans *et al.* (1994) reported that parenteral (s.c.) administration of cadmium did not change intestinal iron, zinc and copper concentrations. At the same time authors found that high dietary concentrations of cadmium increased mucosal metallothionein and cadmium concentrations longitudinally from duodenum to the ileum, while intestinal mucosal concentrations of iron decreased and of copper increased. These findings are consistent with other data suggesting that only non-metallothionein bound cadmium, luminally or superficially present in the intestine, can interfere with absorption of essential elements (Schäfer & Elsenhans 1985; Bremner 1987; Elsenhans *et al.* 1994). However, it is possible that cadmium, iron and other essential elements interact from the cadmium transfer site through the basolateral membrane of the intestinal mucosa. Sugawara & Sugawara (1991) found that even when cadmium was administered parenterally, there was a measurable cadmium concentration in the intestine. This finding speaks in favor that interactions of parenterally administered cadmium and essential elements are possible even on the intestinal level.

After intestinal absorption, an early burst of cadmium appears in blood where it behaves much like free cadmium ions injected parenterally (Bhattacharyya 2000). From this point on we can follow fate of cadmium in the body irrelevant of the route of exposure. Three major biochemical reactions involving cadmium in blood have been hypothesized: liver deposition (possibly from circulating

Table 4. Tissue zinc concentrations on gestation day 19 in rats fed on diet with 240 mg iron (Fe)/kg feed (*High Fe diet*) or 10 mg Fe/kg feed (*Low Fe diet*) and exposed to cadmium (Cd) (3 or 5 mg Cd/kg body wt) either by sc. implanted osmotic mini pumps from gestation day 1–19 (*Subchronic Cd exposure*) or by a single sc. injection on gestation day 15 (*Acute Cd exposure*)

Group	N	Tissue cadnium concentrations (μg/g wet wt)							
		Mother rat				Placenta		Fetus	
		Blood	Liver	Kidney	Ovary[#]	Maternal portion	Fetal portion	Whole body	Fetal liver
					Subchronic Cd exposure				
High Fe diet									
Control	16	5.97±0.110	29.1±0.476	24.8±0.563	11.7	17.3±0.543	(15) 15.8±0.294	16.0±0.383	43.1±1.42
Cd 3 mg/kg	8	6.31±0.202	39.5±0.710*	34.5±0.922*	35.2	17.6±0.518	16.1±0.203	11.9±0.525*	41.8±1.95
Cd 5 mg/kg	7	5.97±0.113	50.1±1.37*	32.3±1.35*	20.6	17.6±0.444	14.9±0.191	14.2±0.351*	36.4±0.632
Low Fe diet									
Control	14	5.46±0.113	32.9±1.68	24.2±0.834	17.0	(13) 17.2±0.247	(13) 15.8±0.620	14.1±0.612	51.3±1.24
Cd 3 mg/kg	7	5.19±0.278	45.6±6.14*	30.7±0.920*	19.1	17.6±0.637	15.2±0.350	11.2±0.802*	44.9±3.85*
Cd 5 mg/kg	8	5.11±0.154	59.2±3.52*	31.7±1.46*	18.4	16.5±0.724	13.2±0.658*	14.3±0.266	39.1±2.32*
MAIN EFFECT			a,b	b			a,b	a,b	a,b
					Acute Cd exposure				
High Fe diet								b	
Control	6	5.93±0.265	24.7±0.567	24.0±0.927	16.8	18.5±0.453	14.7±0.168	16.5±0.592	n.d.
Cd 3 mg/kg	8	5.88±0.426	48.9±0.863*	31.1±1.27*	21.8	(5) 17.4±0.546	(5) 16.4±0.698	(6) 13.5±1.08*	n.d.
Cd 5 mg/kg	9	7.18±1.48	54.2±3.67*	32.0±0.367*	21.5	-[§]	-[§]	(2) 12.7±1.13	n.d.
Low Fe diet									
Control	7	5.79±0.680	24.2±1.34	24.7±2.65	16.4	21.8±4.61	17.4±2.48	16.7±0.631	n.d.
Cd 3 mg/kg	7	5.58±0.082	54.6±1.87*	28.7±1.00*	19.4	(2) 16.9±0.310	(2) 16.3±0.997	(4) 13.5±0.915	n.d.
Cd 5 mg/kg	8	5.07±0.245	62.5±5.30*	33.1±0.997*	23.5	-[§]	-[§]	-[§]	n.d.
MAIN EFFECT			b	b				b	

Results are presented as means ± S.E.M. (N in brackets if different than shown in second column); n.d. – not determined.
[#]Pooled samples of left ovaries from each group;
[§]No data due to high fetal mortality at Cd 5 mg/kg dose.
Significant differences (at $P<0.05$ by ANOVA): *different from respective control value; [a]main effect of Fe; [b]main effect of Cd.

cadmium-albumin), kidney deposition from circulating cadmium-metallothionein and cadmium transfer to red blood cells. Other unidentified reactions are possible, as most of the cadmium that enters blood has already been deposited in tissues. After oral exposure, the small intestine, as a primary organ, may be more important in handling cadmium then the liver, and after parenteral administration, the kidney rapidly but not extensively takes up cadmium ions. Cadmium is generally taken by all segments of the proximal tubules (Dorian *et al.* 1995), and 80–90% goes to the liver. The liver plays a very active and important role in rapidly clearing cadmium ions from the blood (Crowe & Morgan 1997; Jin *et al.* 1998; Bhattacharyya 2000). This mechanism is considered to serve as an important protective pathway for the body, sparing organs such as kidney and bone from continued exposure to cadmium. During pregnancy, in cadmium-injected rats, hepatic cadmium could be mobilized and transferred to the kidney and placenta by an increase in plasma cadmium-metallothionein (Chan & Cherian 1993). Thus pregnancy may induce a high risk of cadmium nephrotoxic effects in women with previous chronic cadmium exposure, similar to Itai-itai disease cases in Japan (Tschuchiya 1978; Bhattacharyya 2000).

Cadmium accumulation and disposition under our experimental conditions generally followed the pathways described above. Concentrations of parenterally administered cadmium in tissues were ordered: kidney>liver>fetal placenta>maternal placenta> blood>>fetal liver>whole fetus by subchronic exposure, and liver>kidney>fetal placenta>maternal placenta>blood>>whole fetus by acute exposure of cadmium. Cadmium accumulated in highest proportion in the kidney after longer (subchronic) and in the liver followed by the kidney after short-term (acute) exposure that is consistent with the data reported in the literature (*e.g.*, Crowe & Morgan 1997; Jin *et al.* 1998; Bhattacharyya 2000). Of the reproductive tissues, fetal part of the placenta accumulated more cadmium than the maternal portion, and fetal liver and whole fetus had least cadmium concentrations, as has been observed in earlier investigations (*e.g.*, Sonawane *et al.* 1975; Ahokas & Dilts 1979; Piasek & Laskey 1994; Trottier *et al.* 2002).

In our investigation cadmium accumulation and distribution were evaluated under condition of latent iron deficiency. Low iron in diet was associated with significant and dose-related increase in cadmium concentration in the maternal liver. This finding indicates that parenterally administered cadmium interacts with dietary iron on the level of cadmium accumulation in the liver. Other investigators have also found increased cadmium body burden mostly in the liver of cadmium-exposed human and animal females associated with iron deficiency (Hamilton & Valberg 1974; Ragan 1977; Flanagan *et al.* 1978; Kollmer & Berg 1989; Schäfer *et al.* 1990; Tandon *et al.* 1993, 1994).

Iron metabolism is influenced differentially by different administration route of cadmium. In chronically cadmium-exposed male mice, Sugawara *et al.* (1984) found that concentration of iron in the liver was decreased after oral cadmium administration (up to 200 mg Cd l for 45 days in drinking water). After parenteral cadmium exposure (to 1 mg Cd kg week during 7 weeks by s.c. injection), liver iron was increased. Only in the rats parenterally exposed to cadmium, ceruloplasmin activity was stimulated with concomitant increase in both hepatic copper and iron. Fetal liver accumulation of iron has also been found in rats after maternal low-protein diet during pregnancy (Naismith & Binns 1978). Ashby *et al.* (1980) also found that liver iron tended to increase with parenteral cadmium dosage. In a recent paper Oishi *et al.* (2000) reported the differences in hepatic iron related to the route of cadmium administration in female rats. They found decreased iron concentrations after oral and not affected iron concentrations in the liver after parenteral (intravenous) cadmium exposure. Our results on effect of parenteral cadmium exposure on tissue iron concentrations corroborate the above-presented data.

Iron and zinc compete during intestinal absorption, but their post-absorptive interactions are less clear (Lönnerdal 2000; Donangelo *et al.* 2002). Iron can have a negative effect on zinc absorption if given together in a supplement, and no effect was observed when the same amounts are present in a meal as fortificants. Recently Donangelo *et al.* (2002) reported that the use of iron supplements in women with marginal iron status improves iron indices with no effect on zinc concentrations. They also found that modest use of zinc supplement improves zinc indices but also appears to induce a cellular iron deficiency and may further reduce iron status. Effects of dietary iron on tissue zinc concentrations were found in our investigation (in the first experiment) in both maternal and fetal compartments.

Interactions of cadmium and zinc have been widely studied in experimental animals under condition of oral ingestion of cadmium. It has been shown that cadmium may inhibit zinc activities at many stages,

interfering with its absorption, distribution to different tissues, transport into cells and/or transport into several intracellular structures (Petering *et al.* 1979; Bin & Garfinkel 1994; Oishi *et al.* 2000; Lönnerdal 2001; Satarug *et al.* 2001). Summarizing data on cadmium-zinc interactions in the organism, based mainly on the experimental data from literature, Brzóska & Moniuszko-Jakoniuk (2001) concluded that these interactions were found even at exposure levels and tissue concentrations corresponding to environmental and occupational exposure in humans. Interactions of cadmium and zinc in *postmortem* human tissue samples were evaluated only in a few studies. Blanuša *et al.* (1985) found significantly higher concentrations of cadmium and zinc in human kidney cortex in cigarette smokers (including previous smokers) than in non-smokers (mean ± SD of cadmium and zinc concentrations in smokers were 225±87 and 328±65, and in non-smokers 74±33 and 234±48 μg g dry tissue wt). Recently Satarug *et al.* (2001) assessed changes in zinc and copper concentrations in tissues of human livers, kidneys and lung associated with environmental exposures to cadmium. The results indicate increased sequestration of zinc and copper in the liver having more than 1 μg g wet tissue wt of cadmium, and in kidney cortex having more than 26 μg g wet tissue wt of cadmium.

Results from our earlier investigations and other authors' data in the pregnant rats exposed to cadmium *per os* show cadmium accumulation and zinc retention in maternal organs accompanied by zinc deprivation in fetal and/or pup organs and impaired fetal growth (Sorell & Graziano 1990; Piasek *et al.* 1996; Whelton *et al.* 1997; Brzóska & Moniuszko-Jakoniuk 2001). Cadmium-induced increase of zinc concentrations in maternal liver and kidneys is possibly due to metallothionein induction, and the effect of maternal cadmium exposure on fetal and neonatal weight may be due to fetal cadmium accumulation, fetal zinc deprivation, or both. Present results on disposition of zinc in maternal and fetal tissue compartments after parenteral cadmium exposure corroborate the above findings in cadmium-exposed humans and animals. Ashby *et al.* (1980) found that parenteral acute administration of cadmium to male rats resulted in increased liver zinc concentrations directly in response to cadmium administration at all applied doses (0.1–1.5 mg kg s.c.). Oishi *et al.* (2000) observed elevated cadmium and zinc concentrations in the liver and kidneys of female rats regardless of cadmium dose and route of exposure.

In conclusion, our results show that parenteral route of cadmium exposure during pregnancy causes perturbations in essential trace elements in both maternal and fetal organs. Acute cadmium exposure poses a risk for fetal viability in the last trimester of gestation, especially when combined with low iron in maternal diet.

Acknowledgements

The major part of the investigations was conducted in, at that time, Health Effects Research Laboratory of the United States Environmental Protection Agency in Research Triangle Park, North Carolina, USA, during the fellowships of Martina Piasek sponsored by NIH Fogarty International Center, Bethesda, Maryland, USA. Our special thanks are due to Ms Janet M. Ferrell (US laboratory) for her skilled technical assistance and to Ms Đurđa Breški and Ms Marija Ciganović (Croatian laboratory) for the expert metal analyses.

The Croatian Ministry of Science and Technology in part financially supported the research (grant No. 00220102).

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