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The effect of dietary selenium supplementation on cadmium absorption and retention in suckling rats

Maja Lazarus · Tatjana Orct · Jasna Jurasoviæ · Maja Blanuša

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Abstract Selenium (Se) reduces cadmium (Cd) toxicity in adult animals, but its effects in newborn animals are still unknown. This study investigated Cd (as $CdCl_2$) absorption, distribution, and retention in suckling rats receiving oral Se supplementation (as Na_2SeO_3) in equimolar doses (8 μmol Cd and/or Se per kg b.w./day). Selenium was given either before and during Cd exposure (Se_{pre} + Cd group; pre-treatment group) or only during Cd exposure (Se + Cd group). Rats were treated from postnatal day (PND) 6–14 as follows: controls (H_2O, PND 6–14), Se (PND 10–14), Cd (PND 10–14), Se_{pre} + Cd (Se PND 6–14 + Cd PND 10–14) and Se + Cd (Se + Cd PND 10–14). Selenium supplementation, especially pre-treatment, decreased Cd levels in the blood, brain, liver and kidney of suckling rats. Selenium levels in plasma, brain, and kidney also decreased. These findings suggest that higher Se intake could efficiently reduce Cd retention during the suckling period.

Keywords Cadmium · Selenium · Interaction · Suckling rat

M. Lazarus (✉) · T. Orct · J. Jurasoviæ · M. Blanuša
Institute for Medical Research and Occupational Health, Zagreb, Croatia
e-mail: mlazarus@imi.hr

Introduction

Infant development is recognised as highly susceptible to environmental hazards with even subtle changes in specific organs and tissues causing life-long functional deficits and increased susceptibility to disease (WHO 1986; Grandjean et al. 2008). Although low, transplacental transfer of Cd is reported from many animal experiments (Sonawane et al. 1975; Dési et al. 1998). However, much greater portion of maternal burden is transferred during lactation (Whelton et al. 1993). Similar to Cd retention by the placenta, mammary tissue accumulates Cd during lactation (Bhattacharyya et al. 1986), thus disturbing fatty acid composition in milk and liver of lactating rats and in the brain of their suckling pups (Petersson Grawé et al. 2004a). In newborns exposed to Cd via maternal milk Pillet et al. (2005) found sex-specific delays in the development of female animals and changes in the immune function, even in the absence of measurable Cd accumulation in the kidneys. Since a low percentage of mothers exclusively breast-feed their children beyond 6 months (WHO 2003), Cd from additional nutrition, e.g. infant formulas (Eklund and Oskarsson 1999) and from oral exploration and hand-to-mouth activity will contribute much more to the ingested burden than Cd from milk (WHO 1986). Consequently, more pronounced adverse effects can be expected. The physiological state, nutritional status and specific nutrient deficiencies (Fe, Ca) can also significantly influence the response to toxic substance (WHO 2001; JECFA

2001). Developing brain is particularly sensitive to Cd (Nagymajtényi et al. 1997; Dési et al. 1998; Antonio et al. 2003) that crosses immature blood-brain barrier acting as an endogenous ligand (Aschner and Kerper 2000). Even low-dose Cd exposure during lactation resulted in altered serotonin concentrations in the brain and increased motor activity of pups (Andersson et al. 1997; Petersson Grawé et al. 2004b).

Animal exposure to Cd from drinking water was often used as a model of human exposure to Cd (Leret et al. 2003; Brzóska and Moniuszko-Jakoniuk 2004, 2005; Jihen et al. 2008). Because Cd absorption in the gastrointestinal tract of rats is markedly lower than in humans, rat models simulating human exposure need to increase exposure doses to be higher than the real daily human intake of Cd (WHO 1992; Rogalska et al. 2009). To investigate the effects of Cd in suckling rats and avoid the limitations of the rat model of exposure through maternal milk, we fed the pups artificially (Kostial et al. 1971; Matek Sarić et al. 2002). We did that to avoid exposure of dams to doses that could have toxic effects, and which would be needed to adjust for the limited transfer of Cd and selenium (Se) to milk (Bhattacharyya 1983; Andersson et al. 1997; Dorea 2002) and for detection limits of instruments and methods used for measuring metals. By feeding Se and Cd solutions to suckling rats in between suckling, we had a better control of Se and Cd intake and their absorption coincided with the absorption of suckled milk.

Higher gastrointestinal uptake of Cd in newborns on milk diet than in the weaning animals (Kostial et al. 1978; Eklund et al. 2001, 2004) prolongs Cd transport to the systemic circulation. Known adverse effects of Cd at such an early age prompted studies on trace element additives (Fe, Zn, Mn, Cu, Ca) to decrease Cd absorption in suckling rats (Kostial et al. 1980; Matek Sarić et al. 2002; Öhrvik et al. 2007). The interaction between Cd and Se is well-documented in studies with adult animals, although the mechanisms underlying this interaction and detoxification have not yet been entirely clarified. Cadmium is proved to form an equimolar complex with Se in the form of selenide in plasma, which then binds to selenoprotein P, and as a high molecular-weight protein complex changes the metabolism of Cd (Sasakura and Suzuki 1998). Furthermore, according to some authors, Se seems to reduce oxidative stress in tissue caused by Cd (Ulusu et al. 2003; Santos et al. 2005; Newairy et al. 2007). Following chronic (Andersen and Nielsen 1994; Jamba et al. 1997; Jihen et al. 2008), subchronic (Santos et al. 2005; El-Sharaky et al. 2007), and acute (Wahba et al. 1993; Sarkar et al. 1997; Štajn et al. 1997) exposure to Cd in adult animals, contradictory results concerning Cd organ redistribution are reported when animals are supplemented with Se.

In our study, we tested the hypothesis that Se supplementation changes absorption, inter-organ distribution, and retention of Cd, and therefore reduces its toxic effects in sucklings. As no such data on sucklings are available, we also investigated which of the two experimental designs for Se supplementation used in adult animals is more effective in reducing Cd in sucklings: treatment before and during Cd exposure or only during Cd exposure. We exposed suckling pups to both Se and Cd orally to mimic infant exposure conditions from additional nutrition while still on a milk diet. Interaction between Cd and metalloenzymes of the newborn may also affect essential metal metabolism (Brzóska and Moniuszko-Jakoniuk 2001; Ishitobi and Watanabe 2005). This is why the study also included the analysis of zinc (Zn), copper (Cu), and iron (Fe) in the pup's organs.

Materials and methods

Animals

For this experiment 20 female Wistar rats bred in the Laboratory Animal Unit of the Institute for Medical Research and Occupational Health, Zagreb, Croatia were mated with males in the ratio 3:1. Four females with deliveries on the same day and their pups were used in this experiment. The study was performed on six-day-old pups of both sexes. The animals were maintained in a 12 h light/dark cycle, at room temperature of 21 ± 1°C, and constant humidity of 40%. Each litter was in an individual polycarbonate cage (26.5 × 20.7 × 14.0 cm) with stainless steel lid. The cages were cleaned and pine shaving bedding changed daily. All research procedures were carried out in accordance with the national law on protection of animal welfare and approved by the Croatian Ministry of Agriculture, Forestry, and Water Management.

Experimental design

The pups ($n = 32$) were randomly assigned to four litters (of four mother rats) with eight pups per litter (four males and four females) on postnatal day 2 (PND 2; day of birth = PND 0). One or two pups from each litter formed one of the five experimental groups with six or seven animals per group (Table 1): Controls received distilled water for 9 days; the Se and Cd group received either 8 μmol of Na_2SeO_3 or 8 μmol of $CdCl_2$ a day for 5 days; the Se_{pre} + Cd group received 8 μmol of Na_2SeO_3 for 9 days + 8 μmol of $CdCl_2$ a day for 5 days; and the Se + Cd group received 8 μmol of Na_2SeO_3 + 8 μmol of $CdCl_2$ a day for 5 days.

Water, Se and/or Cd solutions were administered to pups orally using the method of artificial feeding introduced by Kostial et al. (1971). Every morning before the first administration, each pup was weighted. The daily dose was freshly prepared and administered in two portions (at 9:00 a.m. and 2:00 p.m.) drop-by-drop with an automatic pipette (25 μl), four drops a day in total. In between administrations, all pups were returned to their lactating mother rats and allowed to suckle. Pups always received Se before Cd, 15 min apart. Selenium-supplemented (Se and Se + Cd group) animals were given sodium selenite (p.a. grade, Sigma–Aldrich Co.) at a daily dose of 0.632 mg Se kg^{-1} b.w. for five consecutive days (PNDs 10–14). Animals pre-treated with Se (Se_{pre} + Cd group) were receiving 0.632 mg Se kg^{-1} b.w. a day for nine consecutive days (PNDs 6–14). This procedure is called pre-treatment because the Se_{pre} + Cd pups had been receiving Se for 4 days before they were supplemented with Se for the following 5 days, just like the pups from the Se and Se + Cd group. The daily dose of Se was calculated to achieve an equimolar ratio to the daily dose of Cd (Cd:Se, 1:1). Cadmium was administered as cadmium chloride (p.a. grade, Kemika Co.) at a daily dose of 0.9 mg Cd kg^{-1} b.w. for five consecutive days (PND 10–14). The same Cd dose was given to the Cd alone, the Se_{pre} + Cd, and the Se + Cd experimental group. The daily dose of Cd was chosen to meet the following requirements: no adverse effects on the pup development, sufficient tissue element levels for analytical measurement and relevance to human exposure (Matek Sarić et al. 2002).

On PND 15, pups were anaesthetised (Narketan 0.8 ml kg^{-1}b.w. plus Xylapan 0.6 ml kg^{-1}b.w., Vetoquinol AG; i.p.) and were dissected in the same sequence for each animal. For technical reasons (pups are too small and mother-dependent) urine and faeces could not be sampled in the metabolic cages. Therefore, urine was taken directly from the urinary bladder by a syringe and faeces collected from the rectum (content 5 cm proximal to the anus). We are aware that urine and faeces measurements are but rough estimations of pup Cd and Se excretion. While still under anaesthesia, blood samples were collected from the heart in BD Vacutainer® tubes with lithium heparin anticoagulant. Plasma was obtained by centrifugation of collected blood samples (3000 rpm, for 15 min). Animals were killed by bleeding from the abdominal aorta and the organs were dissected (stomach, small and large intestine, kidneys, liver and brain). The stomach was cut open and a section of duodenum with proximal jejunum (14 cm distal to the pyloric

Table 1 Experimental protocol

Group	Number of pups per group	Treatment during postnatal day	Duration of treatment (days)	Total dose received (mg kg^{-1} b.w.)
Control	6	6–14	9	–
Se	6	10–14	5	3.2 of Se
Cd	6	10–14	5	4.5 of Cd
Se_{pre} + Cd	7	6–14	9	5.7 of Se
		10–14	5	4.5 of Cd
Se + Cd	7	10–14	5	3.2 of Se
		10–14	5	4.5 of Cd

Suckling rats were treated with selenium as sodium selenite and/or cadmium as cadmium chloride from postnatal day (PND) 6–14. Rats received deionised water (control group), Cd, Se or Se + Cd orally in a water solution at 9:00 am and 2:00 pm. Cadmium and selenium daily doses were equimolar (8 μmol/kg b.w./day)

sphincter) rinsed with cold deionised water. Wet weight of all collected samples were recorded and tissues stored at −20°C until element analysis.

Analysis of elements

Cadmium and Se mass fractions were analysed from the same thawed tissue samples (rinsed stomach and duodenum, right kidney, part of liver, part of brain and faeces) digested in closed tubes in a Digestion System (DS-40, Tecator, Sweden) after adding 1–2 ml of concentrated HNO_3, 65% p.a. grade (Merck). Digested samples and thawed urine were analysed using graphite furnace atomic absorption spectrometry (GFAAS) (Perkin Elmer AAnalyst 600, Perkin Elmer, Shelton, USA) with Zeeman background correction. For Zn, Cu, and Fe analysis, the left kidney and a part of the liver and brain were dried at 105°C and dry-ashed overnight in a muffle furnace at 450°C. The ash residues were then dissolved in concentrated HNO_3, heated and filled up to 10 ml with deionised water. Essential metals were determined by aspirating prepared sample solution into the air-acetylene flame of a Varian AA-375 atomic absorption spectrometer (Mulgrave, Victoria, Australia). The reliability of analytical methods was evaluated using standard reference materials: bovine liver 1577b (NIST, USA), horse kidney H8 (IAEA, Austria), Seronorm™ Trace Elements Whole Blood (Sero AS, Norway), and Seronorm™ Trace Elements Urine (Sero AS, Norway). The results of our analysis were within ±10% of the certified values. Once a year, our laboratory takes part in the proficiency testing for trace elements in food (National Food Administration, Sweden). Satisfactory results for the elements concerned with Z-score <2 were obtained.

Statistical procedure

Results are presented as arithmetic mean and standard error of the mean (S.E.M.). Statistical analysis was performed using Statistica for Windows software (StatSoft, Inc. 2004, release 7.0) after log-transformation of elementary data. When data showed equal variance (Bartlett) and followed a normal distribution (Shapiro-Wilk), one-way analysis of variance (ANOVA) with post-hoc analysis (Tukey's HSD test) was used to determine significant differences between the groups. In other cases, when the criteria for parametric methods were not fulfilled, the effect of treatment was assessed using the Kruskal–Wallis test with different subsets identified by the Mann–Whitney *U*-test. Differences were considered significant at $P \leq 0.05$.

Results and discussion

The main goal of this study was to investigate if Se interacted with Cd in the early postnatal suckling period, as many experiments on adult animals produced contradictory results. The experimental period (PND 6–15) was chosen to make up for the maturation differences between the rat and human newborn. Namely, Miller (1983) has proposed that a 7–10-day-old rat neonate may be more comparable to the human at birth. By choosing PND 15 for the last day of the experiment, we wanted to exclude the intake of any solid food by the pups (supplied to mother rats in the cage), which we noticed to occur in the late pre-weaning period (weaning at PND 21).

The mean daily dose of Cd given to pups was in the range of those ingested by adult rats chronically exposed to 5–25 ppm of Cd in drinking water (Brzóska and Moniuszko-Jakoniuk 2005; Lafuente et al. 2005; Rogalska et al. 2009). According to Brzóska and Moniuszko-Jakoniuk (2005) treatment with these doses reflects real exposure level in moderately to heavily polluted areas, in active tobacco smokers or under occupational exposure conditions. Other authors used similar doses in their experimental designs (Leret et al. 2003; Petersson Grawé et al. 2004a; Fowler et al. 2004; Pillet et al. 2006). The total dose of Se (5.7 mg or 3.2 mg/kg body weight, Table 1) in our experiment was 54% or 30% the LD_{50} dose, respectively, reported for adult female rats (WHO 1987). No data for selenite LD_{50} have been reported for rat pups, so we monitored their general appearance and body weight gain. We did not notice significant differences in organ weights, body weight gain (Fig. 1) and general appearance of the suckling rats between the groups on the last day of the experiment (PND 15). Average pup body weight gain was 2.04 g per day.

This study focused on the interaction of Se and Cd, so we used slightly higher doses, than one would expect to occur in children, which still fit the range of human exposure. Furthermore, our daily Cd dose was

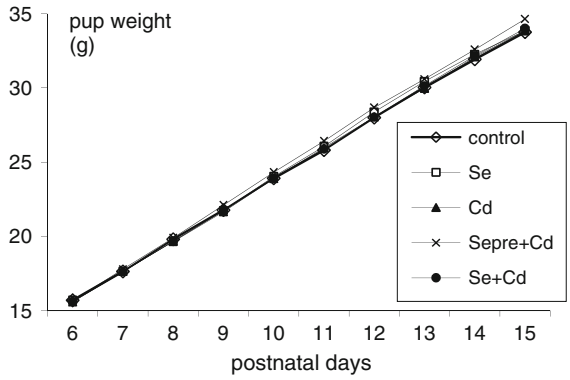

Fig. 1 Body weights (g) of pups measured throughout the experiment (PND 6-15). Each point represents the mean of 6 (control, Se, Cd group) or 7 (Se_{pre} + Cd, Se + Cd group) pups in the experimental group at respective postnatal day

much lower than the doses used in recent studies of Se and Cd interaction with chronic and subchronic exposures in adult animals (Rana and Verma 1996; Jamba et al. 1997; Štajn et al. 1997; Santos et al. 2005; Newairy et al. 2007; El-Sharaky et al. 2007; Ognjanović et al. 2008; Jihen et al. 2008).

We found much lower mass fraction and retention of Cd in the stomach than in the duodenum (Table 2). This is due to a higher pH in the intestines, resulting in a more soluble Cd (Oskarsson et al. 1998). High and prolonged absorption of Cd, characteristic for newborn animals, is evident from the high level of Cd in the duodenum 24 h after the last Cd administration. Duodenum has been identified as the segment with the highest gastrointestinal Cd absorption in both adult (Sørensen et al. 1993) and newborn animals (Eklund et al. 2001). By tracking the retention of oral ^{109}Cd dose given in water or infant formula to 11-day-old rats, Eklund and co-workers also confirmed milk diet as a platform for higher Cd absorption and retention than water and cereal- or soy-based diets. This dietary factor, observed even earlier by other authors (Sasser and Jarboe 1977; Kostial et al. 1981), is partly responsible for the high Cd absorption in suckling rats. Other reason can be the physiology of a newborn organism. High pinocytotic activity in newborn mammals enables non-selective uptake of macromolecules; this means that Cd bound to smaller polypeptides of proteins can enter enterocytes (Zalups and Ahmad 2003). In addition, naturally elevated concentrations of metallothionein (MT) in immature rats (Goering and Klaassen 1984) may trap more Cd ions entering enterocytes after oral exposure, and result in longer retention in the mucosa of the small intestine (Zalups and Ahmad 2003). From the mucosa, Cd is slowly transferred into systemic circulation and distributed to target tissues, primarily liver and kidney (Bhattacharyya et al. 2000). The highest increase in Cd levels was found in the liver followed by the kidney and the brain of pups exposed to Cd when compared to the control group (Table 2). Although pups in this study were exposed to Cd for 5 days, the liver-to-kidney ratio in the Cd group of 4.6:1 reflects acute exposure, and is similar to the findings of Sørensen et al. (1993) in mice 24 h after a single oral Cd dose. After long-term oral administration Cd preferentially accumulates in the kidney (WHO 1992).

Our study shows that Se supplementation (pre-treatment and treatment only during Cd exposure) affects Cd distribution to critical organs of suckling rats (Table 2). The retention of Cd in the stomach and duodenum did not change with Se because Cd and Se seem to interact only after they enter the bloodstream. Selenium ingested as selenite is reduced to selenide in the red blood cells and is complexed with Cd in an equimolar plasma ratio (Sasakura and Suzuki 1998). This complex (Cd–Se) then binds to a specific plasma protein, selenoprotein P (Sel-P), the most common Se-containing protein important for whole-body Se supply, especially brain and testis (Schomburg et al. 2003). Since Shigeta and coworkers (2008) found that the Sel-P is a major pathway for the transfer of Se in mice from 6 to 72 h after injection of selenite, we suggest that the metabolism of the ternary complex $\{(Cd–Se)_n\}_m$-Sel P formed in plasma of suckling rats may be similar. The formation of a complex, i.e. the diversion of Cd to a Se-containing protein in the plasma of Se-supplemented rats was reported long ago by Chen et al. (1975) and Parizek et al. (1968). Chen and co-workers also found that Se pre-treatment diverted Cd—otherwise bound to the metallothionein (MT), a low-molecular-weight protein in liver and kidney—to a larger molecular weight protein. This diversion in some reports decreased the ability of Cd to induce MT (Gambhir and Nath 1992), whereas Chmielnicka et al. (1983) found that Se supplementation in the presence of Cd did not change MT levels.

In our experiment Se and Cd interaction decreased whole blood Cd and plasma Se (Tables 2 and 3) compared to the pups receiving Cd or Se alone. It is possible that at the moment of sampling the Cd/Se complex was already eliminated from the bloodstream,

Table 2 Gastrointestinal, blood and tissue cadmium retention after two different selenium supplementation treatments in suckling rats exposed to cadmium

Group	Cd µg/kg wet weight (% dose)[1]					
	Rinsed stomach	Duodenum	Blood	Brain	Liver	Kidney
Control	43.8 ± 5.4^{a}	3026 ± 323^{a}	1.00 ± 0.19^{a}	0.700 ± 0.033^{a}	29.2 ± 2.8^{a}	46.7 ± 4.4^{a}
Se	35.8 ± 4.7^{a}	2983 ± 456^{a}	1.09 ± 0.42^{a}	0.457 ± 0.031^{a}	20.2 ± 1.3^{a}	41.9 ± 5.1^{a}
Cd	339 ± 28^{b} (0.054)	7919 ± 1057^{bc} (0.887)	18.6 ± 1.1^{b} (0.005)	14.0 ± 1.6^{b} (0.013)	1492 ± 135^{b} (1.04)	1651 ± 219^{b} (0.224)
Se_{pre} + Cd	239 ± 28^{b} (0.037)	10021 ± 868^{b} (0.887)	4.30 ± 0.32^{c} (0.002)	3.66 ± 0.67^{c} (0.003)	324 ± 21^{c} (0.229)	571 ± 18^{c} (0.081)
Se + Cd	253 ± 67^{b} (0.039)	6161 ± 900^{c} (0.605)	5.91 ± 0.79^{c} (0.002)	4.29 ± 0.59^{c} (0.004)	509 ± 72^{d} (0.333)	698 ± 37^{d} (0.094)

Suckling rats received deionised water (control group) or Se or Se and Cd orally in a water solution at 9:00 am and 2:00 pm. Selenium supplement was given as sodium selenite and cadmium as cadmium chloride. Rats were treated from postnatal day (PND) 6–14 as follows: Control (H_2O, PND 6–14), Se (PND 10–14), Cd (PND 10–14), Se_{pre} + Cd (Se PND 6–14 + Cd PND 10–14), and Se + Cd (Se + Cd PND 10–14). Cadmium and selenium daily doses were equimolar (8 µmol/kg b.w./day)

[1] The results are presented as mean ± SEM, n = 6–7. The percent of the initial Cd dose in parentheses is calculated from total cadmium in tissue/total cadmium administered × 100

Values with the same superscript letter within a column are not significantly different ($P < 0.05$)

as reported by Ohta et al. (1988), who found this complex very unstable and who reported that Cd rapidly decreased in the complex with time. However, if this protein complex is distributed to organs, it is probably quickly metabolized in the liver. The greatest accumulation of Se was observed in the liver of the Se group (Table 3) and its distribution did not change when Cd was co-administered, while Cd retention in the liver of pups from the Se_{pre} + Cd and Se + Cd group (Table 2) decreased markedly compared to the Cd group. This different effect of Se supplementation on the levels of Cd and Se in the liver suggests that their complex formed in the plasma is broken down in the liver. As the liver is the main organ for Se accumulation (Shigeta et al. 2008), it is no surprise that Se levels were higher there than in the kidney and brain even when the pups were exposed to Cd. Some authors propose that new Cd/Se complexes are formed in the liver and kidney, which then bind to specific tissue proteins (Jamall and Smith 1985), possibly enhancing Cd removal from these organs. In our study, Se supplementation reduced Cd levels in the liver and kidney of pups, as reported before for adult animals (Chen et al. 1975; Wahba et al. 1993; Rana and Verma 1996; Jamba et al. 1997). However, due to differences in the administered doses, in the mode of application, and in exposure duration, many authors failed to see any effect of Se and Cd interaction (Gambhir and Nath 1992; Sidhu et al. 1993; Andersen and Nielsen 1994; Ognjanović et al. 1995, 2008) or reported higher Cd levels in Se-supplemented animals (Chmielnicka et al. 1983, 1985; Jamall and Smith 1985).

As far as we know, our study is the first ever to report a decrease in the brain Cd of Se-supplemented pups (Table 2). The reason for this is that most studies investigating Se and Cd interaction involved adult animals whose blood-brain barrier, unlike in newborn animals, does not allow the uptake of Cd in brain cells (Aschner and Kerper 2000). Therefore, these authors mainly focused on their effects on the kidney and liver. Cadmium has been found to accumulate in the brain of newborn animals. Recent findings have revealed behavioural, neurochemical, and neurotoxicological changes in foetus and newborn rats exposed to Cd (Leret et al. 2003; Petersson Grawé et al. 2004a, b; Minetti and Reale 2006).

Judging by the decrease in tissue (Table 2) and whole body Cd levels (unpublished results M.L.), one would

Table 3 Gastrointestinal, plasma and tissue selenium retention after two different selenium supplementation treatments in suckling rats exposed to cadmium

Group	Se (μg/g wet weight[1])					
	Rinsed stomach	Duodenum	Plasma[2]	Brain	Liver	Kidney
Control	0.169 ± 0.007[a]	0.182 ± 0.010[a]	109 ± 6[a]	0.113 ± 0.004[a]	0.454 ± 0.016[a]	0.617 ± 0.009[a]
Se	1.95 ± 0.12[b]	0.851 ± 0.058[b]	811 ± 62[b]	0.191 ± 0.005[b]	7.93 ± 0.87[b]	2.57 ± 0.07[b]
Cd	0.140 ± 0.012[a]	0.209 ± 0.007[a]	108 ± 8[a]	0.102 ± 0.003[ac]	0.443 ± 0.023[a]	0.569 ± 0.019[a]
Se_{pre} + Cd	1.54 ± 0.10[b]	1.73 ± 0.14[c]	/	0.176 ± 0.007[bc]	10.2 ± 1.2[b]	2.61 ± 0.15[bc]
Se + Cd	1.47 ± 0.20[b]	1.07 ± 0.09[b]	442 ± 29[c]	0.157 ± 0.005[c]	7.97 ± 0.92[b]	2.06 ± 0.09[d]

Exposures as in Table 2

[1] The results are presented as mean ± SEM, $n = 6–7$

[2] Se expressed in μg/l. Because of a technical mistake, data for Se in plasma for Se_{pre} + Cd group are not available

Values with the same superscript letter within a column are not significantly different ($P < 0.05$)

expect enhanced Cd excretion in Se-supplemented pups. However, our results show somewhat lower Cd levels in urine and faeces (Table 4), which confirms an earlier conclusion of Wahba et al. (1993) that greater Cd elimination is not a mechanism through which Se reduces Cd toxicity. It remains to be seen whether our sampling method is too coarse to measure fine differences in urine Cd between the groups or the excretion takes place before our sampling time.

The faeces of pups in the Cd group contained the highest amounts of Cd (Table 4), which confirms that most of the ingested Cd just passes through the intestines, and is excreted in faeces. The average oral absorption of Cd is low, up to 5% in adult humans (WHO 1992). Data for human infants are not available, but animal experiments suggest somewhat higher absorption in newborns (Kostial et al. 1978; Nordberg et al. 1978; Eklund et al. 2001). Lower levels of Cd (not significantly) and higher levels of Se in both urine and faeces of pups supplemented with Se (both Se_{pre} + Cd and Se + Cd group, Table 4) imply separate metabolism of Cd and Se after the formation of equimolar complex in plasma. Addition to here presented excretion data would be an experiment where the sampling of urine and faeces would take place in few hour intervals after the oral Cd administration to pups. Such an investigation is left to be done in the future research. Selenium is excreted mostly in the urine in the form of selenosugars (Francesconi and Pannier 2004). Cadmium is eliminated mostly in the faeces, comprising Cd transferred via the intestinal mucosa and bile, and in the urine associated with the levels in the renal cortex (WHO 1992; ATSDR 1999).

Quantification of Se and Cd in the faeces of pups exposed to both elements (Table 4) also showed that

Table 4 Excretion of cadmium and selenium after two different selenium supplementation treatments in suckling rats exposed to cadmium

Group	Urine (μg/l)		Faeces (μg/g wet weight)	
	Cd	Se	Cd	Se
Control	0.205 ± 0.040[a]	29.0 ± 3.4[a]	4.07 ± 0.79[a]	1.49 ± 0.13[a]
Se	0.355 ± 0.131[a]	2173 ± 228[b]	9.03 ± 0.65[b]	50.4 ± 6.8[b]
Cd	1.92 ± 0.41[b]	39.6 ± 5.5[a]	324 ± 44[c]	1.85 ± 0.31[a]
Se_{pre} + Cd	1.37 ± 0.29[b]	2681 ± 207[b]	198 ± 32[c]	84.4 ± 10.1[c]
Se + Cd	1.53 ± 0.40[b]	2805 ± 403[b]	318 ± 56[c]	72.5 ± 10.6[bc]

Exposures as in Table 2

[1] The results are presented as mean ± SEM, $n = 6–7$

Values with the same superscript letter within a column are not significantly different ($P < 0.05$)

there was no binding of added chemicals. Namely, the formation of a complex would result in a vast elevation of Cd and Se levels in the faeces of pups from groups exposed to Se and Cd compared to the pups exposed to Se or Cd alone. Instead, we measured only greater Se level in faeces of $Se_{pre} + Cd$ group. The same results were reported by Magos and Webb (1976) when they injected 8 μmol Cd^{2+}/kg with an equimolar dose of selenite, and found no retention of Cd^{2+}, but some of Se at the injection site. The other proof that there was no binding of added Se and Cd compounds is that in an aqueous solution and at physiological pH, Se from selenite is predominantly present as $HSeO_3^-$ (Gailer 2007), and therefore can not form a complex with Cd ions.

Cadmium retention data (Table 2) imply that pre-treatment with Se is more effective in reducing Cd retention than supplementation only during Cd exposure (Se + Cd group). This effect is the most visible in the liver and kidney. In addition, pre-treatment with Se redistributed Cd between the liver and kidney. This is evident from the liver-to-kidney ratios (calculated from the percent of dose) in the Cd, the $Se_{pre} + Cd$, and the Se + Cd group (4.6:1, 2.8:1 and 3.5:1, respectively).

The disturbances of Zn levels in the liver and kidney in the pups exposed to Cd (Fig. 2) are in accordance with reported Cd-induced changes in Zn homeostasis (Brzóska and Moniuszko-Jakoniuk 2001; Bridges and Zalups 2005; Nakazato et al. 2008). We can not provide a good explanation for the elevated Fe level after Se supplementation, as no significant interaction between these elements was reported in the literature. Other essential elements were not affected by Se and/or Cd treatment.

This study has shown that Se supplementation markedly decreases Cd retention in the blood, brain, liver and kidneys of suckling rats. These results are particularly important in view of a number of studies estimating Cd in human infants and unsuccessfully seeking an efficient and harmless Cd antagonist among essential elements. Our results are add to the existing knowledge about Se and Cd interaction, and are not directly comparable with studies on adult animals. They are unique inasmuch as no such investigation has been done before on newborn rats.

In conclusion, our results suggest that Se supplementation (Se and Cd in equimolar ratios), especially pre-treatment, can decrease Cd retention in the tissues of suckling rats, and consequently reduce possible

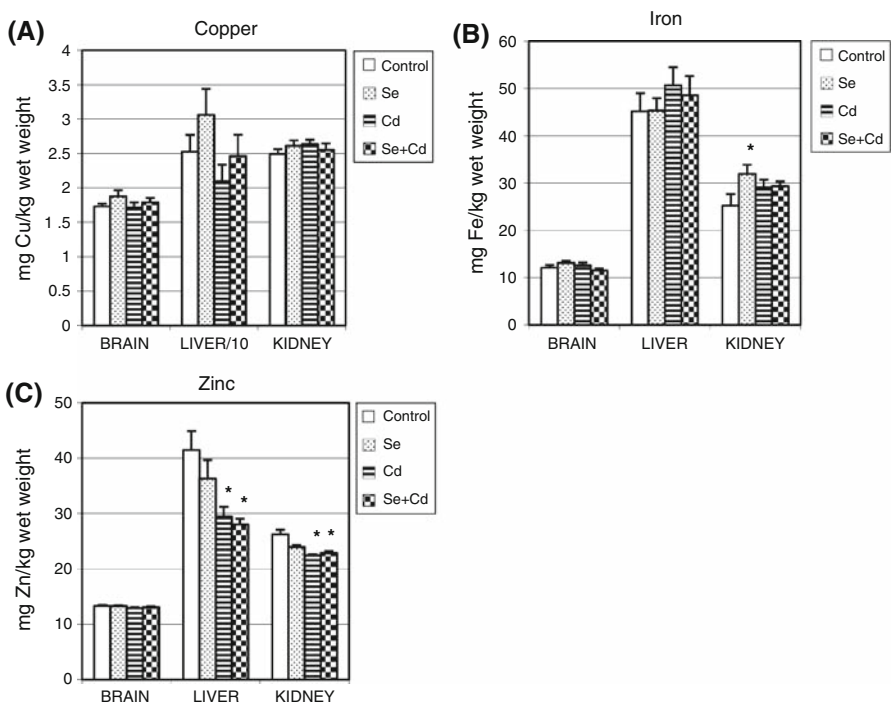

Fig. 2 The effect of cadmium and/or selenium oral exposure on copper (**A**), iron (**B**), and zinc (**C**) levels in the brain, liver, and kidney of suckling rats. Exposures as in Table 1, without the pre-treated group (Sepre + Cd). The data are expressed as mean ± SEM; $n = 6–7$. *Asterisks* indicate statistically significant differences from the control group ($P < 0.05$)

toxic effects of Cd. However, our experimental design could not give information about the mechanism of Se and Cd interaction so further investigation is needed to clarify this mechanism.

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