Bioavailability of cadmium from infant diets in newborn rats

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Abstract Infants are exposed to higher levels of cadmium (Cd) from infant and follow-on formulas than from breast milk. We studied the bioavailability of $^{109}\text{CdCl}_2$ from cows’ milk formula, soy formula, wheat/oat/milk formula, wholemeal/milk formula and water in 11-day-old rat pups. The pups received a single oral dose of one diet labelled with $^{109}\text{Cd}$, 0.1 or 0.3 mg Cd/kg body weight. After 2 or 24 h or 4, 9 or 12 days the fractional retention of $^{109}\text{Cd}$ in the whole body, in segments of rinsed small intestine and in tissue was measured in a gamma counter. Pups receiving $^{109}\text{Cd}$ in water or cows’ milk formula had the highest mean whole-body retention. It ranged from 67% of the dose in the water group to 52% in the wholemeal/milk formula group 4 days after dosing. The retention of $^{109}\text{Cd}$ in the rinsed small intestine was significantly higher in the water group and the cows’ milk formula group than in the cereal-based formula groups at 24 h and 4 days after dosing. It was still high in all groups on day 9, ranging from 26 to 11%. Initially most of the $^{109}\text{Cd}$ was retained in the duodenum but by day 4 it had moved further down into the jejunum. In the liver, the highest and lowest retention on day 4 was 16% and 3% of the dose in the water group and wholemeal/milk formula group, respectively. In the kidney, $^{109}\text{Cd}$ was still increasing 12 days after exposure in all groups. Whole-body retention and tissue levels were higher than previously reported in adult animals. The lower bioavailability of $^{109}\text{Cd}$ from the cereal-based formulas compared to water and cows’ milk formula on the longer survival times is most likely explained by Cd binding to dietary fibre and phytic acid in the cereal-based formulas reducing the intestinal binding and decreasing the bioavailability of Cd. The high retention of $^{109}\text{Cd}$ in the small intestine, leading to a prolonged absorption period, emphasizes the importance of extending studies on neonatal Cd absorption over a long time period in order to detect for example, endpoints, accumulation of Cd in the kidney.

Keywords Absorption · Cadmium · Infant formula · Newborn

Introduction

Infants are exposed to the toxic metal cadmium (Cd) from infant and follow-on formulas. The levels are higher in soy- and cereal-based formulas than in cows’ milk formulas. In a recent study, we have found 1.5 to 21 times higher mean Cd levels in Swedish cereal-based formulas compared to cows’ milk formulas (Eklund and Oskarsson 1999). Cd is taken up by growing crops from the soil through the root system. Wheat has the highest concentration among the different grains (Jorhem et al. 1984; Sillanpää and Jansson 1991). Since Cd accumulates in the outer part of the grain, including the bran, the highest levels of endogenous Cd are usually found in wholemeal products and consumption of these products results in a higher Cd intake (Jorhem and Sundström 1993). During the lactation period the mammary gland functions as a barrier, restricting transfer of Cd into the milk (Bhattacharyya et al. 1981; Bhattacharyya et al. 1982; Pietrzak-Fis et al. 1978). Low lactational Cd transfer has also been shown in cattle (Smith et al. 1991), and the Cd levels in infant formulas based on cows’ milk are generally low (Dabeka and McKenzie 1987; Eklund and Oskarsson 1999). Yet the intake of Cd by the infant can be twice as high from cows’ milk formula than from breast milk, and from soy formula up to 12 times higher (Eklund and Oskarsson 1999).

Cd is normally poorly absorbed in the gastrointestinal tract. In adult humans the absorption is approximately...
5% (WHO 1992). The site of gastrointestinal uptake has been studied in adult mice by Sørensen et al. (1993) and Andersen et al. (1994), who found a high deposition of Cd in the duodenum, indicating that this is probably the major site of absorption. The bioavailability of Cd from food is dependent on the composition of the diet and on the age and nutritional status of the individual. Lower fractional absorption of Cd has been reported in adult rats on a whole-wheat diet compared to rats on an endosperm wheat diet (Moberg Wing 1993). Wholemeal diets are rich in dietary fibre and phytic acid, which probably bind Cd thus reducing its intestinal binding and retention and decreasing its bioavailability.

Studies in experimental animals have shown that a milk diet (Engström and Nordberg 1978; Kello and Kostial 1977) and young age (Kostial et al. 1983; Sasser and Jarboe 1977) are factors that increase gastrointestinal Cd absorption. Recently, the recovery of unabsorbed $^{109}$Cd in bulked faeces from infants given a test meal of porridge containing the stable isotope has been reported to be 63–96%, indicating a high gastrointestinal Cd uptake in infants (Crews et al. 2000). Despite higher Cd levels in infant formulas than in breast milk, Cd levels in the serum of formula-fed and breast-fed infants are reported to be similar, about 0.4 μg/l, which is higher than in healthy adults (0.2 μg/l) (Krachler et al. 1999). Thus, the bioavailability of Cd from breast milk may be higher than from formulas.

An expert group within the WHO/FAO has set a provisional tolerable weekly intake (PTWI) of Cd of 7 μg/kg body weight to avoid Cd concentrations in the kidney cortex exceeding 50 mg/kg. The expert group recognizes that the exposure will not be uniform with age and the PTWI takes into account the higher Cd intake, on a body weight basis, by infants and children (WHO 1989). Little is known about Cd toxicity in infants and children. Knowledge of the bioavailability of Cd from infant diets during the neonatal period is insufficient yet essential for the risk assessment of Cd in infants and children. Scandinavia has a long tradition of feeding infants liquid formulas based on cows’ milk and cereals as weaning food. In a previous study, we estimated the mean Cd intake in infants given Swedish wheat/oat/milk formula to be about 50% of the established PTWI (Eklund and Oskarsson 1999).

In the present study the bioavailability of $^{109}$CdCl$_2$ from water and four infant formulas with different compositions was studied in a suckling rat model by comparing Cd uptake and retention in the rat pups. The pattern of intestinal retention of Cd from the diets was also investigated in relation to the uptake of Cd.

### Material and methods

#### Animals

A group of 15 Sprague-Dawley rats with litters, bred at Charles River (Uppsala, Sweden), were delivered on postnatal day 7 (PND 7). The litters were randomly standardized to ten pups per dam and the animals were allowed to acclimatize for 4 days before the experiments began. The dams with litters were housed in individual cages at 24°C and 41% humidity under a 12-h light/dark cycle and maintained on a commercial pelleted diet (Ewos, Södertälje, Sweden) and tap-water ad libitum. The pups were weighed on arrival (PND 7) and before they were killed. The animal experiments were approved by Uppsala Ethics Committee of Animal Experiments (permit no. C 253/97).

#### Isotopes and formulas

Two batches of $^{109}$CdCl$_2$ from Du Pont De Nemours (Brussels, Belgium) were used in the experiments. The specific activities were 1.19 mCi/mg Cd and 2.41 mCi/mg Cd. The infant formulas tested were soy formula, cows’ milk formula, wheat/oat/milk formula and wholemeal/milk formula, recommended from 0, 4, 6 and 12 months, respectively. The soy formula was a semiconcentrated liquid (25% dry weight) while the others were powdered. The respective endogenous Cd levels in the formulas were 1.6, 0.9, 18.5 and 22.7 μg/kg liquid or powder determined by graphite furnace atomic absorption spectrometry (Eklund and Oskarsson 1999). Since the diets were commercial infant formulas, the exact compositions were not known, but the ingredients were given on the packaging in weight order. The cereals in the wholemeal/milk formula were oatmeal, wheat flour, whole-wheat meal, rye flour and rice flour. Deionized water of Milipore quality was used as a control diet in the experiment.

#### Preparation of the diets

The formulas were prepared in proportions recommended by the manufacturers. To approximately 1.5 g of the different formula powders was added 9 ml deionized water and to 5 ml semiconcentrated soy formula was added 4 ml deionized water. One isotope (1.19 mCi/mg Cd) was diluted with deionized water to 200 μCi/ml, and 1 ml of the diluted isotope solution was added to each diet to give a volume of 10 ml test diet containing 0.017 mg Cd/ml (20 μCi/ml) for administration to survival groups 4, 9 and 12 days. The diets for the survival groups 2 and 24 h were prepared in the same manner. To 1 g formula powder was added 6.4 ml deionized water and to 3.2 ml soy formula was added 3.2 ml deionized water, and 10 μl of the other isotope (2.41 mCi/mg Cd) was added to each diet to give 0.007 mg Cd/ml (16 μCi/ml) test diet. The contribution of Cd from the formulas was negligible compared to the contribution from the isotopes. The infant diets and water controls were incubated with gentle shaking for 24 h at 4°C. Aliquots (0.2 ml) of the incubated diets were transferred to test tubes and the radioactivity was determined in a gamma counter.

#### Experimental design

On PND 11 the pups were separated from the dams for 2 h and given 0.5 ml radiolabelled diet by gastric intubation. The administration volume was not expected to influence the kinetics as it only contributed approximately 6% of the daily milk intake of 10-day-old rat pups (Bornschein et al. 1977). Each pup received a dose of 0.1 mg Cd/kg body weight (8 μCi) (survival groups 2 and 24 h) or 0.3 mg/kg body weight (10 μCi) (survival groups 4, 9 and 12 days). After gastric intubation the pups were returned to the dams. On PND 20 (9 days after administration of the diets) the pups were weaned. The pups in the longest survival groups (12 days) were maintained on the same commercial pellets as the dams and tap-water for the last 3 days.

The pups were anaesthetized with methoxyflurane (Metofane; Mallinkrodt Veterinary, Mundelein, Ill.) and killed 2 or 24 h (n = 5 per diet) or 4, 9 or 12 days (n = 6 per diet) after intubation by heart puncture and the blood was collected into heparinized syringes. The whole-body retention of $^{109}$Cd was measured in a whole-body gamma counter (NaI well crystal, diameter 80 mm, depth 120 mm).