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Joint effects of dietary cadmium and polychlorinated biphenyls on metallothionein induction, lipid peroxidation and histopathology in the kidneys and liver of bank voles ☆

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Abstract

Free-living bank voles have been shown to be more sensitive to cadmium (Cd) toxicity than the rodents exposed to Cd under laboratory conditions. The present study was designed to find out whether polychlorinated biphenyls (PCBs), common environmental co-contaminants, increase susceptibility to Cd toxicity through inhibition of metallothionein (MT) synthesis—a low molecular weight protein that is considered to be a primary intracellular component of the protective mechanism. For 12 weeks, the male bank voles were provided with diets containing Cd (0.05 μg/g (control) and 10 μg/g dry wt) and PCBs (0, 10 and 50 μg/g dry wt) alone or in combination under long (16 h) and short (8 h) photoperiods. At the end of exposure period, histological examinations and analyses of MT, Cd, Fe and lipid peroxidation in the kidneys and liver were carried out. Dietary PCBs did not affect Cd inducibility of renal MT, but decreased it significantly in the liver; however, no signs of Cd toxicity (measured by histopathology) occurred in both organs. On the contrary, PCBs at the highest dose increased significantly lipid peroxidation in the kidneys and liver (4-fold) only in the bank voles raised under a long photoperiod; the PCB-induced hepatic lipid peroxidation was accompanied by extensive histopathological changes including hepatocyte enlargement, necrosis and steatosis. Co-treatment with dietary Cd significantly suppressed the increase in lipid peroxidation and apparently reduced hepatic damage. These data indicate that (1) dietary PCBs do not enhance Cd toxicity in the kidneys and liver of bank voles and (2) dietary Cd suppresses PCB-induced hepatotoxicity that appears to be photoperiod-dependent.

Keywords: Cadmium; Polychlorinated biphenyls; Metallothionein; Lipid peroxidation; Histopathology; Kidneys; Liver; Iron

1. Introduction

Cadmium (Cd) is an industrial and environmental pollutant that is toxic to humans and animals (Goering et al., 1995). Chronic Cd exposure produces damage to the entire kidney, including tubular degeneration, tubular cell apoptosis, interstitial inflammation and glomerular swelling (Groten et al., 1994; Liu et al., 1998b). Chronic exposure to Cd results also in liver injury including non-specific chronic inflammation and apoptosis (Habeebu et al., 2000; Włostowski et al., 2004). During chronic exposure, Cd-induced toxicity in the kidneys and liver is dependent on renal and hepatic Cd concentrations. In laboratory rodents, renal injury occurs when the Cd concentration reaches 90–120 μg/g (Groten et al., 1994; Liu et al., 1998a), while liver damage is observed at the Cd concentration higher than 150 μg/g (Habeebu et al., 2000). Surprisingly, in the wildlife such as roe deer, moose, Algerian mice, yellow-necked mice and bank voles the renal and hepatic injury occurs at the Cd levels as low as 1–22 μg/g (Leffler and Nyholm, 1996; Beiglböck et al., 2002; Damek-Poprawa and Sawicka-Kapusta, 2003, 2004; Pereira et al., 2006). So far, the reason for this difference in susceptibility to Cd toxicity is not known and remains to be elucidated.

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It is well known that susceptibility to Cd toxicity increases dramatically in animals that are unable to synthesize metallothionein (MT), a low-molecular weight protein that is induced by and bound to the metal (Klaassen et al., 1999; Nordberg and Nordberg, 2000). Notably, in MT-null animals the kidney and liver injury occurs at the Cd concentrations lower than 10 μg/g (Liu et al., 1998b; Habeebu et al., 2000). Sensitivity to Cd toxicity is also enhanced by some organic environmental co-contaminants through the reduction of tissue MT levels (Hurk et al., 2000; Sogawa et al., 2001). For instance, a non-toxic dose of bisphenol A has been shown to reduce Cd-induced expression of hepatic MT through estrogen receptor, which results in Cd-induced damage to the liver (Sogawa et al., 2001). Thus, an appropriate amount of MT is required to provide protection against Cd-induced tissue injury. When the amount of Cd in the kidneys and liver exceeds the binding capability of MT, the non-MT-bound Cd ions are believed to cause nephro- and hepatotoxicity (Goyer et al., 1989; Nomiyama and Nomiyama, 1998). One mechanism by which these ions can produce injury is thought to be through generation of reactive oxygen species (ROS) and lipid peroxidation, which in turn depress renal and hepatic functions (Nomiyama and Nomiyama, 1998; Shaikh et al., 1999; Thevenod and Friedmann, 1999).

Polychlorinated biphenyls (PCBs) are also ubiquitous environmental co-contaminants which are resistant to biodegradation and remain widely distributed along with heavy metals such as Cd in the environment (Batty et al., 1990; Alleva et al., 2006; Wilcke et al., 2006). PCB exposure has been associated with neurobehavioral, immune, developmental, reproductive and hepatic abnormalities in both humans and experimental animals (Whysner and Wang, 2001; Jacobson and Jacobson, 2003; Ulbrich and Stahlmann, 2004; Branchi et al., 2005). It is assumed that PCB toxicity is mediated through oxidative stress induced by these chemicals (Krishnamoorthy et al., 2005; Lee et al., 2006). Furthermore, many of the adverse effects can result from hormonal (including estrogenic) and anti-hormonal activities of PCBs and their metabolites (Ulbrich and Stahlmann, 2004; Mi and Zhang, 2005), which may also interfere with Cd-induced synthesis of MT as shown in the case of bisphenol A (Sogawa et al., 2001). However, it is not known whether PCBs affect Cd inducibility of MT in the kidneys and liver, and whether this effect (if any) is responsible for the enhancement of Cd toxicity.

Therefore, in the present study we decided to examine the effect of PCBs on Cd inducibility of MT in the kidneys and liver of a small rodent, the bank vole (*Clethrionomys glareolus*), which appeared to be highly vulnerable to Cd toxicity when free-living in a contaminated environment (Damek-Poprawa and Sawicka-Kapusta, 2004); at the same time the toxicity was evaluated by measuring lipid peroxidation and histopathology. Since the bank voles are sensitive to changes in daylight and photoperiod affects MT induction and Cd toxicity (Włostowski et al., 2004), the animals were exposed chronically to environmentally relevant concentrations of dietary Cd and PCBs under both long and short photoperiods.

2. Materials and methods

2.1. Animals and experimental design

Male bank voles (1 month old, weighing 10–13 g), being the F_1 offspring of the wild-caught population (Knyszyn Old Forest near Białystok, northeastern Poland), were used throughout the study. The bank voles were randomly assigned into two groups according to photoperiod: (1) 16 h light/8 h dark (long-photoperiod group) and (2) 8 h light/16 h dark (short-photoperiod group). Each photoperiod group was divided into six subgroups ($n = 6$ each) according to dietary Cd and PCBs: (1) control, (2) Cd 10 μg/g–PCB 0 μg/g, (3) Cd 0 μg/g–PCB 10 μg/g, (4) Cd 10 μg/g–PCB 10 μg/g, (5) Cd 0 μg/g–PCB 50 μg/g, and (6) Cd 10 μg/g–PCB 50 μg/g dry wt. The bank voles were housed for 12 weeks in groups of two in stainless-steel cages at 18–20 °C and at 50–70% relative humidity. They received ad libitum distilled water and control or Cd- and PCB-containing whole wheat grains, which appeared to be an adequate quality food for these rodents (Włostowski et al., 2004). Prior to the experiment the grains were contaminated with Cd (soaked in $CdCl_2$ solution) (Włostowski et al., 2004) and then 1 kg mixed with 10 mL of corn oil containing 0, 10 or 50 mg PCBs (Aroclor 1254- NSI Solutions Inc., Raleigh, NC, USA). Atomic absorption spectrophotometry (AAS) analysis of the grains revealed that actual levels of Cd were between 9 and 12 μg/g (Cd groups) and less than 0.05 μg/g dry wt (control). The chosen doses of dietary Cd and PCBs were environmentally relevant (Huang et al., 1998; Zakrzewska et al., 2002; Boonstra and Bowman, 2003). In addition, an identical quantity of apple was offered to all voles (3 g/vole/week), who ate it completely. The food intake was monitored throughout the experiment. The experimental protocols were approved by the local ethical committee for conducting an experimental study on laboratory animals (Medical Academy in Białystok).

2.2. Assays

At the end of the 12-week exposure period, the bank voles were weighed, euthanized by cervical dislocation and the liver, both kidneys and testes were removed, rinsed in ice-cold saline and blotted dry. A portion of the fresh liver (0.25 g) and one kidney were transferred to 2.0 mL chilled 0.25 M sucrose and homogenized with a Teflon pestle in a glass homogenizer. Aliquots (0.2 and 1.0 mL) of the homogenate were taken for determination of lipid peroxidation and metal concentrations, respectively. The remaining homogenate was centrifuged at 20,000*g* for 20 min at 4 °C, and the resulting supernatant was removed for MT assays.

MT content in the kidneys and liver was determined by a Cd-saturation method (Onosaka and Cherian, 1982) with minor modification (Włostowski and Krasowska, 1999). Briefly, a 0.1-mL sample was incubated in a 1.5-mL vial for 10 min at room temperature with 1.0 mL Tris-HCl buffer (0.03 M, pH 7.8) containing 1.0 μg Cd/mL. To remove non-MT-bound Cd, bovine hemoglobin (Sigma) (0.1 mL of a 5% solution in H_2O) was added and the sample was heated for 1.5 min at 95 °C, cooled, and centrifuged for 5 min at 10,000*g*. Addition of hemoglobin, heating, and centrifugation of the sample was repeated three times. Cd bound to MT in the resulting clear supernatant was determined by electrothermal AAS. MT content was expressed in micrograms of the protein per gram of wet tissue, assuming that 1 mol of MT (6600) binds 7 mol of Cd (Winge and Miklossy, 1982). Cd inducibility of MT was expressed as μg MT/μg Cd.

Cadmium and iron determinations were carried out as described elsewhere (Włostowski et al., 2004). The homogenate (1.0 mL) was placed in a glass tube with 2.0 mL of concentrated nitric acid. After 20 h of sample digestion at room temperature, 72% perchloric acid (0.5 mL) was added and the mixture was heated at 100 °C for 3 h. Finally, the temperature was raised to 150–180 °C and digestion continued for another 4 h. Deionized water was added to the residue after digestion to a volume