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Effect of cadmium on bone tissue in growing animals

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ABSTRACT

Accumulation of cadmium (Cd), an extremely toxic metal, can cause renal failure, decreased vitamin D synthesis, and consequently osteoporosis. The aim of this work was to evaluate the effect of Cd on two types of bone in growing Wistar rats. Sixteen 21-day-old male Wistar rats were assigned to one of two groups. The Cd group subcutaneously received 0.5 mg/kg of $CdCl_2$ 5 times weekly for 3 months. The control group similarly received bidistilled water. Following euthanasia, the mandibles and tibiae were resected, fixed, decalcified and processed histologically to obtain sections for H&E and tartrate-resistant acid phosphatase (TRAP) staining. Photomicrographs were used to determine bone volume (BV/TV%), total growth cartilage width (GPC.Wi) hypertrophic cartilage width (HpZ.Wi), percentage of yellow bone marrow (%YBM), megakaryocyte number (N.Mks/mm^2), and TRAP + osteoclast number (N.TRAP + Ocl/mm^2). Results were statistically analyzed using Student's *t* test. Cd exposed animals showed a significant decrease in subchondral bone volume and a significant increase in TRAP+ osteoclast number and percentage of yellow bone marrow in the tibia, and an increase in megakaryocyte number in mandibular interradicular bone. No significant differences were observed in the remaining parameters. The results obtained with this experimental design show that Cd would seemingly have a different effect on subchondral and interradicular bone. The decrease in bone volume and increase in tibial yellow bone marrow suggest that cadmium inhibits differentiation of mesenchymal cells to osteoblasts, favoring differentiation into adipocytes. The different effects of Cd on interradicular bone might be due to the protective effect of the mastication forces.

1. Introduction

In recent years, Cadmium, an extremely toxic metal, has attracted considerable interest as an environmental pollutant. Cadmium is released into the environment mainly from uncontrolled e-waste recycling (Guo et al., 2010; Leung et al., 2008), fertilizers, cigarette smoke, leather industry effluents, and even some metals used in jewelry electroplating (Weidenhamer et al., 2011). It must be pointed out that the half life of cadmium in the body is approximately 10–30 years (Jarup and Akesson, 2009).

According to clinical studies, exposure to cadmium causes mainly renal failure and osteoporosis (Engström et al., 2012; Jin et al., 2004; Sughis et al., 2011). Two mechanisms of action have been proposed for cadmium: a direct and an indirect mechanism (Kazantzis, 2004). The indirect mechanism suggests that Cd is taken up by cells in the proximal convoluted tubules of the kidney and accumulates in mitochondria, generating disruption of the respiratory chain and causing oxidative stress due to generation of reactive oxygen species (ROS) (Bertin and Averbeck, 2006; Johri et al., 2010). These ROS would seemingly block production of an enzyme responsible for vitamin D activation, thus decreasing calcium absorption in the digestive tract. The ensuing hypercalciuria, caused by the inability of the kidneys to reabsorb calcium in addition to decreased calcium absorption in the duodenum, would deleteriously affect bone formation and mineralization (Alfvén et al., 2000; Horiguchi et al., 2005).

The direct mechanism suggests that Cd exerts an effect on bone tissue cells (Chen et al., 2009). Biochemical assays performed *in vivo* in rats showed that consumption of 1 mg of $CdCl_2$/L of drinking water for 24 months affects the bone remodeling process by decreasing alkaline phosphatase activity and increasing serum levels of C-terminal cross-linking telopeptide of type I collagen (Brzóska and Moniuszko-Jakoniuk, 2004). *In vitro* studies in human cell cultures have shown that Cd induces activation of caspase-3 and −8, both involved in osteoblast apoptosis (Coonse et al., 2007).

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In addition, cell exposure (RAW264.7 mouse monocytes/macrophage cell line (TIB-71; ATCC) to Cd in the presence of receptor activator of nuclear factor kappa-B ligand (RANKL) was found to increase levels of tartrate- resistant acid phosphatase (TRAP) activity and formation of TRAP+ cells, suggesting that Cd could increase bone mass loss by inducing osteoclast formation and differentiation (Chen et al., 2011a).

Toxic agents affect the entire skeletal system, including the jaw bones. Alveolar bone is a part of the jaw bones that forms the primary supporting structure for teeth. Although alveolar bone and long bones are composed of cortical or compact bone and trabecular bone, their different origin and function determine different responses to the same stimulus (Sodek and McKee, 2000). Studies by Aghaloo et al. (2010) showed that mesenchymal cells derived from mandibular bone marrow have a greater osteogenic potential than those derived from long bone marrow cells. Because of the small size and anatomical complexity of alveolar bone in experimental animals, there are few experimental toxicological studies in the literature using this type of bone.

Given the distribution of Cd in the environment and its use in a number of applications, people are exposed to this metal throughout life. According to the literature, mean U-Cd values per μg/g of creatinine range between 0.32 and 0.4 in areas with low contamination (Wang et al., 2014a, 2014b), and can be higher than 10 μg/g of creatinine in highly contaminated zones (Nakagawa et al., 2006).

Although studies in the literature have reported several effects of this toxic metal on a number of body tissues and organs, there is not sufficient evidence of the effect of Cd on different types of bone in growing animals. Thus, the aim of the present work was to evaluate the effect of Cd on two types of bone in growing animals, a long bone (tibia) and mandibular bone (interradicular bone at the first lower molar).

2. Materials and methods

2.1. Experimental animals

Sixteen healthy male Wistar rats aged 21 days and weighing 65 ± 10 g were assigned to one of two groups: Control and Cd. The rats were housed in galvanized steel cages, 4 animals per cage, at 21–24 °C and 52–56% humidity, under 12 h light/dark cycles. The animals had free access to food (Standard diet rat-mouse chow, Cooperación, Argentina) and water; they were fed standard chow containing 23% protein, 1-1.4% calcium and 0.5-0.8% phosphorus (Bernhart and Tomarelli, 1966). The animals in group Cd were subcutaneously administered a 0.5 mg/kg dose of $CdCl_2$ (Sigma-Aldrich, US) 5 times weekly for 3 months (Chen et al., 2011b). The animals in the control group were similarly treated with vehicle (bidistilled water).

Body weight was recorded weekly. Three months after the onset of the experiment, the animals were weighed, anesthetized by intraperitoneal injection of xylazine (5 mg/kg, König Laboratory, Argentina) and ketamine (50 mg/kg, Holliday Laboratory, Argentina), and euthanized by intracardiac injection of 0.2 ml of euthanyl (Brouwer Laboratory, Argentina). The experimental protocol was approved by the Ethics Committee of School of Dentistry (28/11/2012-38), University of Buenos Aires, and is in keeping with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

The mandible and tibiae were excised from all the animals. The tibiae were measured using a Vernier type caliper, and were weighed using a precision balance (E. Mettler, Zürich). All the material was fixed in a 4% formaldehyde-buffer solution pH 7.4, decalcified in 10% EDTA pH 7 for 30 days, and processed histologically for embedding in paraffin. Longitudinal sections were obtained from the tibia, and mesio-distally oriented sections were obtained from the first lower molar in order to evaluate the interradicular bone. Interradicular bone sections and one set of tibia sections, approximately 7-8 μm thick, were stained with hematoxylin-eosin.

In addition, one longitudinal section of each tibiae was processed for TRAP staining (Minkin (1982)Minkin C, 1982). Briefly, the method involves incubation in a TRIS buffer solution (pH 5) containing naphtol phosphate AS-BI (Sigma-Aldrich, Switzerland), fast red violet (Sigma, Germany), and sodium tartrate (Sigma, Germany).

Bone histomorphometry studies (Demster et al., 2013) were performed using Image Pro Plus 4.5 software.

The following parameters were measured in the areas of subchondral bone shown in Fig. 1a and b:

- BV/TV(%): Bone volume: percentage of bone tissue in the studied area
- Tb.N (1/mm): Trabecular number
- Tb.Wi (mm): Trabecular width
- Tb.Sp (mm): Trabecular spacing
- GCP.Wi (μm): Total growth cartilage width (Fig. 1b)
- HpZ.Wi (μm): Hypertrophic cartilage width (Fig. 1b)
- N.Mk/mm^2: Number of megakaryocytes in bone marrow (Fig. 1a*)
- YBM (%): Percentage of yellow bone marrow (Fig. 1a*)

The number of TRAP+ osteoclasts (NTRAP + Oc/mm^2) was determined in a region immediately below the growth cartilage, using a light field microscope at 400X magnification. Cells presenting 2 or more nuclei and that were close to trabeculae were considered osteoclastic cells.

The following histomorphometric parameters were assessed in the areas of interradicular bone of the first lower molar shown in Fig. 2a and b:

- BV/TV(%): Bone volume: percentage of bone tissue in the total studied area.
- N.Mk/mm^2: Number of megakaryocytes in bone marrow (Fig. 2b*)
- YBM (%): Percentage of yellow bone marrow (Fig. 2b*)

Statistical analysis

The results are expressed as mean ± standard deviation. The data were statistically analyzed by Student's *t* Test, using "Primer of Biostatistics" software. Values of p below 0.05 were considered statistically significant.

3. Results

Animal body weight and length were recorded throughout the experiment. As shown in Fig. 3, body weight and size were similar in all animals throughout the study, and no significant differences were observed between the Cd and the control groups. Likewise, no significant differences were found in tibia weight (g) (control: 0.978 ± 0.068; Cd: 0.908 ± 0.068) or length (cm) (control:4.28 ± 0.118; Cd:4.33 ± 0.131) three months post-exposure. Although no significant morphometric differences were observed, the histomorphometric study showed a decrease in subchondral bone volume and an increase in the percentage of yellow bone marrow in the tibia of Cd exposed animals. Significant differences ($p < 0.05$) in tibial trabecular bone volume were observed between groups, as a result of lower trabecular number Tb.N (1/mm) (control: 2.07 ± 0.76; Cd: 1.31 ± 0.26) ($p<0.05$) and greater trabecular spacing