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Quantitative characterization of changes in bone geometry, mineral density and biomechanical properties in two rat strains with different Ah-receptor structures after long-term exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin

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ABSTRACT

Background: Both industrial chemicals and environmental pollutants can interfere with bone modeling and remodeling. Recently, detailed toxicological bone studies have been performed following exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which exerts most of its toxic effects through the aryl hydrocarbon receptor (AhR).
Objectives: The aims of the present study were to quantitatively evaluate changes in bone geometry, mineral density and biomechanical properties following long-term exposure to TCDD, and to further investigate the role of AhR in TCDD-induced bone alterations. To this end, tissue material used in the study was derived from TCDD-exposed Long–Evans (L–E) and Han/Wistar (H/W) rats, which differ markedly in sensitivity to TCDD-induced toxicity due to a strain difference in AhR structure.
Methods: Ten weeks old female L–E and H/W rats were administered TCDD s.c. once per week for 20 weeks, at doses corresponding to calculated daily doses of 0, 1, 10, 100 and 1000 ng TCDD/kg bw (H/W only). Femur, tibia and vertebra from the L–E and H/W rats were analyzed by peripheral quantitative computed tomography (pQCT) and biomechanical testing at multiple sites. Dose–response modeling was performed to establish benchmark doses for the analyzed bone parameters, and to quantify strain sensitivity differences for those parameters, which were affected by TCDD exposure in both rat strains.
Results: Bone geometry and bone biomechanical parameters were affected by TCDD exposure, while bone mineral density parameters were less affected. The trabecular area at proximal tibia and the endocortical circumference at tibial diaphysis were the parameters that showed the highest maximal responses. Significant strain differences in response to TCDD treatment were observed, with the L–E rat being the most sensitive strain. For the parameters that were affected in both strains, the differences in sensitivity were quantified, showing the most pronounced (about 49-fold) strain difference for cross-sectional area of proximal tibia.
Conclusion: The study provides novel information about TCDD-induced bone alterations at doses, which are of relevance from a health risk assessment point of view. In addition, the obtained results provide further support for a distinct role of the AhR in TCDD-induced bone alterations, and suggest that the benchmark dose modeling approach is appropriate for quantitative evaluation of bone toxicity parameters.

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1. Introduction

Epidemiological studies indicate that exposure to environmental levels of food-derived organohalogen contaminants such as polychlorinated chlorinated biphenyls (PCBs) (Hodgson et al., 2008) or dichlorodiphenyldichloroethylene (DDE) (Beard et al., 2000; Glynn et al., 2000) modulate bone quality as assessed by altered bone mineral density. Furthermore, high-level, accidental dietary exposure to hexachlorobenzene has resulted in severe osteoporosis (Cripps et al., 1984; Gocmen et al., 1989), and infants exposed in utero to high concentrations of PCBs and polychlorinated dibenzofurans (PCDFs) were shown to develop irregular calcification of their skull bones (Miller, 1985). Bone quality effects have also been reported in wildlife from organohalogen contaminated environments (Fox et al., 2008; Lind et al., 2003; Sonne et al., 2004; Rodriguez-Navarro et al., 2006), and experimental studies have demonstrated that bone modeling and remodeling are sensitive endpoints of organohalogen exposure both in adult animals following long-term exposure (Alvarez-Lloret et al., 2009; Andrews, 1989; Badraoui et al., 2007; Jämsä et al., 2001; Lind et al., 1999, 2000a,b, 2004; van der Ven et al., 2006,) and in the offspring following maternal exposure (Hermsen et al., 2008; Miettinen et al., 2005; van der Ven et al., 2009).

The most detailed toxicological bone studies have been performed with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the organohalogen pollutant model compound, which exerts most of its toxic effects through activation of the aryl hydrocarbon receptor (AhR). Through the use of rat lines with different sensitivities to TCDD toxicity, the role of AhR as a determinant of sensitivity to the observed bone effects has been demonstrated (Jämsä et al., 2001; Miettinen et al., 2005). Following *in utero*/lactational TCDD exposure, altered bone geometry, bone mineral density and biomechanical strength were observed in a sensitive rat line with normal AhR, whereas the more resistant rat line with a mutated AhR were completely resistant to TCDD exposure within the practically feasible dose range (Miettinen et al., 2005). Also results from studies of bone cells suggest an important role of AhR in the toxicity of potent AhR-ligands. It has been shown that 3-metylcholantren, which is an AhR activator, retard proliferation and differentiation of osteoblasts in vitro and ossification of mouse embryos in vivo (Naruse et al., 2002), and that the AhR antagonist reservatrol reverse dioxin-induced inhibition of osteodifferentiation in vitro (Singh et al., 2000). Further, TCDD was shown to decrease the expression of differentiation markers in osteoblasts from wild-type mice, but had no effect on cells from AhR-knockout mice (Korkalainen et al., 2009). Central elements of the AhR signaling pathway is present already in developing bone (Abbott and Probst, 1995), and studies on bone cell cultures indicate that AhR is strongly expressed in osteoblasts (Ilvesaro et al., 2005; Korkalainen et al., 2009; Ryan et al., 2007) as well as in osteoclasts (Ilvesaro et al., 2005).

The Long–Evans (L–E) and Han/Wistar (H/W) rat strains are known to exhibit remarkable differences in sensitivity to dioxin-induced lethality, liver toxicity, liver foci development and several other endpoints of toxicity, while sensitivity to hepatic CYP1A induction, thymic atrophy and some developmental defects are quite similar (Pohjanvirta et al., 1993; Unkila et al., 1994). The sensitivity differences between the strains have been ascribed to the structurally aberrant AhR exhibited by the more resistant H/W rat strain (Tuomisto et al., 1999). The AhR of H/W rats harbor a point mutation in intron sequence, leading to an insertion/deletion type of alteration at the 3′ end of the coding region of cDNA and a smaller receptor size (Pohjanvirta et al., 1998). Based on sensitivity differences observed in short-term studies, the AhR-mediated effects of dioxin have been classified into two categories (Simanainen et al., 2002). Type I effects are not affected by the strain difference and hence difference in the AhR, while type II effects are less responsive in rats with the altered H/W-type AhR (Simanainen et al., 2002; Tuomisto et al., 1999). In the present study, the benchmark dose (BMD) methodology (Sand et al., 2008) was used to quantitatively evaluate changes in bone geometry, mineral density and biomechanical properties following long-term exposure to TCDD, and to further investigate the role of AhR in dioxin-induced bone alterations by quantifying the difference in sensitivity to TCDD-induced bone effects between the L–E and H/W rat strains.

2. Materials and methods

2.1. Animals and treatments

The experimental protocol, including animal housing and care during the study, was approved by the Animal Experiment Committee at the University of Kuopio and the Kuopio Provincial Government. The study design has previously been described in detail (Viluksela et al., 2000). Ten weeks old female H/W (Kuopio) and L–E (Turku/AB) rats were administered TCDD in corn oil by s.c. injection (2 ml/kg) once per week for 20 weeks; control rats received corn oil only. To achieve rapidly the kinetic steady state, in order to mimic adult human steady state exposure, the first treatment was a loading dose 5 times higher than the 19 subsequent weekly maintenance doses (Flodström and Ahlborg, 1989). The total doses, selected in order to cover the whole spectrum of biological effects in both strains, were 0, 0.17, 1.7, 17 and 170 μg TCDD/kg bw (H/W only), corresponding to calculated daily doses of 0, 1, 10, 100 and 1000 ng TCDD/kg bw. Exposure at the low dose, i.e. 1 ng TCDD/kg bw, resulted in animal tissue levels comparable to the polychlorinated dibenzo-p-dioxins (PCDD)/polychlorinated dibenzofuran (PCDF) concentrations observed in exposed humans (Jämsä et al., 2001; Viluksela et al., 2000), which makes the applied dose levels of relevance from a health risk assessment point of view.

The right femur (8–10 samples per dose group), right tibia (8–10 samples per dose group) and distal lumbar vertebra (L4) (8–10 samples per dose group) were dissected, cleaned of muscle and soft tissue and put in test tubes with Ringer solution (1 l contains 0.3 g Tris; 0.24 g $CaCl_2(H_2O)_2$; 0.4 g KCl; and 2.05 ml 1 M HCl; pH 7.4) and stored at −20 °C until analysis. For biomechanical testing of lumbar vertebra, the vertebral endplates were removed by cutting a 4.5-mm high section using a diamond saw with two parallel blades. Thus, a bone cylinder consisting of central trabecular bone surrounded by cortex, without endplates, was retrieved. The transverse processes of vertebrae were removed with a knife. On the day of analysis the bones were thawed at room temperature and stored moistened in closed plastic tubes until examination. The bone material was derived from a study originally designed to investigate liver foci development (Viluksela et al., 2000), which means that the rats at the age of 5 weeks were partially (2/3) hepatectomized and initiated 24 h later with a single dose of nitrosodiethylamine (30 mg/kg) ip. As illustrated in Table 1, partial hepatectomy and nitrosodiethylamine initiation did not change the pattern of TCDD-induced effects on cortical area or bone mineral density. Therefore, further investigation of geometry, density and biomechanical properties of bone from hepatectomized/initiated rats were considered appropriate and used in the present study.

2.2. Bone geometry, mineral density and biomechanical properties

The total length (mm) of femur, tibia and lumbar vertebra was measured using an electronic sliding caliper (IP65, Sylvac SA, Crissier, Switzerland) to the nearest 0.02 mm. The femoral bone length was measured from the top of the caput femoris to the distal point of the condylus medialis. The tibial bone length was measured from the proximal point of the medial tibial condyle to the medial malleolus. The height of the vertebral body was measured from its upper to lower surface.

The excised femur, tibia and lumbar vertebra were scanned with a peripheral quantitative computed tomography (pQCT) system (Stratec XCT Research SA+ with software version 5.50R, Norland Stratec Medizintechnik, GmbH, Birkenfeld, Germany). Calibration of the machine was performed with a phantom provided by the manufacturer. A single tomographic slice perpendicular to the long axis of the femoral and tibial shaft of 1.0 mm was acquired at a voxel size of 0.148 mm × 0.148 mm × 1 mm. The scans of the distal femur and proximal tibia were performed at sites distanced 20% of the length from the distal end of femur and the proximal end of tibia (Fig. 1). The scans of femoral and tibial diaphysis were performed at sites distanced 50% of length from the end of the bone. The positions were verified using scout views and one slice perpendicular to the long axis of the bone shaft was acquired. For the femoral neck, the scan perpendicular to the neck axis was made at 50% of distance between the base of femoral head and great trochanter. The scan of distal lumbar vertebra was made at 50% of the total vertebral body height, perpendicular to the long axis. Image processing and the calculation of numerical values were performed using the manufacturer's software package. The cross-sectional area (mm^2) was determined after detecting the outer bone contour at a threshold of 710 mg/cm^3. Total bone mineral density (mg/cm^3) was defined as the mean density of the total cross-section. At proximal tibia and distal femur, trabecular area was calculated, using a contour mode of 1 and a peel mode of 2. Thresholds of 400 mg/cm^3 as an upper limit and 200 mg/cm^3 as a lower limit was