EFFECTS OF TCDD ON BONE QUALITY IN A TCDD-SENSITIVE AND A TCDD-RESISTANT RAT STRAIN

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Introduction

Bone development and maintenance are strictly controlled by a complex network of hormonal interactions. Dioxins are known to disturb the balance of several hormonal systems, and therefore they may potentially interfere with the bone modelling and remodelling processes. However, effects of dioxins on bone are largely unknown.

Sensitivity to dioxin-induced toxic effects is highly variable among animal species and even strains of the same species. An animal model based on >1000-fold sensitivity difference in acute lethality of TCDD between two rat strains has been developed in our laboratory[1]. Long-Evans (L-E) rat is the most TCDD-sensitive rat strain, while Han/Wistar (H/W) rat is the most resistant mammal to the acute lethality of TCDD. H/W rats are also exceptionally resistant to some other endpoints of dioxin toxicity. AH receptor (AHR) of H/W rats was recently shown to harbor a point mutation resulting in an insertion/deletion type alteration at the 3'end of the coding region of cDNA, and an altered transactivation domain[2]. Although the deviant AHR seems to account for the resistance of H/W rats to acute lethality[3], liver toxicity and some hormonal alterations, H/W rats and L-E rats show nearly similar sensitivity to induction of CYP1A1 activity, thymic atrophy, embryotoxicity or decreases in serum thyroxine and melatonin levels[1]. In this study we utilized L-E and H/W rats to examine the effects of TCDD on rat long bones.

Materials and Methods

Ten weeks old female L-E (*Turku/AB*) and H/W (*Kuopio*) rats were assigned into treatment groups of 5 animals. The rats were given weekly s.c. doses of TCDD for 20 weeks. The first treatment was a loading dose with a 5 times higher dose than the subsequent weekly maintenance doses. Total doses were 0, 0.17, 1.7, 17 and 170 (H/W only) μg/kg. One week after the last maintenance dose the animals were killed and stored in plastic bags at -20°C until analysis. The carcasses were thawed at room temperature, bones harvested and soft tissue removed immediately before analysis.

Bone mineral density (BMD) and cross-sectional geometry of tibial diaphyses were evaluated using a peripheral quantitative computed tomograph (pQCT) system (Stratec XCT 960A, Norland Stratec Medizintechnik GmbH, Birkenfeld, Germany) as previously described[4], using

a pixel size of 0.148 x 0.148 mm^2. The mechanical properties of the tibial diaphyses were tested with a three-point bending test[4,5] , and the breaking force and stiffness defined.

Results and Discussion

Tibial length and the cross sectional area of diaphysis were lower in L-E rats at the two highest dose levels (1.7 and 17 μg/kg; $p<0.01$) and in H/W rats only at the highest dose level (170 μg/kg; $p<0.05$). Total BMD of tibial diaphysis was significantly higher at 17 μg/kg in both strains ($p<0.001$), and in H/W rat also at 170 μg/kg ($p<0.01$). In H/W rats the medullary area was decreased ($p<0.01$), but the cortical area did not show any changes even at the highest dose level. In L-E rats also the cortical area was dimished ($p<0.01$). Thus, TCDD treatment resulted in smaller diaphyseal cross-section with slightly denser bone.

In biomechanical testing the bending breaking force of tibial diaphysis was decreased in L-E rats at 17 μg/kg ($p<0.01$) and in H/W rats at 170 μg/kg ($p<0.05$). Furthermore, the stiffness of tibial diaphysis showed statistically significant and dose-dependent decreases both in L-E and in H/W rats (Fig. 1.). The data indicate that exposure to TCDD results in decreased mechanical strength of rat long bones.

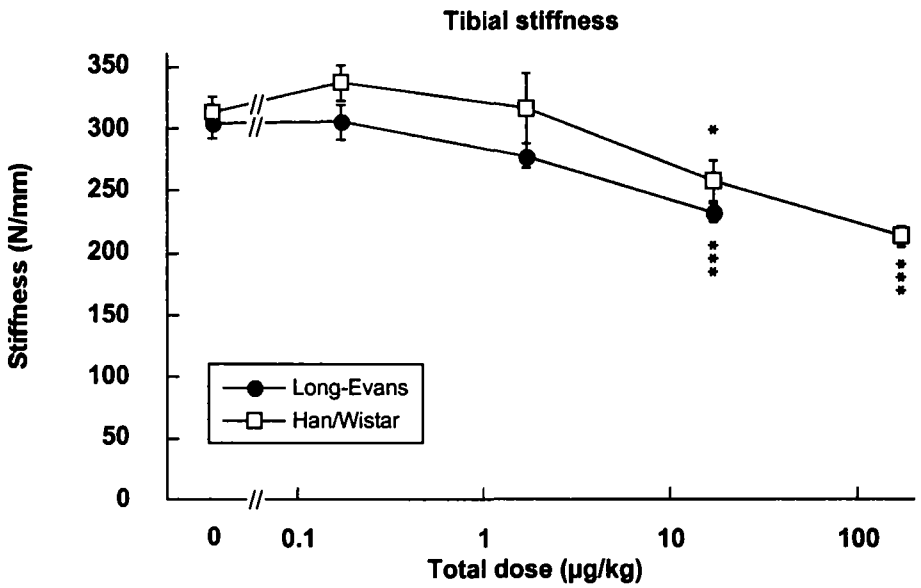

Figure 1. Effect of TCDD on the stiffness of tibial diaphysis in Long-Evans and Han/Wistar rats (mean ± SE, n = 5). Statistics: *$p<0.05$, ***$p<0.001$ vs controls; ANOVA followed by an independent *t*-test.

Effects of TCDD on bone have been previously examined only in a few studies. TCDD was shown to inhibit the differentation of cultured rat osteoblasts[6]. Osteolysis of alveolar bone associated with proliferation of periodontal squamous epithelium was recently reported in minks exposed to high dietary concentration (5 ng/g diet) of TCDD for 6 months[7]. Similarly with our results treatment of ovariectomized rats with coplanar PCB126 for 3 months resulted in decreased tibial lenght and increased BMD[8]. These changes, however, were not seen in sham operated rats treated with PCB126.

In conclusion, our data indicate that TCDD interferes with bone modelling inducing dose-dependent changes in bone size and density, as well as in decreased mechanical strength. L-E rats were affected at the total dose level of 1.7 μg/kg and above, while in H/W rats the effects were seen at 17 or 170 μg/kg.

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