IN UTERO / LACTATIONAL TCDD EXPOSURE IMPAIRS THE MOLAR TOOTH DEVELOPMENT IN RATS

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Introduction

Developmental defects caused by TCDD are gaining increasing concern since they occur at low dose levels and are usually permanent. Exposure to the higher end of the current background levels of polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs) via mother's milk was recently associated with tooth mineralization defects in children[1].

Although the mechanisms are not thoroughly known, it is clear that the AH receptor (AHR) is involved in dioxin induced toxicity. Typically, sensitivity to some but not all endpoints varies greatly among different species and strains. Han/Wistar (*Kuopio*; H/W) rats are exceptionally resistant to acute lethality and some other endpoints of dioxin toxicity, while Long-Evans (*Turku*/AB; L-E) rats are sensitive.

The resistance of H/W rats is associated with a mutated AHR allele (Ahr^{hw})[2] and another, currently unidentified allele B^{hw}. The resistance alleles were recently segregated in new rat lines[3]. Line A has the mutated Ahr^{hw} allele, line B the B^{hw} allele and line C none of these. The lines exhibit highly different LD_{50} values, line A being the most resistant and C the most sensitive line. The purpose of this study was to examine the effects of low dose *in utero*/lactational TCDD exposure on rat tooth development, and to define the roles of the resistance alleles in sensitivity to these effects. Rat third molar development spans from perinatal period to about 6 weeks after birth. In this study, eruption of third molar, its size and mineralization were assessed.

Methods

A, B and C rats were obtained from the SPF barrier unit of the National Public Health Institute (Kuopio, Finland). Pregnant females were administered a single oral dose of 0.03-1 μg TCDD/kg in corn oil on gestation day (GD) 15 (GD 0 = sperm positive). Control animals received corn oil. There were 5-8 pregnant dams at each dose level. On postnatal day (PND) 1, offspring number was recorded and litters were adjusted to 3 males and 3 females, if possible. On PND 28, offspring were weaned and housed with same-sex littermates. On PND 35 and 70, female and male pups, respectively, were sacrificed, the jaws collected and preserved in formaline.

The jaws were examined using a stereo–microscope (Olympus SZH-ILLB). The developmental stage of the third molars was assessed based on their eruption, which is visible as a penetration of the tooth through the gingival tissue and on the height of the tooth. The jaws were radiographed to confirm the presence (mineralization of dentin and enamel) of the mandibular and maxillary third molars. The size of the developing mandibular third molar was estimated from the radiographs by measuring the height (from the top of the crown to the tip of the root) and the mesio-distal width. The radiograph was done with an experimental X-ray unit (Phoenix, Radiante Oy, Finland) with 40 cm focus-film distance, 20 kV tube voltage, 0.4 mA tube current and exposure times from 0.9 to 1.5 s, depending on the size of the jaws. The jaws were placed on the surface of the 18 x 24 cm KodakMinR cassette equipped with only one intensifying screen, at back side of the cassette. Kodak Ektascan NB film with single emulsion layer was used. Mandibles were split into two halves and radiographed with their buccal sides facing the film cassette. Maxillas were radiographed in an axial projection, teeth facing the surface of the film cassette.

Results and Discussion

At 1 μg/kg TCDD completely prevented the mineralization of the third mandibular molar in 59% of line C female rats and in 60% of line C male rats, but only in 5% and 6% in line A and line B female rats, respectively. In these rats neither molars nor tooth rudiments could be seen in stereomicroscopy or in X-ray examination. In line A and B males all third mandibular molars were seen at 1 μg/kg. The third molars were seen in all control rats and all TCDD-treated rats at 0.3 μg/kg or below.

In female rats sampled on PND 35 the third mandibular molar was barely or completely erupted in 94% of line A control rats, but only in a few line B and C control rats. Therefore, the effect of TCDD on molar eruption could be examined only in line A females. TCDD-treatment resulted in dose-dependently-decreased proportion of erupted third molars in line A rats, and the decrease was statistically significant at 0.3 and 1 μg/kg (Fig. 1). Completely erupted third molars were not seen at 1 μg/kg. In male rats sampled on PND 70 all existing molars had mineralized and erupted.

TCDD treatment also dose-dependently diminished the height and the mesio-distal width of the existing third molars in both genders of all rat lines. In males the decrease was statistically significant at 0.1 μg/kg and above in line B and at 0.3 μg/kg in all lines (Fig. 2.).

This study demonstrates a spectrum of effects of TCDD on the rat third molar development, ranging from delayed eruption and diminished size to complete block of development observed only at the maternal dose level of 1 μg/kg. The resistance alleles Ahr^{hw} and B^{hw} seem to have some influence on the sensitivity to these effects, because only a single case of blocked molar development was observed both in line A and in line B compared to 60% in line C.

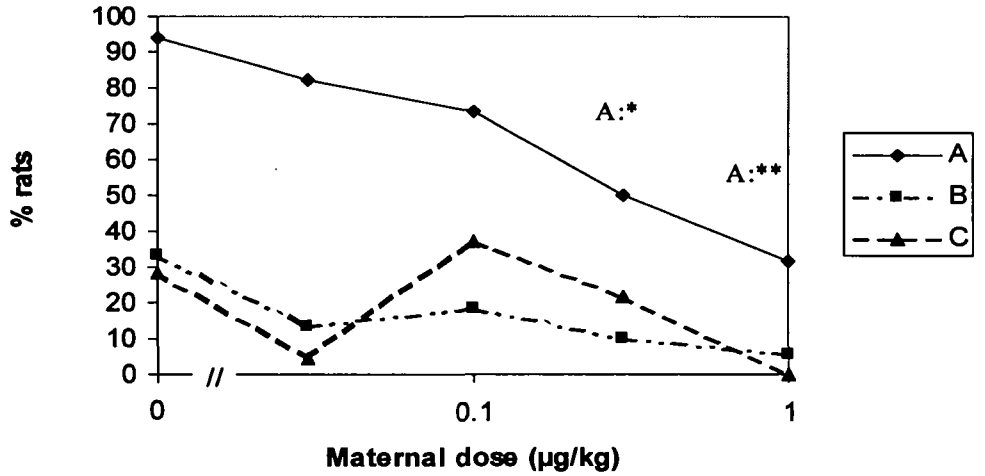

Figure 1. Effect of TCDD on the proportion of the rats whose both mandibular third molars are at least barely erupted in female rats on PND 35. Statistics: *p<0.05, **p<0.01 vs controls; Fisher's Exact Test.

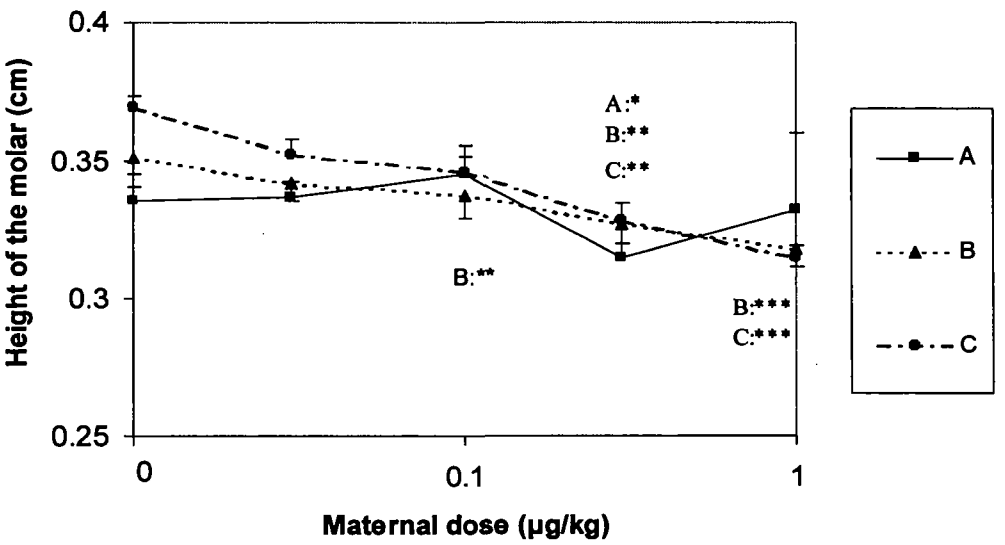

Figure 2. The height of the third mandibular molar in male rats (mean ± SE). Statistics: *p<0.05, **p<0.01, ***p<0.001 vs controls; ANOVA + LSD test. The missing third molars of line C males at 1µg/kg were excluded from the analysis.

In a previous study an extremely high dose of TCDD (1000 μg/kg) given to adult TCDD-resistant H/W rats was shown to induce perforation of the lingual surfaces, pulpal necrosis and defective dentin formation of the continuously erupting incisors[4]. In man, however, exposure to as low as the higher European background levels of PCDD/Fs via mother's milk was associated with an increased incidence of mineralization defects of the first permanent molars that undergo mineralization during the first year of life[1]. Based on this finding it was suggested that tooth development is a sensitive biomarker of dioxin exposure in man. In accordance, our data indicate that molar development is among the most sensitive endpoints of *in utero* / lactational TCDD exposure in rats. It is also interesting to note that a relatively low dose of TCDD given on GD 15 is able to block the development of a whole organ. Further studies to clarify the mechanisms of these effects are therefore warranted.

Acknowledgements

We thank Ms. Arja Tamminen and Ms. Minna Voutilainen for excellent technical assistance. This study was financially supported by the Academy of Finland, Research Program for Environmental Health (Contracts No 42551 and 43353) and the European Commission (Contract No QLK4-1999-01446).

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