available at www.sciencedirect.com

ScienceDirect

journal homepage: www.intl.elsevierhealth.com/journals/arob

Qualitative effects of dioxin on molars vary among inbred mouse strains

J.M. Keller *, *Y.M. Huet-Hudson*, *L.J. Leamy*

Department of Biology, University of North Carolina at Charlotte, Charlotte, North Carolina 28223, United States

ARTICLE INFO

Article history:
Accepted 21 October 2006

Keywords:
Dioxin
TCDD
Inbred strains
Molars
Mice

ABSTRACT

Objective: We evaluated the effects of different levels of the potent environmental toxicant and teratogen, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), on molar development in mice in six inbred strains, all with TCDD responsive *Ahr* alleles.

Design: Pregnant females were exposed on gestation day 13 to 4 different levels of TCDD (control, 0.01, 0.1 and 1.0 μg/kg) and their offspring were examined for the frequency of missing third molars (M3s) and for differences in first mandibular molar (M1) cuspal morphology.

Results: Missing M3s were prevalent only in mice in two strains, C3H/HeJ and CBA/J, and their frequency significantly increased with increasing TCDD exposure. The frequency of the M1 variant was high in mice in only one strain, C57BL/10J, and was significantly higher in the treated compared with the control group.

Conclusions: Inbred mice strains exhibited differential responses to TCDD suggesting that there is a genetic component, beyond *Ahr* differences, mediating the effects of TCDD on molar development.

1. Introduction

Embryonic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a potent environmental toxicant, has been shown to interfere with tooth development. In humans, increased incidences of dental defects have been associated with childhood TCDD exposure.[1] In addition, in vitro studies have found that TCDD exposure alters dental cell organization, enamel and dentin deposition, and cuspal morphology in cultured embryonic molar teeth.[2,3] Similarly, the continuously erupting incisors of rats exposed to TCDD from 10 to 20 weeks of age exhibited dose-dependent changes in dental tissues.[4]

While tooth development, in general, appears to be sensitive to TCDD's effects, strain-specific differences in sensitivity have been attributed primarily to differences in the TCDD binding affinity of different alleles at the aryl hydrocarbon receptor (AHR) locus.[5,6] We therefore began an investigation into the effects of varying prenatal exposures of TCDD on molar size and shape in mice from six different inbred strains, all with high affinity *Ahr* alleles. During the molar digitization process, we discovered a number of mice with missing third molars as well as some mice with an unusual morphological variant of the first mandibular molar (M_1). We decided to test whether prenatal exposure to TCDD might be responsible for these effects and if so, whether the

* *Corresponding author at*: University of North Carolina at Charlotte, 9201 University City Blvd., Woodward Hall, Charlotte, NC 28223-0001, United States. Tel.: +1 704 687 8671; fax: +1 704 687 3128.
E-mail address: jmkeller@email.uncc.edu (J.M. Keller).

Abbreviations: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; M1, first molar; M_1, first mandibular molar; M3, third molar; M_3, third mandibular molar; M^3, third maxillary molar; AHR, aryl hydrocarbon receptor

0003–9969/$ – see front matter
doi:10.1016/j.archoralbio.2006.10.017

effect of TCDD on these characters depended on strain. This paper reports the results of that investigation.

2. Materials and methods

2.1. Population

Six inbred mouse strains (C57BL/6J, BALB/cByJ, A/J, CBA/J, C3H/HeJ, and C57BL/10J) possessing the high affinity ligand binding *Ahr* allele (*b*) were purchased from Jackson Laboratories (Bar Harbor, Maine). Each strain was maintained and bred separately in the University of North Carolina at Charlotte vivarium. All animals were provided Purina Mouse Chow (Formula Number 8604 or Formula Number 2014 for pregnant and nursing females; Harlan Teklad, Indianapolis, IN) and water ad libitum. Each night, a number of females from a subset of the six strains were caged with males of the same strain. The following morning, each of these females was examined for the presence of a vaginal plug, which was taken as an indication of pregnancy and marked the beginning of gestation (gestation day 0; GD0).

Thirteen days after the start of gestation (GD13), each pregnant female was placed into one of four groups and dosed via oral gavage. Treatment group 1 (T1) received a dose of 0.01 μg TCDD/kg body weight, treatment group 2 (T2) received 0.1 μg TCDD/kg body weight, and treatment group 3 (T3) received 1.0 μg TCDD/kg body weight. All three treatment solutions were derived from an initial stock solution of TCDD (Sigma–Aldrich Inc., St. Louis, MO) and corn oil that was serially diluted with additional corn oil to produce mixtures with final concentrations that allowed all groups to receive similar gavage volumes (approximately 6–11 μl). The control group (C) was given an equal volume of corn oil without TCDD. Dose selection for each mother was based on the current distribution of dosage groups within and between strains. GD13 was chosen for dosing because while the first morphological signs of tooth development are seen on GD11, the first visible signs of the M1 occur on GD13–14, and final cuspal morphology is not determined until after GD15.[7]

The F_1 offspring of the females from each strain and treatment group were weaned and separated by sex at 28 days of age, euthanized at 70 days of age, and then skeletonized. All procedures involving the treatment of animals were approved by the Institutional Animal Care and Use Committee at the University of North Carolina at Charlotte.

2.2. Traits

Each mouse was examined for the presence or absence of both maxillary (M^3) and mandibular third molars (M_3) on both the left and right sides. In addition, all mice were scored as either normal or variant in M_1 morphology for both molar rows. Fig. 1 contains examples of both the normal (A) and

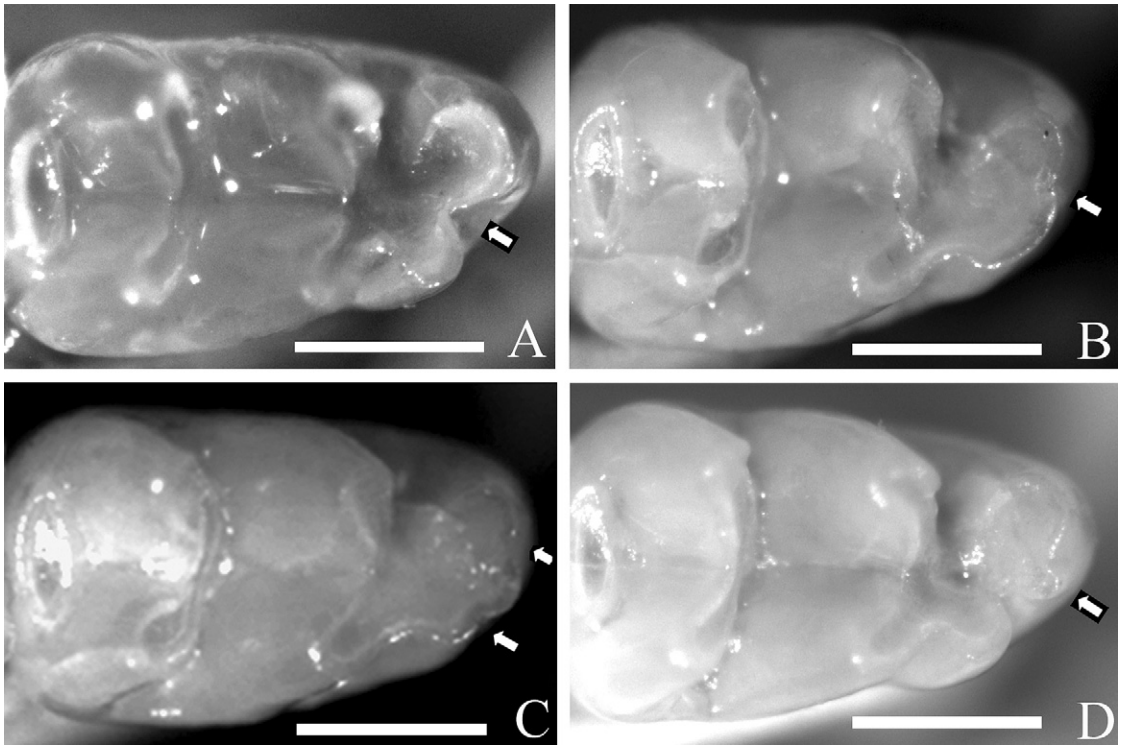

Fig. 1 – Morphological variation among first mandibular molars of C57BL/10J mice. (A) Normal morphology of right first mandibular molar. An obvious cleft is visible between the first buccal and lingual cusps (arrow). (B–D) Variant morphologies of right first mandibular molars. (B) No significant cleft is present between the first buccal and lingual cusp (arrow). (C) No significant cleft is present between the first buccal and lingual cusp, but two subtle indentations (arrows) are visible suggesting the development of an additional cusp. (D) A small additional cusp is present between the typical buccal and lingual cusps (arrow). Bars: 500 μm in A–D.