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Exposure to a continuous low dose of tetrachlorodibenzo-p-dioxin impairs the development of the tooth root in lactational rats and alters the function of apical papilla-derived stem cells

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ABSTRACT

Objectives: Ubiquitous environmental pollutants such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) cause abnormalities in reproduction and development. TCDD inhibits the development of teeth, and its effects depend on its dose and the developmental stage of the tooth. Our aim here was to investigate the effect of lower doses of TCDD on the development of the tooth root *in vivo* and *in vitro*.

Design: We observed tooth root development in lactational rats exposed to continuous low doses of TCDD starting on postnatal day 6 using Mico-CT analyses and histopathological examinations. And then the characteristics of stem cells derived from the apical papilla (SCAPs) were evaluated and compared with SCAPs induced by lower doses of TCDD both *in vitro and in vivo*.

Results: The results of experiments showed that rat pups exposed to low dose TCDD at prenatal stage developed, dentine hypoplasia, and hypomineralization. Further, TCDD impaired the functions of SCAPs *in vivo* by inhibiting cell proliferation and osteogenic and odontogenic differentiation. The impairment of SCAPs after TCDD exposure was accompanied by increased expression of AHR, down-regulation of the expression of

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Runx2, and alkaline phosphatase, suggesting that the AHR pathway mediated the effects of TCDD.

Conclusion: These results provide the first insights into the toxicity of TCDD, which adversely affects the development of the tooth root through indirectly altering the function of SCAPs.

1. Introduction

Prenatal development is highly sensitive to the effects of environmental contaminants. Dioxins contaminate the environment and exert a wide range of adverse effects on humans and animals. One of the most toxic dioxins is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which is generated by combustion, resists biological degradation, and accumulates in animal tissues.[1] Environment contamination by dioxins has rapidly increased in China and represents a significant risk to public health and safety.[2] For example, the development of hard tissue, the tooth in particular, is highly sensitive to dioxin toxicity which is readily transmitted to offspring during gestation or by breast milk.[3]

Tooth and bone development are genetically programmed but are susceptible to environmental factors. Because teeth cannot be remodelled in contrast to bone, developmental defects caused by genetic or environmental abnormalities are usually permanent. For example, TCDD causes a variety of toxic effects on teeth in both developing and adult animals. The adverse effects of TCDD on developing human teeth include blocked eruption, mineralization defects, abnormally shaped roots, and loss of permanent teeth.[3,4] Exposure to high doses of dioxins impairs periodontal tissues and perturbs amelogenesis in rhesus macaques.[5] A single high dose of TCDD administered to healthy adult rats or lactating pups causes chronic defects in erupting incisors and blocks the development of third molars of offspring.[6,7] During pregnancy, TCDD exposure interferes with tooth morphogenesis, cell differentiation, and root development, which leads to tooth malformation in the offspring.[8–10]

TCDD mediates numerous adverse biological effects, mainly through the aryl hydrocarbon receptor (AHR). AHR is a cytosolic receptor and a member of the basic helix-loop-helix/PAS protein family. AHR dimerizes with the aryl hydrocarbon nuclear translocator, and this complex binds to dioxin-responsive elements in the genome, which initiates a cascade of events, including proliferation, differentiation, and apoptosis.[11,12] Evidence indicates that the epidermal growth factor receptor signalling pathway may mediate the toxicity of TCDD by an unknown mechanism.[13,14]

The major risk for embryos and juvenile offspring is generally a low dose of TCDD, which may be present in the environment, and the defects induced by TCDD may be irreversible.[14] At birth, tooth root development is still incomplete and continues after the tooth erupts into the oral cavity. Stem cells (SCAPs) isolated from the apical papilla of immature teeth generate pulp tissue and roots.[15,16] Moreover, the impairment of tooth development by high doses of TCDD occurs in the embryo and continues to adulthood; however, whether continuous low exposure to TCDD causes defects in root development is unknown. Therefore, the aims of the present study were to analyse tooth development in lactational rats exposed to continuous low doses of TCDD starting on postnatal day 6 as well as to determine the effects of TCDD on the proliferation and differentiation of SCAPs derived from the apical papilla.

2. Materials and methods

2.1. Animals and experimental design

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of Fourth Military Medical University. The protocol was approved by the Committee on the Ethics of Animal Experiments of Fourth Military Medical University (Permit Number: IRB-REV-2013007). All nude mice and Sprague Dawley rats were purchased from the Laboratory Animal Center of The Fourth Military Medical University. One male was mated with 1–2 females at 12 weeks of age. Postnatal 0 day (PND0) indicates the day of birth. We randomly picked up 30 healthy offspring and divided them into three groups of 10 pups. On PND6, rats in each group were administered intraperitoneal injections of dimethylsulfoxide (DMSO), 1 μg/kg, or and 5 μg/kg TCDD (Sigma–Aldrich, St. Louis, MO, USA) twice weekly for five weeks.[17] The offspring was rendered unconscious with CO_2 and then euthanized by cervical dislocation on postnatal weeks.

2.2. Micro-computed tomography (CT) and histomorphometric analyses

The maxillofacial regions were scanned using the Inveon micro-CT system (Siemens AG, Germany) to detect abnormalities of structure and mineralization using an 80-kV, 500-mA microfocus X-ray source. The first molars with alveolar bone were fixed with 4% paraformaldehyde (PFA), decalcified with 17% EDTA (pH 7.0), and embedded in paraffin. For histological and morphometric analysis, the tissue sections were deparaffinized and stained with haematoxylin and eosin (H&E).

2.3. Isolation and culture of stem cells isolated from the apical papilla (SCAPs)

Referring to the previous studies,[18,19] the apical papilla tissue from the immature roots of the first molars were removed on PND6 and minced into approximately 1-mm^3 fragments. The SCAPs were purified from the apical papilla using the Percoll