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Research paper

Prenatal low dosage dioxin (TCDD) exposure impairs cochlear function resulting in auditory neuropathy

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ABSTRACT

2,3,7,8-tetrachorodibenzo-p-dioxin (TCDD), a ubiquitous and persistent environmental contaminant, is a potent teratogen. Whereas developmental TCDD toxicity is mediated by the aryl hydrocarbon receptor (AhR), the normal function of the AhR is poorly understood. We tested whether dioxin exposure during a critical period of hair cell development disrupts cochlear function in three mouse strains, (C57BL6, BalbC, and CBA) that contain high affinity AhR-b alleles. C57BL/6, BalbC, and CBA dams were exposed to 500 ng/kg TCDD or olive oil (vehicle) on embryonic day 12 by gavage. Cochlear function was analyzed at 1.5 months of age by measuring 1) auditory brainstem response (ABRs) to tone pips from 5.6 to 30 kHz, and 2) distortion-product otoacoustic emissions (DPOAEs) evoked by primaries with f_2 at the same frequency values. Cochlear threshold sensitivity following TCDD exposure was significantly elevated in both female and male mice in the C57BL/6 strain, carrying the Ahb-1 allele, but not significantly elevated in the BalbC or CBA strains, carrying the Ahb-2 allele. These ABR threshold deficits in mice carrying the Ahb-1 allele parallels the cleft palate incidence to higher TCDD exposures, suggesting that ABR testing could serve as a sensitive indicator of TCDD toxicity in at-risk children. Moreover, DPOAEs were not affected following TCDD exposure in any of the mouse strains, suggesting that following TCDD exposure mice with the Ahb-1 allele exhibit a mild auditory neuropathy. The causes of many auditory neuropathies are unknown, yet a developmental exposure to dioxin may be a risk factor for this condition.

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1. Introduction

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, dioxin), the most potent teratogen belonging to the halogenated aromatic hydrocarbon (HAH) family, is a ubiquitous and persistent environmental contaminant. Toxicity associated with TCDD exposure is mediated through the arylhydrocarbon rececptor (AhR), a ligand-activated transcription factor whose normal function is not well characterized. When activated, the AhR translocates to the nucleus, where it dimerizes with the aryl hydrocarbon receptor nuclear translocator (Arnt), and interacts with xenobiotic response elements (XREs) in 5′-regulatory regions of target genes, which include xenobiotic metabolizing enzymes and growth regulatory molecules (Bock and Kohle, 2006; Wilson and Safe, 1998; Bock, 2013). Bemis et al. (2005) developed a lacZ reporter mouse model driven by activation of the AhR, and found that exposure to TCDD on embryonic day 14 resulted in strong activation of the lacZ transgene in numerous tissues, including the inner ear otocyst. Interestingly, TCDD and other coplanar polychlorinated biphenyls (PCB) induced similar patterns of AhR activation in the inner ear otocyst. Moreover, prenatal exposures to PCB mixtures containing both non-coplanar and coplanar PCBs have caused auditory deficits in rats (Powers et al., 2006).

A known teratogenic effect of high doses of prenatal dioxin exposure is an increased incidence of cleft palate. In order to determine if cleft palate incidence segregates with the allelic binding affinities of the AhR, Powers et al. (2006) took advantage of the known mouse strain differences in AhR alleles, with the b alleles (Ahb-1, Ahb-2, Ahb-3) characterized as having a high binding affinity for dioxin, while the d allele (AhD) has a low binding affinity (Poland et al., 1994). In this study, the strain distribution of cleft palate formation, following a single prenatal TCDD high dose exposure of 30 μg/kg to the dam on embryonic day 10 (ED10), was examined in ten mouse strains containing either the high affinity b allele for AhR, or the lower affinity d allele (Poland and Glover,

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1980). Of the five stains with the b allele, four of the strains, including c57Bl/6 (Ahb-1) showed a high occurrence of cleft palate formation, while the CBA strain (Ahb-2) and mouse strains carrying the d allelle showed no incidence of cleft palate.

To examine potential role of prenatal TCDDs in mediating ototoxicity and if ototoxicity segregated with AhR alleles, pregnant mouse dams were given a single low dose (0.5 μg/kg) of TCDD to evaluate whether prenatal exposure (ED12) disrupted auditory system development and cochlear function. Using both auditory brainstem response (ABR) and distortion-product otoacoustic emissions (DPOAE) to assess cochlear function, we found that only the c57Bl/6 mouse strain, carrying the Ahb-1 allele, upon TCDD exposure had elevated ABR thresholds with no concomitant change in DPOAE responses or cochlear structure, whereas stains carrying the Ahb-2 allele were not affected. Elevated ABR thresholds, without concommitant DPOAE losses, is a phenotype reflective of auditory neuropathy (Menten et al., 2014; Ptok, 2000). Collectively our results suggest that a subclinical gestational TCDD exposure may a risk factor for and contribute to auditory neuropathy.

2. Methods

2.1. Animals

All animal work was conducted under University of Rochester UCAR approved animal protocols in accordance with the AAALAC guidelines. Wildtype c57Bl/6, CBA mice and Balb/C mice, 6–8 weeks of age, were purchased from Jackson Laboratories for breeding. Males were removed the day after pairing, and the day of removal was considered embryonic day (ED) 0. Dams were assumed pregnant if a weight gain of greater than 1.5 g was observed by ED 9. Fifty-eight c57Bl/6 mice (29 M:29 F); 49 BalbC mice (24 M; 25 F) and 76 DBA mice (38 M, 38 F) were used in this study.

2.2. Dioxin exposure

Pregnant c57Bl/6 dams, CBA dams and Balb/C dams were exposed to 500 ng/kg TCDD or olive oil (vehicle control) on ED 12, see Fig. 1, which corresponds to the day before hair cell progenitors become committed hair cells. TCDD or olive oil were absorbed onto a vanilla wafer and administered orally to dams.

2.3. Cochlear function analysis

Auditory nerve and outer hair cell function were assessed using auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs). Cochlear function was assessed in mice at 6 weeks of age and mice were sedated with ketamine (40 mg/kg ip) and acepromazine (1 mg/kg ip) prior to testing. Intelligent Hearing Systems (IHS) Smart EP and Smart DPOAE software were used for recording auditory data and subsequent analysis.

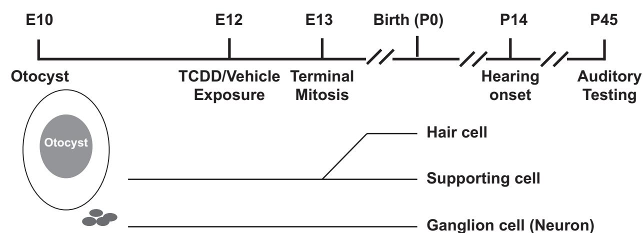

Fig. 1. Time course of cochlear development and study design. Dams were exposed to either TCDD or vehicle on embryonic day 12, which is approximately 1.5 days before terminal mitosis of the hair cell-supporting cell precursor cell pool begins. In the mouse, hearing onset occurs at postnatal day 14, and we assessed cochlear function in the pups at postnatal day 45. We also assessed cochlear function in the adult dam that were exposed to TCDD and vehicle. In all instances, at least three different litters were exposed to vehicle and at least four different litters were exposed to TCDD.

2.4. Auditory brainstem response (ABR) testing

To obtain ABR measurements, needle electrodes were inserted subcutaneously: the ground electrode above the tail, the positive electrode at the vertex and the negative electrode behind the left ear, and the microphone was inserted in the animal's left ear canal. ABR potentials were evoked with 5 ms tone pips (0.5-ms rise-fall with a $\cos^2$ onset envelope, delivered at 35/s) at 6 log-spaced frequencies: 5.6, 8, 11.3, 16, 24.4 and 30 kHz. The response was amplified (10,000×), filtered (100 Hz–3 kHz) and averaged using IHS Smart EP software. Sound level was decreased in 10 dB steps from 80 dB to 20 dB. Two waveforms per frequency per sound level were recorded at 512 averaged samples per waveform. Artifacts were rejected based on peak-to-peak amplitudes in response waveforms exceeding 15 mV. Threshold was determined by visual inspection as the sound level at which peaks were no longer present or the two waveforms were no longer synchronous. Wave 1 amplitudes and peak latencies were also determined.

2.5. Distortion Product Otoacoutic Emissions (DPOAE) testing

F_1 and f_2 primary tones for measurement of the $2f_1{-}f_2$ DPOAE were presented in a ratio of $f_2/f_1 = 1.2$ with unequal levels ($L_1 = 10$ dB + L_2). The microphone was inserted in the animal's left ear. In order to obtain moderate level DPOAE measurements, data was obtained at $L_1 = 70$ dB, $L_2 = 60$ dB for each f_2 frequency, which corresponded to the same 6 log-spaced frequencies used in ABR testing; 5.6, 8, 11.3, 16, 24.4, and 30 kHz. Ear canal sound pressure waveforms were amplified and digitally sampled at 4 ms intervals. Fast Fourier Transforms were computed from averaged waveforms and DPOAE amplitudes and noise floor sound levels were extracted. DPOAEs were rejected if amplitudes were less than 3 dB above the noise floor. DP-grams were assessed as a function of f_2 at $L_2 = 60$ dB.

2.6. Cochlear histology

Mice were perfused intracardially with 4% paraformaldehyde at 8 weeks of age. Cochleae were removed and post-fixed overnight at 4° C followed by decalcification for 72 h in 0.12 M EDTA at room temperature. Cochleae were then either prepared for plastic sectioning or microdissected for staining. Cochleae from three C57Bl/6 male animals that received TCDD exposure were compared with three C57Bl/6 male animals that received vehicle exposures.

2.7. Plastic sectioning

Cochleae were removed from EDTA and rinsed in PBS. They were then osmicated for 1 h with 1% OsO4 in 0.1 PBS, followed by PBS rinses. Cochleae were then subjected to a series of ethanol dehydrations: in 70% EtOH, 90% EtOH, 95% EtOH and 100% EtOH. Dehydrations were followed by exchanges in 100% propylene oxide, 1:1 mixture of Araldite and propylene oxide, and an incubation in a 2:1 mixture of Araldite to propylene oxide overnight. Cochleae were then removed from the mixture and placed in coffin beds with fresh Araldite and baked at 60° C. All steps were performed at room temperature unless otherwise noted. Mid-modiolar sections of about 40 μm thickness were cut using a diamond knife on a microtome. Sections were mounted on glass slides and imaged with a Zeiss Axioplan microscope outfitted with a Zeiss digital

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