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Chronic lead exposure induces cochlear oxidative stress and potentiates noise-induced hearing loss

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ABSTRACT

Acquired hearing loss is caused by complex interactions of multiple environmental risk factors, such as elevated levels of lead and noise, which are prevalent in urban communities. This study delineates the mechanism underlying lead-induced auditory dysfunction and its potential interaction with noise exposure. Young-adult C57BL/6 mice were exposed to: 1) control conditions; 2) 2 mM lead acetate in drinking water for 28 days; 3) 90 dB broadband noise 2 h/day for two weeks; and 4) both lead and noise. Blood lead levels were measured by inductively coupled plasma mass spectrometry analysis (ICP-MS) lead-induced cochlear oxidative stress signaling was assessed using targeted gene arrays, and the hearing thresholds were assessed by recording auditory brainstem responses. Chronic lead exposure downregulated cochlear Sod1, Gpx1, and Gstk1, which encode critical antioxidant enzymes, and upregulated ApoE, Hspa1a, Ercc2, Prnp, Ccl5, and Sqstm1, which are indicative of cellular apoptosis. Isolated exposure to lead or noise induced 8–12 dB and 11–25 dB shifts in hearing thresholds, respectively. Combined exposure induced 18–30 dB shifts, which was significantly higher than that observed with isolated exposures. This study suggests that chronic exposure to lead induces cochlear oxidative stress and potentiates noise-induced hearing impairment, possibly through parallel pathways.

1. Introduction

Exposure to lead, a persistent environmental pollutant that is ubiquitous in air, water, and soil, is a major public health hazard. It accumulates in soil and water over a long period of time and is absorbed by humans, predominantly, by inhalation and ingestion. Though regulatory measures prevent environmental exposures above toxic levels, sub-toxic exposures are generally unavoidable as lead is found in lead-based paints in older homes, batteries, solder, pipes, pottery, roofing materials, and some cosmetics. Exposure to even low levels of lead can cause adverse effects in multiple tissues and organs such as blood, liver, brain, and kidney (Ercal et al., 2000; Rio et al., 2001; Sanders et al., 2009; Senut et al., 2012; Song et al., 2017; Witzmann et al., 1999). In addition, exposure to lead has been reported to disrupt the structure as well as the function of the auditory system. Blood lead levels ≥2 μg/dl, which is well below the current action level (5 μg/dl) recommended by the Centers for Disease Control and Prevention (CDC advisory committee report, 2012), were associated with higher odds of high-frequency hearing loss (Shargorodsky et al., 2011). Exposure to low levels of lead during development decreased the expression of voltage-dependent anion channel proteins and disrupted the monoaminergic system in the auditory brainstem (Fortune and Lurie, 2009; Prins et al., 2010a,b). Furthermore, lead exposure induced degeneration of sensory receptor cells in the cochlea, affected auditory nerve conduction velocity, disrupted cochlear blood-labyrinth barrier, and caused vestibular dysfunction (Jones et al., 2008; Klimpel et al., 2017; Lasky et al., 1995; Liu et al., 2013; Yamamura et al., 1989). Despite these overwhelming evidences indicating the ototoxic effects of lead, the underlying mechanism is yet to be fully understood.

Generally, oxidative stress plays a critical role in auditory dysfunction as it activates cochlear cell death pathways in hearing loss associated with aging, exposure to noise, organic solvents, heavy metals such as cadmium, ototoxic drugs such as aminoglycosides and cisplatin, and radiation (Bottger and Schacht, 2013; Huth et al., 2011; Jamesdaniel et al., 2016; Kim et al., 2008; Poirrier et al., 2010; Samson et al., 2008; Warchol, 2010; Wong and Ryan, 2015). Oxidative stress-

Abbreviations: SPL, sound pressure level; ICP-MS, inductively coupled plasma mass spectrometry; PCR, polymerase chain reaction; OSHA, occupational safety and health administration; ABR, auditory brainstem response

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induced damage has been detected in three discrete regions of the cochlea, namely, the sensory epithelium, the lateral wall, and the modiolus, in acquired hearing loss. Though lead exposure also damages the sensory, vascular, and neuronal components of the cochlea, it is not known whether oxidative stress mediates its ototoxic effects. However, exposure to lead has been reported to cause oxidative damage in other tissues and organs by inducing lipid peroxidation and compromising the antioxidant defense systems (Ahamed and Siddiqui, 2007; Ercal et al., 1996; Roy et al., 2015). Lead-induced oxidative damage has been reported to disrupt the functions of the reproductive, cardiovascular, urinary, and nervous systems (Ding et al., 2001; Hsu et al., 1998; Patra et al., 2001; Patrick, 2006). Therefore, this study sought to investigate whether chronic exposure to lead acetate in drinking water induces oxidative stress in the cochlea.

Environmental exposures to ototoxicants are usually complex and therefore, potential interactions between multiple toxicants at different levels are unavoidable. Combined exposures, even at sub-toxic levels, may have additive or synergistic interactions and can cause auditory dysfunction. For example, simultaneous exposure to even low levels of ototoxic drugs such as cisplatin and noise caused a greater shift in hearing thresholds and histological damage than that caused by isolated exposures (Boettcher et al., 1987). Similarly, simultaneous exposure to organic solvents and noise potentiated the noise-induced permanent threshold shifts (Steyger, 2009).

Although epidemiological studies have indicated an association between hearing loss and simultaneous exposure to lead and noise (Wu et al., 2000) the interaction between lead and noise exposure in inducing hearing loss has not been fully characterized. Combined exposure to lead and noise is expected to have an additive effect in the auditory system because oxidative stress, which plays a pivotal role in mediating noise-induced cochlear cell death, has been reported to facilitate the adverse health effects of lead (Ercal et al., 2001; Henderson et al., 2006; Kopke et al., 2002; Ohlemiller et al., 2000; Samson et al., 2008; Vaziri and Khan, 2007; Yamane et al., 1995). Therefore, we employed a mouse model to test the hypothesis that chronic exposure to lead will induce oxidative stress in the cochlea and potentiate noise-induced hearing loss.

2. Materials and methods

2.1. Animals

Male C57BL/6 mice (4 weeks of age) were purchased from Jackson Laboratories (Jackson Laboratories, Bar Harbor, ME) and allowed to acclimatize for 5 days at the Laboratory Animal Facility of Wayne State University. All animals were housed in a temperature-controlled room with a 12-h light/dark cycle and allowed free access to food and water. The ambient noise in the facility was 50 dB SPL (sound pressure level). Every effort was made to minimize pain and discomfort and all animals were handled and treated according to guidelines established by the National Institutes of Health. Though C57BL/6 strain harbor mutations in Cdh23 and are sensitive to noise, this strain is a good model to study both lead- and noise-induced oxidative stress (Ercal et al., 1996; Samson et al., 2008). The experimental protocol (Fig. 1) was reviewed and approved by the Institutional Animal Care and Use Committee (#16-01-038).

2.2. Lead exposure

After the baseline testing twenty four animals were randomly divided into four groups with six animals in each group. Two groups were exposed to lead through drinking water containing 2 mM lead acetate (Cat # 316512, Sigma-Aldrich, St. Louis, MO, USA), for 28 days while animals in the other two groups were given normal drinking water. Three animals were housed in one cage to ensure adequate and similar exposure for all animals. Animal weight, appearance, and behavior were monitored routinely and the mice were provided free access to standard mouse chow.

2.3. Noise exposure

Awake mice were exposed to broadband white noise at 90 dB SPL, 2 h daily for two weeks, in an acoustic chamber (Model RE-121, Acoustic Systems, Austin, TX). The animals were placed on a slowly revolving platform (1 revolution/min) in a custom designed sub-divided cage with one mouse per division. The noise signal was generated by TDT System3 RZ6 Processor (Tucker Davis Technologies, Alachua, FL), amplified by a QSC GX5 power amplifier (QSC LLC, Costa Mesa, CA) and presented to the animals with an overhead 1000 W TW67 3" titanium tweeter (Pyramid, Brooklyn, NY). Noise level was adjusted using a Model 831 sound level meter (Larson Davis, Depew, NY) equipped with a ½ inch free-field microphone (377B02, PCB Piezotronics, Depew, NY), which was calibrated using a Model CAL200 Precision Acoustic Calibrator (Larson Davis, Depew, NY). The noise levels within the cage ranged from 90 to 93 dB SPL.

2.4. Blood and cochleae collection

Animals were euthanized by CO_2 inspiration. After ensuring that there is no response to a toe pinch blood was collected by cardiac puncture (~100–150 µl) and stored in 1.5 ml Eppendorf tubes containing 10 µl of 0.5 M EDTA. Then the mice were decapitated and the cochleae were dissected out and immediately frozen in liquid nitrogen. Cochleae were kept at −80 °C degrees until further processing.

2.5. Inductively coupled plasma mass spectrometry (ICP-MS)

Blood samples were initially diluted by adding 75 µl of 0.1% TritionX-100 with 75 µl of blood. The samples were further diluted with 300 µl of 2% nitric acid and incubated between 1 and 2 h. Then the samples were centrifuged and diluted again with 2% nitric acid so that the final dilution was 50 folds. A 13-point standard curve was made with different concentrations of lead ranging from 0.05 to 200 µg/L. The analysis was performed on an Agilent 7700 Series ICP-MS. The Pb and ^{209}Bi (internal) standards were purchased from Inorganic Ventures (Christiansburg, VA).

2.6. Cochlear RNA isolation

Frozen cochleae were immersed in RNAlater-ICE (Catalog # AM7030, Life Technologies, Carlsbad, CA) for at least 16 h and then the cochlear tissue was dissected out of the bulla, under the microscope. Cochlear tissue consisting of the sensory epithelium, lateral wall, and modiolus from 2 cochleae were pooled and homogenized in 500 µl QIAzol Lysis Reagent (Catalog # 79306, Qiagen, Valencia, CA). Total RNA was isolated using the RNeasy Microarray Tissue Mini kit (catalog # 73304, Qiagen, Valencia, CA) following the manufacturer's instructions. The quality of the RNA and its concentration were verified using NanoDrop 8000 (Thermo Fisher Scientific, Rockford, IL) and the purity of RNA was determined from A260: A230 and A260: A280 ratios.

2.7. Reverse transcription-polymerase chain reaction (PCR) analysis

First strand cDNA was synthesized from 5 µg RNA using the RT2 First Strand kit (catalog # 330401, Qiagen). Then the cDNA was mixed with RT2 SYBR Green qPCR Mastermix (cat. # 330529, Qiagen) and 25 µl of the mixture was loaded in each well of the Oxidative Stress RT2 Profiler PCR Array (Cat. # PAMM-065Z, Qiagen), following the manufacturer's instructions. Real-time PCR amplification was done using the Step One RT-PCR system (Applied Biosystems, Foster City, CA), which was programed to include a 10 min Hot-Start at 95 °C, followed by 40 cycles of 15 s denaturation at 95 °C and 30 s annealing at 60 °C.