Original Article

Lead neurotoxicity in rat cochlear organotypic cultures

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Abstract Lead is a major environmental toxicant throughout the world. Lead can induce severe neurotoxicity including irreversible hearing impairment. Many in vivo studies have shown that lead damages the auditory nervous system, but has little or no effect on cochlear sensory hair cells. To gain insights on lead ototoxic and neurotoxic effects in vitro, lead acetate (LA) was applied to postnatal day 3–4 rat cochlear organotypic cultures for 24 or 72 h with doses of 0.1, 0.5,1, 2 or 4 mM. After 24 or 72 h treatment with lead acetate, nearly all of cochlear sensory hair cells were intact. However, after 72 h treatment, the peripheral auditory nerve fibers projecting to the hair cells and the spiral ganglion neurons (SGN) were damaged when lead concentration exceeded 2 mM. Our results indicated that 72 h treatment with only the high doses (≥ 2 mM) of lead actate damaged SGNs and peripheral nerve fibers; hair cells remained structurally intact even after 4 mM treatment. These results show that lead primarily damages cochlear nerve fibers andSGNratherthanhaircells.

Keywords lead, cochlea, organotypic culture, spiral ganglion neurons, hair cells.

Introduction

Industrial pollution describes the introduction of foreign substances into the biosphere. Many heavy metals, such as lead, mercury, nickel, zinc, cadmium, chromium and manganese, [1–6] can invade the human system through the food chain to cause multiple toxicities. The most adverse effects of these heavy metals on the nervous system give rise to neurotoxicity.

Lead is one of the oldest-established poisons in heavy metals, and continues to be a major public health problem throughout the world. The toxic effects of lead were involved in learning impairment, [7] cognitive and behavioral deficits, [8] as well as hearing impairment.[9, 10] In the auditory system, lead can result in decrease of auditory evoked potentials and auditory sensitivity by demyelinization and axonal degeneration in the autitory nervous system. [9–12] In addition, the developing central nervous system is particularly more susceptible to environmental lead exposure than adult. [13, 14]

In the central auditory system, the neurotoxic effects of lead can be detected in many portions in central auditory pathway. After acute treatment of lead, significant reduction of glucose metabolism is detected in the medial geniculate bodies, inferior colliculus and auditory cortex. [15] The results in previous studies demonstrate that lead exposure can alter the neuronal structural proteins by increasing phosphorylations in both medium and high-weight forms of neurofilament, which impairs the axonal transport and results in impairments of temporal processing within the auditory brainstem. [15, 16]

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In addition, VMAT2, 5-HT, and $D_{\beta}H$ expression in the superior olivary complex can also be significantly reduced by low dose lead treatment. [17] Since the medial geniculate bodies, inferior colliculus and auditory cortex belong to the auditory afferent system, while the superior olivary complex is the location of cochlear efferent neurons. Above evidence therefore suggests that the neurotoxic actions of lead can affect both afferent and efferent nervous systems in the central auditory pathway.

In the peripheral auditory system, many in vivo studies have demonstrated that lead treatment can cause a significant threshold elevation and a latency prolongation in compound action potential (CAP). [18, 19] However, the endocochlear potential (EP) shows no change. [18] and the distortion product otoacoustic emissions (DPOAE) and the cochlear microphonics (CM) remain normal, suggesting that lead in vivo systemic treatment can only impair the peripheral auditory nervous system, but spares the cochlear sensory hair cells and the stria vascularis .[18, 19] However, it is unknown if lead can injure the peripheral auditory system in cochlear organotypic cultures. The purpose of this study was to investigate the neurotoxic effects of lead to the cochlea in vitro.

Methods and materials

Cochlear organotypic cultures

Cochlear organotypic cultures were prepared from postnatal day 3 SASCO Sprague Dawley rats as described previously [5]. Briefly, to establish a collagen gel matrix, a drop (15μl) of cool rat tail collagen (Type 1, Collaborative Biomedical Products #40236) together with a mixture of 3.76 mg/ml in 0.02 N acetic acid, 10×basal medium eagle (BME, Sigma B9638), and 2% sodium carbonate at a 9:1:1 ratio were placed at the center of a 35 mm diameter culture dish (Falcon 1008, Becton Dickinson) and allowed to form a gel at room temperature for approximately 30 min. When the collagen gel matrix is prepared, 1.3 ml of serum-free medium consisting of 2g bovine serum albumin (BSA, Sigma A-4919),2mlSerum-Free Supplement (Sigma I-1884), 4.8 ml of 20% glucose (Sigma G-2020), 0.4 ml penicillin G (Sigma P-3414), 2 ml of 200 mM glutamine (Sigma G-6392), 190.8 ml of 1×BME (Sigma B-1522) were added to the dish. Sprague-Dawley rat pups at postnatal day 3 were decapitated, and the cochleae were carefully removed in Hank's Balanced Salt Solution (1X GIBCO, 14175, Invitrogen, Carlsbad, CA). The cochlear lateral wall and auditory nerve bundle in the center of modiolus were dissected away respectively and the whole basilar membrane containing the organ of Corti and SGNs was transferred onto a collagen gel matrix as a flat surface preparation. The cochlear explants were maintained in an incubator at 37°C and 5% CO2 overnight. On the following day, fresh medium with or without various concentrations of lead was added.

Lead acetate treatments

On the second day, cochlear explants (n=6/group) were treated with lead acetate (Sigma-316512) at concentrations of 0.1 mM, 1 mM, 2 mM, or 4 mM in standard culture medium for 24 h or 72 h. The cochlear explants from the normal control groups were cultured in the same standard culture medium without lead acetate for same period of 24 h or 72 h respectively.

Histological evaluation

At the end of the experiment, cochlear explants were fixed for 1 h in 10% formalin and subsequently washed with 0.1 M PBS. Cochlear cultures were immersed in a solution containing a monoclonal antibody against neurofilament 200 (Sigma N0142, clone N52) for 48 hours at 4 °C. The antibody was diluted 1:100 in blocking solution containing 1% Triton X-100 and 3% goat serum in 0.1M PBS. The specimens were rinsed with 0.01 M PBS three times and incubated for 1 h with a secondary antibody, goat anti-mouse IgG, (Sigma T5393) labeled with TRITC in centuplicate dilution. To visualize F-actin that is heavily expressed in the cuticular plate and stereocilia bundles of hair cells, specimens were labeled with Alexa 488-labeled phalloidin (Invitrogen A12379, diluted by 1:200) for 30 min. After rinsing with 0.1 M PBS, specimens were mounted on glass slides in glycerin, coverslipped and examined under a confocal microscope (Zeiss LSM-510 meta).