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Evaluation of geranylgeranylacetone against cisplatin-induced ototoxicity by auditory brainstem response, heat shock proteins and oxidative levels in guinea pigs

Wu-Chia Lo[a,b], Chen-Tu Wu[a,c], Hillary Chiao Lee[b], Yi-Ho Young[d], Yih-Leong Chang[a,c,**], Po-Wen Cheng[b,*]

[a] Graduate Institute of Pathology, National Taiwan University College of Medicine, Taipei, Taiwan
[b] Department of Otolaryngology, Far Eastern Memorial Hospital, Taipei, Taiwan
[c] Department of Pathology, National Taiwan University Hospital, National Taiwan University College of Medicine, Taipei, Taiwan
[d] Department of Otolaryngology, National Taiwan University Hospital, Taipei, Taiwan

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ABSTRACT

This study aims to assess whether geranylgeranylacetone (GGA) could reduce ototoxicity induced by cisplatin through upregulation of not only heat shock protein(HSP)-70, but also HSP-27 and HSP-40, and to study if GGA would reduce cisplatin-induced increase in oxidative stress. 48 guinea pigs were used in this study and treated with the following regimen: 0.5% CMC (sodium carboxymethyl cellulose) control for 7 days, GGA (600 mg/kg/d) for 7 days, a combination of GGA (600 mg/kg) for 7 days and then one dose of 10 mg/kg cisplatin (GGA + Cis), and a combination of CMC for 7 days and then 10 mg/kg cisplatin (cisplatin group). Auditory brainstem response (ABR) measurement was performed in each animal at time before treatment and 7 days after the last dose. Additionally, HSPs, nitric oxide (NO), and lipid peroxidation (LPO) levels in cochlear membranous tissues were assessed. The mean ABR thresholds in the cisplatin group were significantly ($p < 0.05$) increased when compared to the other three groups. In guinea pigs receiving both GGA and cisplatin, the mean threshold shift (TS) were smaller ($p < 0.05$) than those of the cisplatin group, but larger ($p < 0.05$) than those of the CMC control or GGA only group with statistical significance. Compared to the GGA only group or the group treated with GGA + Cis, the cisplatin group had the highest ($p < 0.05$) oxidative stress (NO and LPO levels), and the lowest ($p < 0.05$) mean HSPs expression levels. It can be concluded that GGA attenuate ototoxicity induced by cisplatin through upregulation of HSP-27, -40, and -70. Moreover, increased oxidative stress induced by cisplatin in the cochlea membranous tissue could be reduced by pre-treatment of GGA.

1. Introduction

Cisplatin is an effective anti-neoplastic agent widely used for the treatment of various types of cancer, particularly for head and neck, testicular, ovarian, bladder, stomach, and small cell lung cancers (Blumenreich et al., 1985; Veronesi et al., 1985). After patients are exposed to this drug, progressive and bilateral high-frequency sensorineural hearing loss have been reported in subjects (Blumenreich et al., 1985; Sergi et al., 2003; Thomas Dickey et al., 2004). Although lowering its dosage could minimize these side effects, the therapeutic efficacy of cisplatin would also be diminished (Berry et al., 1990). Along with patchy damage of inner hair cells, it has been shown that damage induced by cisplatin in the cochlear membranous tissue is the loss of outer hair cells located in the middle and basal cochlear turns (Campbell et al., 1996; Campbell et al., 2003; Sergi et al., 2003). Morphologically, injury to spiral ganglionic cells and degeneration of the stria vascularis after cisplatin exposure was also stated (Zheng and Gao, 1996; Campbell et al., 1999). The extent of hair cells loss and subsequent increased ABR-measured hearing threshold shifts are dose-dependent (Hoeve et al., 1988, Cheng et al., 2005).

In a healthy and functioning ear, the cochlea generates reactive oxygen species (ROS) as a byproduct of its normal metabolic functions (Kopke et al., 1999). Stresses such as aminoglycoside exposure, acoustic overexposure, ischemia, and cisplatin treatment can result in cellular trauma and increased ROS in cochlea (Kopke et al., 1999). After treatment with cisplatin, it was reported that increased cellular

* Correspondence to: P.-W. Cheng, Department of Otolaryngology, Far Eastern Memorial Hospital, 21, Section 2, Nan-Ya South Road, Banqiao, New Taipei 22061, Taiwan.
** Correspondence to: Y.-L. Chang, Department and Graduate Institute of Pathology, National Taiwan University College of Medicine, 7, Chung-Shan South Rd., Taipei 10002, Taiwan.
E-mail addresses: ntuhylc@gmail.com (Y.-L. Chang), pwchengpw@gmail.com (P.-W. Cheng).

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apoptosis and nitric oxide (NO) synthase levels occurred in guinea pigs (Watanabe et al., 2000). Increased ROS can lead to the depletion of antioxidant enzymes, which lead to a cascade of events, resulting in oxidation of lipids, proteins, DNA, and subsequent apoptosis (Ohlemiller et al., 1999; Simon et al., 2000; Poirrier et al., 2010). One family of proteins that provides protection against oxidative stress-induced damage is heat shock protein (HSP) (Lindquist, 1986). Induction of HSPs has been detected in response to various types of stress such as hyperthermia, ischemia, acoustic overstimulation, and hypoxia (Bruey et al., 2000). The cytoprotective response to these stresses is highly conserved and collectively known as the heat shock response. HSPs function as a molecular chaperone by stabilizing denatured polypeptides, preventing protein aggregation, trafficking proteins to proper localization, and inhibiting apoptosis (Beere et al., 2000). HSPs can be induced in the inner ear and are reported to play a cellular defense role against cochlear toxicity generated by cisplatin (Neely et al., 1991; Gower and Thompson, 1997; Oh et al., 2000).

Exogenous manipulation of the heat shock response could be used to protect the ear from ototoxicity. Numerous substances, such as arsenic, ethanol, and cadmium, have been proposed to induce a heat shock response (Lindquist and Craig, 1988). Nevertheless, these reagents are too damaging and detrimental to be used in protecting the ear. An acyclic polyisoprenoid drug, geranylgeranylacetone (GGA), has the ability to induce HSPs (Hirakawa et al., 1996), and has been used clinically as an oral anti-ulcer drug under the proprietary name Selbex since 1984 (Ishii et al., 2003). GGA has been found to have a protective effect in many tissues, including stomach, liver, brain, heart, and small intestines (Tsuruma et al., 1999; Hirota et al., 2000; Ooie et al., 2001; Fujiki et al., 2003). Additionally, no histotoxic event has been reported in these studies after the administration of GGA. In guinea pigs, GGA was reported to have a protective effect on acoustic injury through increased expressions of HSP-27, -40, and -70 in the cochlea (Mikuriya et al., 2005). There is only one report regarding the prevention effect of GGA against cisplatin-induced ototoxicity by inducing the expression of HSP-70 (Yin et al., 2009). The current study further investigates whether GGA can act as a defensive barrier through HSP-27 or -40 to prevent the ototoxic effect caused after cisplatin application. In addition, we also explore whether GGA's protective effect is in association with oxidative stress changes in the cochlea.

2. Materials and methods

2.1. Animals and experimental groups

Hartley guinea pigs *Cavia porcellus* were used in this study. All guinea pigs (200–250 g body weight) were male and had a normal tympanic membrane. All experimental protocols were reviewed and approved by the Committee for Ethics on Animal Research of Far Eastern Memorial Hospital. Forty-eight animals were used for this study. Each animal was randomly assigned to an experimental group (Fig. 1). Guinea pigs underwent hearing evaluation via auditory brainstem response (ABR) prior to the onset of medication, and ABR was repeated 1 week after completion of the drug regimen. Each guinea pig's body weight was measured at the same time point when performing ABR tests.

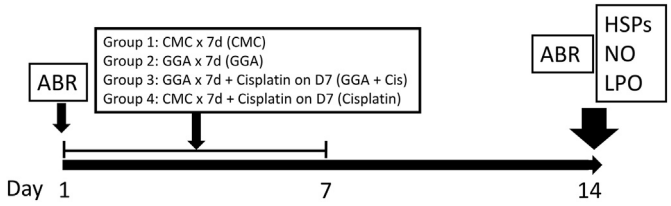

Fig. 1. A timeline of the current experiment indicating exposure and measurement time points.

In the first control group, the guinea pigs were orally administered with 0.5% sodium carboxymethyl cellulose (CMC) once a day for 7 days. CMC is a nontoxic substance used in food as a viscosity modifier or thickener. Guinea pigs in the second group received 600 mg/kg of GGA (Selbex^{R}, Eisai Co., Ltd., Tokyo, Japan) orally once a day for 7 days without following cisplatin application. In order to administer the powder-like substance of GGA to guinea pigs, it was mixed with CMC before being fed orally to the guinea pigs. The GGA dosage was selected according to previous animal studies that HSPs could be induced in cochleae on these treatment conditions (Mikuriya et al., 2005; Nakamoto et al., 2012). The third group of guinea pigs were treated with 600 mg/kg of GGA orally once a day for 7 days before receiving one dose of 10 mg/kg cisplatin via intraperitoneal (IP) injection (GGA + Cis). The guinea pigs in the fourth group were given one dose of 10 mg/kg cisplatin intraperitoneally after pre-treatment of 0.5% CMC orally once a day for 7 days (cisplatin group).

2.2. Assessment of auditory function

The ABR thresholds of all guinea pigs were assessed under 35 mg/kg pentobarbital anesthesia intraperitoneally. The responses were recorded between subcutaneous stainless steel electrodes located at the vertex (positive), the mastoid areas (negative), and the neck served as the ground. The sound stimuli consisted of a spectral peak of 3 to 4 kHz click sounds (rate 57.7/s, duration 100 μsec) through an evoked potential system (Universal Smart Box, Intelligent Hearing System, Miami, FL, USA) in a double-walled room. The stimuli were presented through a tube connecting an earphone (TIP 300, Nicolet, Madison, WI) to the external auditory canal, and 1000 stimulus presentations were averaged to develop the response curve. The stimulus intensity was evaluated with a calibrated B & K precision sound level meter. ABRs were first recorded at 100 dB sound pressure level, and stimulus intensity was reduced in 10 dB steps until the response was no longer identified. Stimulus was then increased in 5 dB steps until a reliable and replicable peak 5 in ABR waveforms was present.

2.3. Biochemical studies

2.3.1. Tissue collection

Guinea pigs were euthanized after injection of 60 mg/kg pentobarbital intraperitoneally following the post-test ABR recordings. Then the bony auditory bulla was opened after decapitation of the animal. The stapes was removed from the oval window and the inner ear was exposed. The cochlear membranous tissues (organs of Corti and the lateral walls) were gathered at 0 °C in 20 mM pH 7.4 tris buffer under the microscope. We preserved cochlear membranous tissues from two animals of each group in 0.3 ml buffer in a microcentrifuge tube and stored at − 10 °C. The collective cochlear membranous tissues were homogenized by sonication (Branson, Digital Sonifier S-450D, Danbury, CT) just before measurement of HSP, NO, and LPO.

2.3.2. Western blot analysis of heat shock proteins

The protein concentrations were determined according to BCA Protein Assay Kit (Pierce, Rockford, IL, USA). In each experiment, equal amounts (5 μg) of protein extracts were subjected to SDS-PAGE, and transferred onto nitrocellulose membranes. The membranes were blotted with antiserum against HSP-70 (Cat. #: ADI-SPA-810-D; Enzo Life Sciences, Farmingdale, NY), HSP-40 (Cat. #: ADI-SPA-400-D; Enzo Life Sciences, Farmingdale, NY), HSP-27 (Cat. #: ADI-SPA-803-F; Enzo Life Sciences, Farmingdale, NY), and β-actin (Cat. #: 3598-100; BioVision, Milpitas, CA, USA). Then, samples were incubated with anti-rabbit (1: 2000; Santa Cruz Biotechnology, Santa Cruz, Calif., USA) horseradish peroxidase immunoglobulin G and served as a second antibody. Finally, the blots were developed using ECL chemiluminescence (Millipore, Billerica, MA), the bands were visualized using FUJIFILM LAS-4000 Imaging System (FUJIFIM, Tokyo, Japan), and