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Allicin protects auditory hair cells and spiral ganglion neurons from cisplatin - Induced apoptosis

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ABSTRACT

Cisplatin is a broad-spectrum anticancer drug that is commonly used in the clinic. Ototoxicity is one of the major side effects of this drug, which caused irreversible sensorineural hearing loss. Allicin, the main biologically active compound derived from garlic, has been shown to exert various anti-apoptotic and anti-oxidative activities in vitro and in vivo studies. We took advantage of C57 mice intraperitoneally injected with cisplatin alone or with cisplatin and allicin combined, to investigate whether allicin plays a protective role in vivo against cisplatin ototoxicity. The result showed that C57 mice in cisplatin group exhibited increased shift in auditory brainstem response, whereas the auditory fuction of mice in allicin + cisplatin group was protected in most frequencies, which was accordance with observed damages of outer hair cells (OHCs) and spiral ganglion neurons (SGNs) in the cochlea. Allicin markedly protected SGN mitochondria from damage and releasing cytochrome c, and significantly reduced pro-apoptosis factor expressions activated by cisplatin, including Bax, cleaved-caspase-9, cleaved-caspase-3and p53. Furthermore, allicin reduced the level of Malondialdehyde (MDA), but increased the level of superoxide dismutase (SOD). All data suggested that allicin could prevent hearing loss induced by cisplatin effectively, of which allicin protected SGNs from apoptosis via mitochondrial pathway while protected OHCs and supporting cells (SCs) from apoptosis through p53 pathway.

1. Introduction

Cisplatin is an effective and broad-spectrum anticancer drug that is commonly used in the treatment of various human cancers, including carcinoma of the head and neck region (Kalcioglu et al., 2005; Goncalves et al., 2013). However, ototoxicity induced by cisplatin leads to irreversible high-frequency sensorineural hearing loss (Hartmann and Lipp, 2003). Studies reported that cisplatin mainly targets three areas in the cochlea: the sensory hair cells in the organ of Corti (Anniko and Sobin, 1986), the lateral wall tissues (Meech et al., 1998) and the spiral ganglion neurons in the modiolus (van Ruijven et al., 2005). Although the cytotoxic mechanism of cisplatin is not fully clear, a large number of studies have proved that the apoptosis is closely involved in DNA damage, oxidative stress and inflammatory factors (Rybak et al., 2007; Jamesdaniel et al., 2008; Park et al., 2009; Schmitt et al., 2009). Therefore, antioxidants and anti-apoptotic agents, as two major groups of molecules, are commonly investigated to evaluate the ototoxicity induced by cisplatin in the cochlea (Duan et al., 2002).

Many studies showed that allicin, a major ingredient of fresh garlic extract, played roles in antimicrobial (Cutler and Wilson, 2004; Canizares et al., 2004), antitumor (Park et al., 2005; Patya et al., 2004), antioxidant (Borek, 2001; Liu et al., 2015a), anti-inflammatory (Lang et al., 2004; Hodge et al., 2002) and anti-apoptosis activities (Zhang et al., 2008). Existing research data have shown that allicin induced the antioxidant and protected cells against oxidative stress, by reducing cytotoxic substances and scavenging free radical (Chan et al., 2013). Recently, it is

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demonstrated that allicin possess neuroprotective activity on traumatic or ischemic neuronal injury which is regulated by oxidative stress and apoptosis pathways (Liu et al., 2015a; Chen et al., 2014a; Zhou et al., 2014). A previous study also confirmed that allicin attenuated spinal cord ischemia–reperfusion injury through improving the function of mitochondrion (Zhu et al., 2012). However, there are no report to date of anti-apoptotic and anti-oxidative effects of allicin on ototoxicity induced by cisplatin. The aim of the present study therefore is to determine whether allicin would be effective in alleviating hearing loss induced by cisplatin.

2. Materials and methods

2.1. Reagents

Cisplatin was purchased from Jiangsu Haosen pharmaceutical Co. Ltd (Jiangsu, China) and dissolved in 0.9%physiological saline. Allicin was purchased from Xuzhou Lai'en Pharmaceutical Co. Ltd. (Shandong, China) and dissolved in 0.9%physiological saline. Antibodies to Bax, Bcl-2, cleaved caspase-3 and cleaved caspase-9 were acquired from Cell Signaling Technology (Beverly, MA, USA). Antibody to Tuj 1 was acquired from Neuromics (Edina, USA). Antibody to cytochrome c, actin and P53 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Click-iT Plus TUNEL assay kits was purchased from Life Technologies (Invitrogen, USA). Superoxide dismutase (SOD) and malonaldehyde (MDA) assay kits were all purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). BCA protein assay kit was a product from Beyotime (Shanghai, China).

2.2. Experimental animals

All experiments were performed according to protocols approved by the Animal Care Committee of Shandong University on the care and use of Laboratory Animal for Research Purposes. C57 mice were purchased from Animal Center of Shandong University (Jinan, China). All animals were bred and housed in standard box cages in a climate-controlled room with an ambient temperature of 23 ± 2 °C and a 12/12 h light/dark cycle. Animals were fed standard laboratory chow, given water freely and were assigned randomly to control or experimental groups. Experiments were performed on age- and sex-matched 7- to 8-week-old mice weighing 17–23 g. The mice were divided into three groups ($n = 30$ each; group 1, 0.9% physiological saline-injected controls; group 2, cisplatin-injected; group 3, cisplatin + allicin-injected). Mice in groups 1, 2 and 3 received intraperitoneal (i.p.) injections of 0.9% physiological saline (0.6 ml/100 g), cisplatin (3 mg/kg) or allicin (18.2 mg/kg), respectively. Group 1 mice were administered with 0.9% physiological saline (0.6 ml/100 g i.p.) for seven consecutive days. Group 2 mice were administered with cisplatin (3 mg/kg i.p.) for seven consecutive days. To evaluate the effects of allicin on cisplatin-induced ototoxicity, groups 3 mice were given 18.2 mg/kg allicin i.p. one day ahead and at 2 h before the daily injection of cisplatin.

2.3. Measurement of auditory brain stem response

The auditory brain stem response (ABR) was measured before and after seven day injection. TDT system hardware and software (Tucker-Davis Technologies, Alachua, FL,USA) were used to record ABRs, with 1024 stimulus repetitions per record. Mice were anesthetized with a chloral hydrate (400 mg/kg) and kept warm during ABR recordings. The record electrode was inserted into subcutaneous tissue at the vertex, reference and ground electrodes were placed subcutaneously at ipsilateral mastoid and back, respectively. Tone bursts of 4 ms duration with a rise–fall time of 1 ms at frequencies of 4,8, 12,16, 24 and 32 kHz were presented to the left ear through a metal loudspeaker in the external auditory meatus. The sound intensity was varied at 10 dB intervals near threshold. Threshold Judgment of three groups was made by the same person.

2.4. Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end-labeling (TUNEL) assay

TUNEL were performed according to the manufacturer's instruction of Click-iT Plus TUNEL assay kits. At last, each section were stained with DIPA(1:1000) solution for 15 min at 37 °C and protected from light. After washing with PBS, samples were examined using a laser scanning confocal microscope (Leica, Germany).

2.5. Survival outer hair cell (OHC) and spiral ganglion neuron (SGN) counting

2.5.1. OHC counting

Seven days after drug administration, C57 mice were anesthetized with lethal doses of chloral hydrate and the cochleae were harvested. After fixation for 2 h and decalcification with 0.5 M EDTA for 6 h, cochleae were dissected under a microscope by removing the lateral wall of the cochlea, spiral ligament and modiolus. The remaining tissue was cochlear sensory epithelium. The epithelia were divided into three segments (apex, middle, and base) and stuck to a small glass slide. The OHCs were evaluated in three groups ($n = 4$ per group). The residual OHCs were counted in a 1 mm long strip at the region from base to apex.

2.5.2. SGN counting

Seven days after drug administration, C57 mice were anesthetized with lethal doses of chloral hydrate and the cochleae were harvested and perfused with 4% paraformalde-hyde in PBS. After decalcification and dehydration, The tissues were embedded by OCT in dry ice for frozen section. The spiral ganglion cells were evaluated among three groups ($n = 5$ per group). The spiral ganglion cell countings were performed within the unit area in three sections from each cochlear in apical, middle and basal turns.

2.6. Transmission electron microscopy (TEM) assessment of outer hair cell and spiral ganglion cell

Briefly, animals were decapitated under deep anesthesia, and the cochleae tissue was removed, washed fast with PBS, immediately placed in 3% glutaraldehyde fixative solution (pH 7.4), the sample block trimming 1 mm × 1 mm × 3 mm, according to the conventional TEM sample preparation method followed by rinsing, 1% osmic acid (OsO4) fixed, rinsing, dehydration, soaked, epon812 embedded; semi- and ultra-thin radial sections were cut from the basal and middle turns with lead citrate and uranyl acetate electron staining. Finally, the sections were observed using a transmission electron microscope (JEOL-1200EX) in JiNan WeiYa Bio-Technology Co, Ltd. (Jinan, China).

2.7. Western blotting

Seven days after drug administration, the proteins from mice cochleae were extracted and expressions of cleaved-caspase-9, cleaved-caspase-3, Bax and Bcl-2 genes were examined by Western blot as described (Liu et al., 2011). Briefly, total protein was extracted from cochleae using radio-immune precipitation buffer protein lysis buffer. The protein content of the samples was measured using the BCA protein assay kit. 30 μg of each protein sample was separated by 12% SDS-PAGE gels. The primary antibodies were (anti-Bcl-2, 1:1000; anti-Bax, 1:1000; anti-cytochrome