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Comparison of the effects of lipoic acid and glutathione against cisplatin-induced ototoxicity in auditory cells

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ABSTRACT

Objectives: The aims of this study were to examine lipoic acid (LA)- or glutathione (GSH)-mediated protection against cytotoxicity following cisplatin exposure in HEI-OC1 auditory cells and measure the potential of LA and GSH to scavenge reactive oxygen species (ROS). This study also compares their protective effects and discusses the determination of a preventive or therapeutic dose.
Methods: HEI-OC1 cells were pretreated with LA or GSH for 24 h and then exposed to 15 μM cisplatin for 48 h. The resulting cytotoxicity was measured using a cell counting kit-8, and intracellular ROS level was measured using flow cytometry. The protective or anti-ROS effects of LA and GSH were compared. Measurement of caspase 3, 8, 9 activity and Western blot analysis of PARP were performed.
Results: Pretreatment with LA at 300 μM and GSH at 3 mM protected HEI-OC1 cells against cisplatin-induced cytotoxicity and significantly reduced the cisplatin-induced increase in ROS. LA showed a significantly more effective protection against cisplatin-induced ototoxicity compared to that shown by GSH (85.4% vs. 73.1% cell viability). Both LA and GSH showed the maximal protective effect at different concentrations in normal or cisplatin-induced cytotoxic conditions. The preventive or therapeutic dose for harmful conditions is quite different for the two drugs and needs careful adjustments.
Conclusion: This comparative study on the protective effects of LA and GSH against cisplatin-induced ototoxicity in an auditory cell line posed many challenges. Although LA and GSH showed a significant protective effect against cisplatin, the LA's effect was superior. The concentration at which the maximal protective effect of LA or GSH was noted was 3 times higher in cytotoxic conditions than in normal conditions, which suggests the need for drug dose adjustments based on the purpose (preventive or therapeutic).

1. Introduction

Lipoic acid (LA), also known as alpha lipoic acid is an antioxidant produced by the body. It is found in all cells, and helps in converting glucose into energy (aerobic metabolism). Recently, LA has gained popularity for exerting anti-aging effects, which are induced by the modulation of signal transduction, thereby improving the antioxidant status of the cell [1,2]. In Asian countries, LA is marketed as an antioxidant and a weight loss and anti-fatigue supplement. LA is both fat and water soluble whereas other antioxidants such as vitamin C and vitamin E act only in water and fat tissues, respectively. Thus, LA can exert its antioxidant mechanisms throughout the body, which seems to be an advantage in serving as an antioxidant or a health supplement [3,4].

Glutathione (GSH) is an important antioxidant that can scavenge reactive oxygen species (ROS), such as free radicals and peroxides, which induce oxidative stress [5]. GSH helps in modulating the human immune system, which can fight infections and prevent cancer [6]. In addition, GSH is also important as a cofactor for the enzyme glutathione peroxidase, in the uptake of amino acids, and in the synthesis of leukotrienes [7]. As a substrate for glutathione S-transferase, GSH reacts with a number of harmful chemical species to form harmless inactive products. GSH is recently considered as a representative of antioxidant and detoxifying enzymes and is even marketed as skin-lightening or whitening drug.

LA and GSH are important antioxidants used in the field of preventive medicine, and both drugs are the most popular in

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aesthetic field. However, their beneficial effects have been challenged, and the preventive doses of antioxidant supplements or therapeutic doses for cytotoxic conditions have not been clearly defined.

Cisplatin (cis-diamminedichloroplatinum II) is a chemotherapeutic agent widely used to treat solid tumors in humans. However, cisplatin has severe adverse effects such as nephrotoxicity, neurotoxicity, and ototoxicity [8,9]. Especially, bilateral, progressive, irreversible, dose-dependent neurosensory hearing loss can be occurred in the patients receiving chemotherapy. In mechanisms, cisplatin may induce cytotoxicity by generating ROS, which depletes glutathione and antioxidant enzymes [10–12]. Thus, animal or cellular models can be used as effective test models for determining the anti-oxidative effect of drugs or supplements [13–15].

This study aimed to examine the protective effects of LA or GSH against cytotoxicity in HEI-OC1 cells following cisplatin exposure. In addition, this study measures the potential of LA and GSH to scavenge ROS. It also compares the protective effects of LA and GSH, and discusses the determination of appropriate preventive or therapeutic dose.

2. Materials and methods

2.1. Chemicals

Cisplatin (CAS no. 15663-27-1, Sigma-Aldrich Chemical Co., St Louis, MO, USA) was diluted in cell culture medium to prepare a 5 mM stock solution and added directly to each culture well. LA and GSH were purchased from Sigma-Aldrich Chemical Co. (St Louis, MO, USA).

2.2. Cell culture

The HEI-OC1 cell line was established from a transgenic immortomouse, and the cell line was provided by F. Kalinec (House Ear Institute, Los Angeles, CA, USA). The HEI-OC1 cells were maintained in high-glucose Dulbecco's Modified Eagle's Medium (DMEM; Gibco BRL, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS; JRH Bioscience, Lexena, KS, USA) and interferon-γ (50 U/mL) without antibiotics at 33 °C and 10% CO_2 in air. HEI-OC1 cells are extremely sensitive to ototoxic drugs and also express several molecular markers characteristic of the organ of Corti sensory cells [16,17].

2.3. Cell viability assay

Cell viability was measured by using Cell Counting Kit-8 (CCK) assay. This is a colorimetric assay used for the determination of viable cell numbers in cell proliferation and cytotoxicity assays. CCK uses a tetrazolium salt, WST-8, which produces an orange colored water-soluble formazan.

HEI-OC1 cells (2×10^4 cells/well of 48-well plate) were incubated in varying lipoic acid (10–500 μM) or glutathione (0.1–5 mM) concentrations for 72 h. The cell populations received 10 μL of the CCK solution was added onto each well of the microplates, and these microplates were incubated for 3 h at 37 °C in a mixture of 5% CO_2 and 95% air. The optical densities were measured using a microplate reader at 450 nm (Spectra Max, Molecular Devices, Sunnyvale, CA, USA).

To examine the protective effect of LA or GSH against cisplatin-induced cytotoxicity, the cells were pretreated with LA or GSH at different concentrations for 24 h and then exposed to 15 μM cisplatin (a concentration which is known to result in 50% of cell viability in our experiments) for 48 h. Following cisplatin exposure, measured by using CCK-8 assay.

2.4. Intracellular ROS measurement

Intracellular ROS production was measured using an automated flow cytometry system (FACScan™ system, BD Biosciences, Heidelberg, Germany) at an excitation wavelength of 488 nm and an emission wavelength of 530 nm (10,000 cells/sample). Intracellular ROS was detected with an oxidation-sensitive fluorescent probe (DCFH-DA, Calbiochem, US). DCFH-DA was deacetylated and oxidized by ROS to form the fluorescent compound 2′,7′-dichlorofluorescein (DCF).

For the assay, HEI-OC1 cells were treated with 15 μM cisplatin for 48 h in the presence or absence of LA (100, 300 μM, 24 h pretreatment), GSH (1, 3 mM, 24 h pretreatment). Following this incubation, the cells were treated with 50 μM DCFH-DA and were further incubated for 30 min. The samples were measured using a FACScan flow and the mean fluorescence intensity was calculated by histogram statistics using BD CellQuest Pro software (BD Biosciences).

2.5. Measurement of caspase 3, 8, 9 activity

The enzymatic activity of caspase-3, 8, 9 was assayed with a caspase 3, 8, 9 fluorometric assay kit (Biovision, Milpitas, California, USA) according to the manufacturer's protocol. Auditory cell line lysate was prepared in a lysis buffer on ice for 10 min and centrifuged for 5 min at 14,000 rpm. The protein concentration in each lysate was measured. Use 50 μg cell lysate and add fluorometric substrate for 2 h at 37 °C. The mixture incubated with no substrate was used as a negative control. The plates were read by microplate reader (Spectra Max, Molecular Devices, Sunnyvale, CA, USA) at a 400 nm excitation filter and a 505 nm emission filter.

2.6. Western blot analysis of PARP

The following primary antibodies were purchased from Cell Signaling Technology (Danvers, MA): poly-ADP-ribose polymerase (PARP), beta-actin. Cell lysates were used in Radio immune precipitation assay (RIPA) buffer (Cell Signaling Technology, Danvers, MA, USA). The proteins (10 μg/sample) were immediately heated for 5 min at 100 °C and were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on gels. Separated

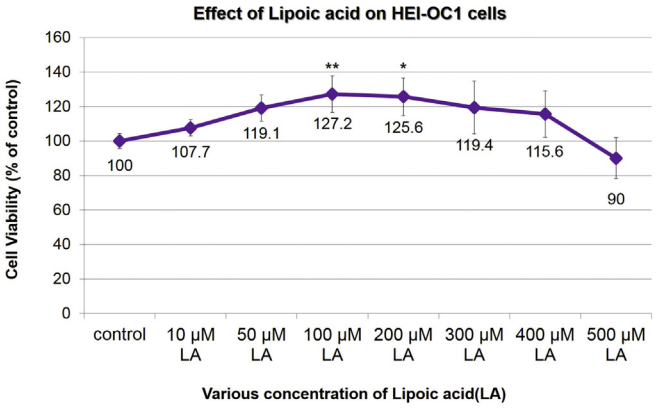

Fig. 1. Effect of lipoic acid on HEI-OC1 cells, an auditory cell line culture. HEI-OC1 cells were treated with variable LA concentrations (10–500 μM) for 72 h. The maximal protective effect of LA was observed at a concentration of 100 μM, and a significant protective effect was observed at 100 and 200 μM of LA of LA compared with the control (**$P < 0.01$, *$P < 0.05$, respectively). At concentrations greater than 500 μM, LA at a concentration greater than 500 μM induced reduced cell viability compared to the control. The results were obtained from 5 separate experiments performed in triplicate.