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Protection against arsenic trioxide-induced autophagic cell death in U118 human glioma cells by use of lipoic acid

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Abstract

Arsenic is an environmental toxicant found naturally in ground water. Epidemiological studies have suggested a correlation between chronic arsenic exposure and potential brain tissue damage in clinical case and animal experiments. Lipoic acid (LA) is a thiol-compound naturally occurring in plants and animals, which is thought to be a strong antioxidant and possess neuroprotective effects. The objective of this study was to determine if the AS_2O_3-induced glial cell toxicity could be prevented by LA. The human malignant glioma cell (U118) was selected as a research model. By using acridine orange staining and flow cytometry analysis, we found that autophagic, but not apoptotic, cell death was significantly induced by AS_2O_3 in U118 cells, and that AS_2O_3-mediated autophagic cell death was nearly completely attenuated by LA. Down-regulation of p53 and Bax proteins and the up-regulation of Bcl-2 and HSP-70 proteins were observed by western blot in AS_2O_3-mediated autophagic cell death. Our results implied that LA completely inhibited U118 cells autophagic cell death induced by AS_2O_3. We suggested that LA may emerge as a useful protective agent against arsenic-induced glial cell toxicity and reversing arsenic-induced damage in human brain.

Keywords: Lipoic acid; Arsenic trioxide; Neurotoxicity; Autophage

1. Introduction

Higher doses or chronic exposure to arsenic is still a global health problem affecting many millions of people (Ratnaike, 2003). Arsenic at a nonlethal level in drinking water consumed over a period of time may result in the manifestations of toxicity in practically all systems of the body (Hantson et al., 2003). Neurotoxic effects have been reported in clinical cases and animal experiments with chronic exposure to arsenic (Rao and Avani, 2004). Epidemiological studies also have suggested a correlation between arsenic exposure and potential neurotoxicity (Hall, 2002). For example, higher concentrations of AS_2O_3 were detected in the plasma and cerebrospinal fluid of Alzheimer's (Basun et al., 1991), mental health burden (Fujino et al., 2004), and Parkinson's disease (Larsen et al., 1981) patients.

Lipoic acid (LA) is a thiol-compound naturally occurring in plants and animals (Sohal et al., 1994). It is consumed in the daily diet, absorbed through the blood-brain barrier, and taken up and transformed in cells and tissues into

Abbreviations: AS_2O_3; arsenic trioxide; DHLA, dihydrolipoic acid; ERK, extracellular regulated kinases; FACS, fluorescence-activated cell sorter; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HSPs, heat shock proteins; JNK, c-jun terminal kinase; LA, lipoic acid; MAP, mitogen-activated protein; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide; ROS, reactive oxygen species; (ST), staurosporine.

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dihydrolipoic acid (DHLA) (Packer et al., 1997). Both LA and DHLA are thought to be strong antioxidants. Aside from acting as a potent antioxidant, LA increases or maintains levels of other low molecular weight antioxidants such as ubiquinone, glutathione, and ascorbic acid (Kozlov et al., 1999). Therefore, it appears that LA could be a potential agent in the prevention of different diseases that may be related to an imbalance of the oxidoreductive cellular status. This occurs in cases of neurodegeneration, ischemia-reperfusion, polyneuropathy, diabetes, AIDS, and hepatic disorder status (Packer et al., 1995).

Human fetal brain explants exposed to arsenic in tissue culture showed the characteristics of cell death, neuronal network damage, loss of ground matrix, cell loss and apoptosis in isolated brain cells and neighboring cells. The arsenic toxicity appears to act through interference the tissue homeostasis in the brain rather than only affect neuron cells (Chattopadhyay et al., 2002). Other studies also indicated that neurological system is the major target of toxic effects of heavy metals such as arsenic (Lee et al., 2001). Pathological alterations of glial cells have also been indicated associated with brain disease. However, the exact mechanism of the toxicity of arsenic in glial cells is not well studied. The objective of this study was to determine if the AS_2O_3-induced glial cell toxicity could be prevented by LA. The human malignant glioma cell (U118) was selected as a research model. We found that autophagic, but not apoptotic, cell death was significantly induced by AS_2O_3 in U118 cells, and that AS_2O_3-mediated autophagic cell death was nearly completely attenuated by LA. Our results provide the molecular basis for the LA prevention of AS_2O_3-induced cell death in U118 cells; such observations may have significance in clinical application.

2. Material and methods

2.1. Cell line and cell culture

Human glioblastoma cell lines U118MG (ATCC HTB-15) and U937 cells, a human pre-monocytic leukemia cell line, were obtained from the American Type Culture Collection (ATCC). U937 cells were cultured in RPMI 1640 medium supplemented with antibiotics containing 100 U/ml penicillin, 100 μg/ml streptomycin (Life Technology, Grand Island, NY), and 10% heat-inactivated fetal calf serum (FCS) (HyClone, South Logan, UT, USA). U118 cells were cultured in DMEM containing supplement as well as U937 cells with additional nonessential amino acids and MEM sodium pyruvate (Gibco, Grand Island, NY). Incubation both cells were performed in a humidified atmosphere containing 5% CO_2 at 37 °C. Exponentially growing cells were detached by 0.05% trypsin-EDTA (Gibco) in DMEM supplemented with 10% fetal bovine serum (Hyclone, Logan, UT) and antibiotics (Life Technologies, Grand Island, NY).

2.2. Determination of cell viability

Human U118 cells were treated with AS_2O_3 (1–50 μM) in the presence or absence of LA (50–100 μM) for 24 h. For the time-dependent study, U118 cells were treated with AS_2O_3 (5–10 μM) for 24, 48 and 72 h. Cell viability was determined at the indicated times based on a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. Briefly, cells were seeded in a 96-well plate at a density of 1×10^4 cells/well and allowed to adhere overnight. After removing the medium, 200 μL of fresh medium per well, containing 10 mmol/L Hepes (pH 7.4), was added. Then, 50 μL of MTT was added to the wells and the plate was incubated for 2–4 h at 37 °C in the dark. The medium was removed, and 200 μL DMSO and 25 μL Sorensens's glycine buffer was added to the wells. Absorbance was measured using an ELISA plate reader at 570 nm.

2.3. Western analysis

Proteins isolated from the U118 cells were loaded at 50 μg/lane on 12% (w/v) sodium dodesylsulfate–polyacrylamide gel electrophoresis, blotted, and probed using antibodies, including anti-caspase-3 (E8), anti-caspase-8 (E20), anti-caspase-9 (H170), cyclin E, p53, p21/Cip1, Bax, Bcl-2, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), (Santa Cruz, Inc. CA), cyclin A, cyclin E, cyclin B, and HSP70 (Transduction Laboratories, Lexington, KY). Immunoreactive bands were visualized by incubation with colorigenic substrates, nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate (Sigma Chemical Co., St. Louis, MO). The expression of GAPDH was used as the control for equal protein loading.

2.4. Determination of apoptosis

Apoptosis was judged by the following criteria: (a) Cell morphology as described previously (Ho et al., 1996). (b) Translocation of phosphotidyl serine to the cell surface detected by an Annexin V-FITC apoptosis detection kit (Calbiochem, Bad Soden, Germany), according to our previous paper (Liu et al., 2003). The U937 cells were selected as a positive control, and treated with either staurosporine (ST) (1 μM) or AS_2O_3 (20 μM) for 24 h, then harvested for Annexin V staining assay. (c) The presence of a sub-G1 peak detected by flow cytometry and measured using a fluorescence-activated cell sorter (Becton Dickinson, Heidelberg, Germany) (Lee et al., 2003). (d) The appearance of DNA fragmentation analyzed by the method described previously (Ho et al., 1996).

2.5. ROS production measurement

ROS production was monitored by flow cytometry using 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA), as described by Zegura (Zegura et al., 2004). This dye is a stable nonpolar compound that readily diffuses into cells and is hydrolyzed by intracellular esterase to yield DCFH, which is trapped within the cells. Hydrogen peroxide or low molecular weight peroxides produced by the cells oxidize DCFH to the highly fluorescent compound 2′,7′-dichlorofluorescein (DCF); thus, the fluorescence intensity is proportional to the amount of peroxide produced by the cells. The cells were incubated with 20 μM DCFH-DA. After 30 min, DCFH-DA was removed and the cells were treated with AS_2O_3 (5 μM) in PBS for 0–60 min. H_2O_2 was added to the U118 cells and incubated for 60 min as a positive control.

2.6. Supravital cell staining with acridine orange for autophagy detection

Cell staining with Acridine orange (Sigma Chemical Co.) was performed according to published procedures (Kanzawa et al., 2003; Traganos and Darzynkiewicz, 1994), adding a final concentration of 1 mg/ml for a period of 20 min. AS_2O_3 (10 μM) was dissolved in DMSO and added to the cells 30 min before the addition of acridine orange. Photographs were obtained with a fluorescence microscope (Axioscop) equipped with a mercury 100-W lamp, 490-nm band-pass blue excitation filters, a 500-nm dichroic mirror, and a 515-nm long-pass barrier filter. Flow cytometric analysis is also available to detect AVO percentage (Kanzawa et al., 2003).

2.7. Statistical analysis

Values are expressed as the mean ± S.E. The significance of the difference of the respective controls for each experimental test condition was