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Dual role of resveratrol in modulation of genotoxicity induced by sodium arsenite via oxidative stress and apoptosis

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ABSTRACT

The potential benefits of resveratrol as an anticancer (proapoptosis) and antioxidant (pro-survival) compound have been studied extensively. However, the role of resveratrol in modulation of the toxicity induced by sodium arsenite ($NaAsO_2$) is still unclear. In the present study, we examined the effects of resveratrol on $NaAsO_2$-induced cytotoxicity, DNA and chromosomal damage, cell cycle progression, apoptosis and oxidative stress in human lung adenocarcinoma epithelial (A549) cell line at concentrations from 1 to 20 μM after 24 h exposure. Our results revealed that at 1 and 5 μM, resveratrol was found to exert benefit effects, promoting cell viability and proliferation over 24 h $NaAsO_2$ exposure, whereas, resveratrol was showed to inhibit cell survival under the same condition at 20 μM. Corresponding to the opposing effect of resveratrol at low vs. high concentrations, DNA and chromosomal damage, cell apoptotic rate and level of oxidative stress were also alleviated by lower concentrations (1, 5 μM) of resveratrol, but exacerbated by higher concentration (20 μM) resveratrol. Our study implicates that resveratrol is the most beneficial to cells at 1 and 5 μM and caution should be taken in applying resveratrol as an anticancer therapeutic agent or nutraceutical supplement due to its concentration dependent effect.

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1. Introduction

Arsenic, as a common metalloid element, is naturally occurring and widely present in the food, water, air and soil. Environmentally relevant states of arsenic are organic and inorganic, and the latter exists predominantly in trivalent (As^{3+}) or pentavalent (As^{5+}) form (Rossman, 2003). Inorganic arsenic is generally considered more harmful than organic forms, and the most toxicologically potent arsenic compounds are in the trivalent oxidation state, such as sodium arsenite ($NaAsO_2$) and arsenic trioxide (Hughes et al., 2011). Epidemiological studies have shown that chronic exposure to trivalent arsenite is associated with the increased incidence of cancer in lung, skin, bladder, and liver (Cui et al., 2008; Rahman et al., 2009; Smith et al., 1992). Being a major form of trivalent arsenite, $NaAsO_2$ contamination of drinking water is a serious environmental problem worldwide due to the particularly high risk of $NaAsO_2$ for inducing human diseases (Brown and Ross, 2002; Smith et al., 1992). A number of experimental theories have also been formulated to explain the toxic effect of $NaAsO_2$ both in mammalian cells and animals (Flora, 2011; Hei and Filipic, 2004; Rossman and Klein, 2011). Several reports have revealed that exposure of human cell lines to $NaAsO_2$ elevated the production of reactive oxygen species (ROS) (Ruiz-Ramos et al., 2009; Zhang et al., 2011), which are responsible for increased intracellular oxidative stress level and result in oxidative DNA damage and apoptosis (Hei and Filipic, 2004; Wang et al., 1996). Therefore, scavengers of ROS or exogenous antioxidants have been proposed to be potentially beneficial in reducing $NaAsO_2$-induced toxicity.

Resveratrol (3,4′,5-trihydroxystilbene) is a natural polyphenolic compound found in grapes, berries, peanuts and several other plants (Bishayee, 2009). Because of its high concentration in grape skin, a significant amount of resveratrol is present in wines, especially red wines, and it may, in part, explain the "French paradox" and be responsible for many health benefits ascribed to the consumption of red wine (Donnelly et al., 2004). Besides acting as a traditional antioxidant, resveratrol is also gaining tremendous importance as it possesses cancer preventive as well as antitumor activities in various biological systems (Aggarwal et al., 2004). In animal study, resveratrol (0.01% in the drinking water) administered for 7 weeks to Min mice was found to prevent the formation of colon tumors and reduce the incidence of small intestinal

Abbreviations: AOD, average optical density; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethylsulfoxide; DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid); FBS, fetal bovine serum; GSH, glutathione; MTT, 3-(45-dimethyl thiazol-2-yl)-25-diphenyl tetrazolium bromide; $NaAsO_2$, sodium arsenite; OTM, olive tail moment; PBS, phosphate buffered saline; PI, propidium iodide; RNase A, Ribonuclease A; ROS, reactive oxygen species; SOD, superoxide dismutase.

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tumors by 70% (Schneider et al., 2001). Similar anticancer effects were observed in another mouse model, in which resveratrol was shown to effectively reduce tumor volume (42%), tumor weight (44%) and metastasis to the lung (56%) at doses of 2.5 and 10 mg/kg (Kimura and Okuda, 2001). Likewise, resveratrol has been reported to possess in vitro cytotoxic effects against a wide variety of human tumor cells, including skin, breast, ovary, prostate, stomach, colon, and liver carcinoma cells (Bishayee, 2009). Interestingly, at low concentrations, resveratrol significantly increased cell proliferation in human breast cancer cell lines (MCF-7, $\leqslant$4 μM; KPL-1, $\leqslant$22 μM), whereas it suppressed cell growth at high concentrations ($\geqslant$44 μM) (Nakagawa et al., 2001). In another cancer cell type, resveratrol (100 or 200 μM) was also found to inhibit proliferation of AH109A hepatoma cells and suppress invasion of the hepatoma cells even at a concentration of 25 μM (Kozuki et al., 2001). Results from these in vitro studies support the view that the effect of resveratrol varied dramatically depending on the concentrations in cancer cells.

Recently, resveratrol has also received considerable attention since its chemopreventive potential role in reducing the toxicity of trivalent arsenite. However, what may be the role resveratrol plays in modulating the toxicity induced by $NaAsO_2$ is still unclear. To our knowledge, there are very few studies that evaluate the protective effect of resveratrol on arsenite toxicity. (i): Pretreatment with resveratrol (3 mg/kg) was revealed to ameliorate the cardiotoxicity in arsenic trioxide-treated mice, manifested by increasing the activities of antioxidant enzymes in heart and anti-apoptotic effects in rat embryonic H9c2 cardiomyocytes (Zhao et al., 2008). (ii) Combining resveratrol (8 mg/kg) with arsenic trioxide has also been postulated to protect arsenic trioxide-treated Wistar rats from the dose-dependent hepatotoxicity by markedly decreasing oxidative stress and arsenic accumulation in the liver (Zhang et al., 2013). (iii) The cyto-protective effect of low doses of resveratrol (0.5 μM) on $NaAsO_2$ toxicity in HaCaT keratinocytes was revealed to be associated with the regulation the Phase II xenobiotic response and the efficiency of DNA base excision repair (Herbert and Snow, 2012). Therefore, based on the previous results so far, we hypothesized that combining resveratrol with $NaAsO_2$ would be a novel strategy with the potential for protecting the toxicity of $NaAsO_2$.

To gain better understanding of the effect of resveratrol in modulation of $NaAsO_2$ toxicity, we assayed cell survival responses, activation of apoptosis, and measured genotoxicity and oxidative stress level after $NaAsO_2$ treatment in the presence and absence of resveratrol at various concentrations (ranging from 1 to 20 μM).

2. Materials and methods

2.1. Cell culture

Human lung adenocarcinoma epithelial (A549) cell line was initially purchased from Gene therapy cancer drug Engineering Research Center, Chengdu Huasun Group Inc., Ltd. (Chengdu, China). Cells were grown in 75 cm^2 cell culture flasks at 37 °C with 5% CO_2, and routinely maintained in high glucose Dulbecco's modified Eagle's medium (DMEM, Gibco Life Technologies, Grand Island, NY, USA) containing 100 units/ml penicillin and 100 μg/ml streptomycin supplemented with 10% (v/v) fetal bovine serum (FBS). After cells reached around 80–90% of confluence, the DMEM media was replaced with the fresh one, and the cell growth was monitored by using microscopic examination (Phenix XDS-200, Phenix optical holding stock Co. Ltd. Jiangxi, China).

2.2. Cell viability measured by using MTT assay

The cell viability was determined by using MTT [3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay, according to the method previously described by Saravanan et al. (2003) with some modification. In brief, cells were seeded at a density of 1×10^4 cells/well (5×10^4 cells/ml, 200 μl/well) in a 96-well microculture plate with complete DMEM media over night. Then cells were treated with desired concentrations of resveratrol (98% purity, Keddia, Chengdu, China; lot number C1206401) or/and 30 μM $NaAsO_2$ (purity $\geqslant$99.0%, Fluka, Buchs, Switzerland) for 24 h. Resveratrol was initially dissolved in dimethylsulfoxide (DMSO) and subsequently diluted with the culture media for treatment of cells at various concentrations while maintaining the concentration of DMSO at $\leqslant$0.25%. The solvent control cells were exposed to 0.25% DMSO in media. After treatment, the cells were incubated in a serum free medium containing MTT solution [100 μl, 0.5 mg/ml in phosphate buffered saline (PBS), pH = 7.4] at 37 °C for 4 h. Subsequently, MTT reagent (Amresco. Co., Ohio, USA) was discarded and 100 μl DMSO was added to the wells to dissolve the formazan crystals. The value of optical density was measured at a test wave length of 570 nm on a Bio-Rad micro-plate reader (Bio-Rad, Hercules, CA, USA). In addition, experiments were conducted in twelve replicates for each treatment, and the DMSO was used as a blank control. The percentage of cell survival for each treatment was calculated by adjusting the control group to 100%.

2.3. Colony formation assay

Plate colony formation assay was performed according to the procedures described by Cordes and van Beuningen (2004) with some modifications. Briefly, exponentially growing cells were seeded into 24-well plate with a density of 200 cells/well and left to adhere in media supplemented with penicillin/streptomycin and 10% (v/v) FBS. Subsequently, cells were treated in triplicate with desired concentrations of resveratrol or/and $NaAsO_2$ for 24 h. After treatments, the cells were rinsed twice with PBS and replaced with fresh DMEM into wells for colony formation. Cultures were incubated for 10 days and then fixed with methanol and stained with 10% Giemsa solution. The number of colonies, defined as more than 50 cells/colony, were counted under a inverted microscope (DMLB2, Leica, Wetzlar, Germany), and the rate of colony formation was calculated according to the formula: Rate of colony formation (%) = (The average number of colonies of each experimental group/200 cells) × 100%.

2.4. Determination of glutathione (GSH) content

The intracellular content of GSH was determined by using the method described previously with some modifications (Beutler et al., 1963). In brief, cells were seeded in 6-well plates at a density of 10^6 per well overnight and then exposed to designed concentrations of resveratrol or/and $NaAsO_2$ for 24 h. After treatments, cells were then detached from dishes with 0.25% trypsin (w/v)/0.02% EDTA (w/v) and lysed in lysis buffer [0.05 mM EDTA, 1% Triton-X100 (v/v), pH = 8.0] for 1 h at 4 °C. Cell suspension was centrifuged at 12,000 rpm for 2 min and the supernatant was added with 1.9 ml freshly prepared disodium hydrogen phosphate buffer and 0.5 ml of 0.004% DTNB reagent [5,5′-dithiobis-(2-nitrobenzoic acid)]. The absorbance was measured by spectrometer (WFJ 7200, Unico Inc. Shanghai, China) at a wavelength of 420 nm. The level of GSH was calculated according to a GSH standard curve, and normalized by the protein concentration, which was detected by Coomassie Brilliant Blue method.

2.5. Measurement of intracellular reactive oxygen species (ROS)

The generation of intracellular ROS were detected by a oxidation-sensitive fluorescent probe 2′7′-dichlorofluorescin diacetate (DCFH-DA, Applygen Technologies Inc., Beijing, China) as described previously (Zeng et al., 2003). Briefly, 10^6 cells/well were cultured on square glass coverslips (24 mm × 24 mm) in 6-well plates and treated with desired doses of resveratrol or/and $NaAsO_2$ for 24 h. Afterwards, cells were incubated with DCFH-DA at a final concentration of 10 μM 37 °C in the dark for 1 h. The cells were observed under a fluorescence microscopy (DMLB2, Leica, Wetzlar, Germany), and the fluorescence density for a single cell was evaluated by using the Image-Pro@Plus 6.0 software (Media Cybemetics Inc. USA). The parameter of average optical density (AOD) was calculated according to the formula as follow: AOD = sum of integrated optical density (IOD)/Sum area.

2.6. Detection of superoxide dismutase (SOD) activity

SOD activity was detected by using a commercially available kit (purchased from Nanjing Jiancheng Bioengineering Institute, Cat. No: A001-1, Nanjing, China), in which the activity of SOD enzyme was evaluated by its ability to inhibit the oxidation reaction of hydroxylamine hydrochloride to nitrite with superoxide. In brief, 10^6 cells/well were exposed to various concentrations of resveratrol or/and $NaAsO_2$ for 24 h. Subsequently, cells were collected using 0.2% EDTA (w/v) and rinsed with PBS. The cell suspension was lysed in lysis buffer [0.05 mM EDTA, 1% Triton-X100 (v/v), pH = 8.0] for 1 h at 4 °C and immediately centrifuged at 3000 rpm for 5 min. The supernatant was used for SOD determination according to the manufacturer's recommendations, and the absorbance was measured at the wavelength of 550 nm with a spectrometer (WFJ 7200, Unico Inc. Shanghai, China). In addition, 50% inhibition was defined as one unit of enzyme activity, and the protein concentration, detected by Coomassie Brilliant Blue method, was used to normalize the total SOD activity.

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