European Review for Medical and Pharmacological Sciences 2015; 19: 3303-3308

Resveratrol caused apoptosis in QGY-7701 cells

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Abstract. – OBJECTIVE: This study aims to evaluate the cytotoxicity of resveratrol in QGY-7701 cells using a cell viability assay and determine genetic damages after 24-h exposure to concentrations of 6, 12, and 24 μmol·L-1.

MATERIALS AND METHODS: Cell viability and genetic damages were determined using a WST-1 assay and Hoechst 33342 staining with single cell gel electrophoresis (SCGE), respectively. Reactive oxygen species (ROS) levels were also determined.

RESULTS: The results demonstrated that resveratrol inhibited QGY-7701 cell growth and decreased their viability in a dose-dependent manner. Furthermore, resveratrol caused nuclear morphological changes and DNA damage, promoted ROS levels, and induced cell apoptosis.

CONCLUSIONS: These results suggest that resveratrol may induce QGY-7701 cell apoptosis by triggering excessive ROS generation and DNA damage.

Key Words:
Resveratrol, Cytotoxicity, Apoptosis, DNA damage, Reactive oxygen species (ROS).

Introduction

The quest to discover an elixir capable of prolonging the average lifespan and retarding aging has been on-going since ancient times[1]. However, numerous diseases such as heart disease, cancer, Alzheimer's disease, and diabetes mellitus are a threat to good health. Cancer is the second leading cause of death after cardiovascular disorders[2], and the number of types and cases has increased over the past decades[3].

Some natural compounds of plant origin with relatively low toxicity can influence carcinogenetic processes including tumor initiation, promotion, and progression[4,5]. Resveratrol is one of the most promising natural chemopreventive products6 and was first extracted from European resveratrol, which is chemically named 3,4',5-trihydroxystilbene[7]. It is a non-flavone polyphenolic molecule found in plants including grapes, peanuts, soybeans, pomegranates, bilberry, Veratrum nigrum, giant knotweed rhizomes, and other plants8. Resveratrol, abundantly present in nature, is a type of phytoalexin, which can be produced by plants under conditions of ultraviolet radiation, pathological changes, or fungal infection. It has been demonstrated to possess multiple bioactivities such as chemopreventive, cytostatic, immunoregulatory, estrogen-like, antiinflammatory, and antioxidant. Previous studies showed that resveratrol has chemopreventive effects via inhibition of tumor initiation, promotion, and progression[9]. However, the results of studies on the inhibitory effect of resveratrol in different cancer cells are not the same[10-12]. Therefore, this study aims to evaluate the cytotoxicity of resveratrol in QGY-7701 cells and determine the genetic damages as well as mechanisms of action involved.

Materials and Methods

Chemicals and Reagents

Resveratrol (purity 99%, Sigma, S. Louis, MO, USA), Roswell Park Memorial Institute (RPMI)-1640 medium and fetal bovine serum (FBS) were obtained from Invitrogen (Shanghai, China). Resveratrol was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 50 mmol·L-1. The 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt (WST-1) cell proliferation and cytotoxicity (catalog # C0036), Hoechst 33342 (catalog # C1025), bicinchoninic acid (BCA) protein (catalog # P0012) and ROS (catalog # S0033) assay kits were purchased from the Beyotime Institute of Biotechnology (Jiangsu, China).

Cell Culture

The QGY-7701 cell line was purchased from the Institute of Cell Biology, Chinese Academy of Science (CAS) and cultured in RPMI-1640 medium supplemented with 10% (v/v) heat-inac-

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tivated fetal FBS. Cells were maintained at 37 °C in an incubator with 5% (v/v) of CO2 and a water-saturated atmosphere.

Cell Viability Assay

Cell viability was measured using a WST-1 cell proliferation and cytotoxicity assay kit, according to the manufacturer's instructions. Briefly, 1 × 104 QGY-7701 cells were seeded in a 96-well plate. Following a 6-h incubation, cells were washed with fresh medium and treated with resveratrol at 1, 2, 4, 8, 16, 32, 64, 80, 128, and 150 μmol·L-1. After 24-h treatment, the cells were incubated in medium containing WST-1 for 4 h. Then, the absorbance of the plate was read at 450 nm using an enzyme-linked immunosorbent assay (ELISA) microplate-reader. Absolute values obtained were normalized to the mean of the QGY-7701 cells grown in RPMI-1640 medium alone (defined as 100%). Three independent experiments were performed. The half-maximal effective concentration (EC50) was calculated using the Matlab software.

Hoechst 33342 Staining

Hoechst 33342 staining was performed to observe the nuclear morphological changes in QGY-7701 cells. The cells were cultured in six-well plates (1 × 106 cells per well) with 0, 6, 12, and 24 μmol·L-1 of resveratrol for 24 h, collected, rinsed twice with phosphate-buffered saline (PBS), and then incubated in Hoechst 33342 (10 μg·mL-1) for 10 min. Then, the cells were washed thrice with PBS and examined under a fluorescence microscope (Nikon, Tokyo, Japan) at an emission wavelength (λem) of 521 nm. Ten random images were acquired from each sample, and 200 nuclei were counted. Cells with nuclear crenation, fractionation, and condensation were considered apoptotic. The percentage of apoptosis was expressed as the ratio of apoptotic to total cells.

Determination of ROS Level

The ROS level was assayed by measuring the oxidative conversion of 2',7'-dichlorofluorescein diacetate to the fluorescent compound dichlorofluorescein (DCF). All procedures were performed according to the manufacturer's instructions. QGY-7701 cells were cultured in six-well plates (1 × 106 cells per well) and exposed to 0, 6, 12, and 24 μmol·L-1 resveratrol for 24 h. After the treatment, the cells were collected, washed twice with PBS, and then incubated with 10 μmol·L-1 2',7'-dichlorofluorescein diacetate for 20 min at 37 °C. Then, the cells were washed with medium (FBS-free) thrice, and the fluorescence was detected using Fluoroskan Ascent FL (λex and λem at 485 and 538 nm, respectively, Thermo Scientific, Waltham, MA, USA). The protein concentration of samples from each treatment group was determined using the BCA protein assay kit. The specific ROS level was normalized to total protein and expressed as a fold change of the baseline ROS level of control cells cultured in Roswell Park Memorial Institute-1640 (RPMI)-1640 medium without resveratrol.

Single cell Gel Electrophoresis (SCGE) Detection

Cellular DNA damage was detected using single cell gel electrophoresis (SCGE) as described in a previous study13. Briefly, QGY-7701 cells in the logarithmic growth phase were digested with trypsin, transferred to a 12.5-cm2 flask, incubated for 6 h, and then treated with resveratrol (0, 6, 12, and 24 μmol·L-1). After 24-h treatment, the cells were collected, washed twice with PBS, and then monoplast suspensions were prepared with PBS.

A total of 100 μL of 0.1% normal melting point agarose (NMPA) prepared in Ca2+- and Mg2+-free PBS was preheated to 45 °C, dripped onto a clean frosted slide, and then immediately covered with a clean coverslip. After a 10-min condensation at 4 °C, the coverslip was removed. The cell suspension to be tested (20 μL) and 0.8% low-melting point agarose (LMPA, 80 μL) were separately preheated to 37 °C and then mixed. The solution was dripped onto the first gel layer and immediately covered with another clean coverslip. After a 10-min condensation at 4 °C, the coverslip was removed, and the slide was instantly submerged in cell lysate pre-chilled to 4 °C for 2 h of splitting. The slide was then removed, washed with PBS for 1 min, and placed in freshly prepared electrophoresis buffer for 20 min, to untangle the DNA double strands sufficiently. Electrophoresis was performed stably at 25 V for 30 min, followed by 20 min of neutralization with pre-chilled Tris buffer. EB (Engerix B) at 20 μg mL-1 (30 μL) was dripped on the sample, which was then coverslipped for 20 min of staining. Afterward, the tailing rate and tail length of the cells were observed under a fluorescence microscope. For each sample, 50 cells were randomly selected for tailing percentage calculation.

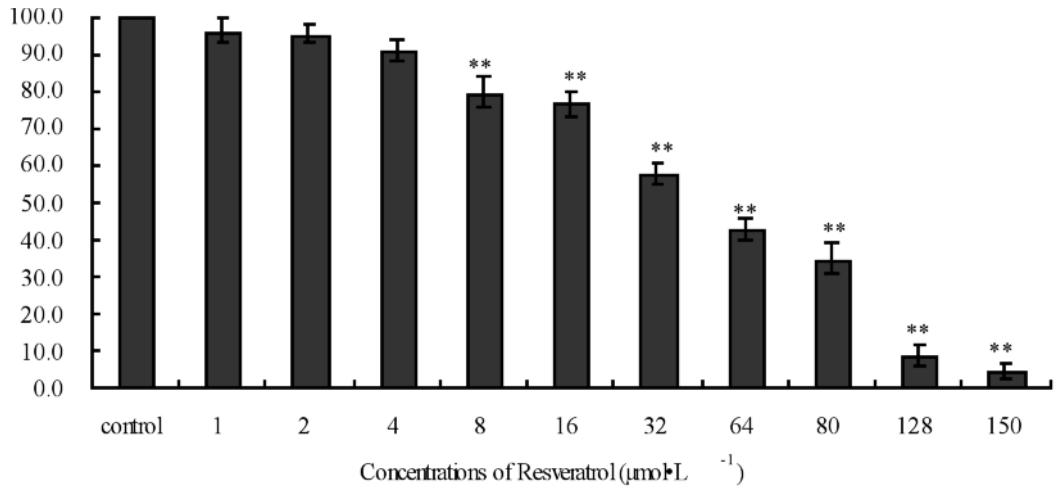

Figure 1. Effect of Resveratrol on cell viabilities in QGY-7701 cells. Data are shown as means ± SD from three independent experiments. Asterisk denotes a response that is significantly different from the control (**$p < 0.01$).

Statistical Analysis

Data are presented as means ± standard derivation (SD) and analyzed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) version 13.0 software. Statistical comparisons were performed using one-factor analysis of variance (ANOVA). Differences of $p < 0.05$ were considered statistically significant.

Results

Resveratrol Significantly Reduced QGY-7701 cell Viability

The viability of QGY-7701 cells is shown in Figure 1. After 24-h treatment with resveratrol, the differences were not significant between the lower concentration (1, 2, and 4 μmol·L-1) and control groups ($p > 0.05$) while concentrations higher than 8 μmol·L-1 showed remarkable differences (Figure 1). The 24-h EC50 of resveratrol in QGY-7701 cells was calculated to be approximately 48 μmol·L-1. In addition, the cytotoxicity of resveratrol against QGY-7701 cells was dose-dependent.

Effect of Resveratrol on Nuclear Morphology

Varying concentrations of resveratrol changed the nuclear morphology to different extents, and the results are shown in Figure 2. The quantitative analysis revealed that the percentage of

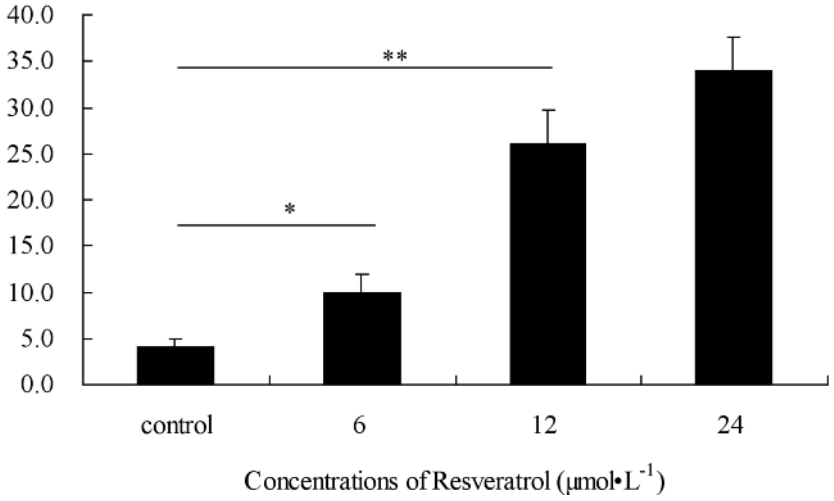

Figure 2. Nuclear morphological alternation of QGY-7701 cells exposed to Resveratrol. Data are shown as means ± SD from three independent experiments. Asterisk denotes a response that is significantly different from the control (*$p < 0.05$, **$p < 0.01$).

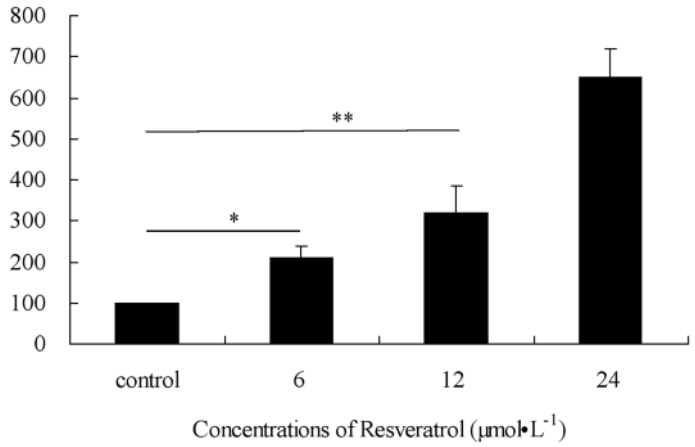

Figure 3. The ROS level in the Resveratrol-treated QGY-7701 cells. Data are shown as means ± SD from three independent experiments. Asterisk denotes a response that is significantly different from the control (*$p < 0.05$, **$p < 0.01$).

QGY-7701 apoptotic cells characterized by cell shrinkage, chromatin condensation, and fragmented fluorescent nuclei increased significantly after 24-h exposure to resveratrol. This result indicates that resveratrol induces apoptosis of QGY-7701 cells.

Effect of Resveratrol on ROS Level

The results of the DCF fluorescence assay showed that the intracellular ROS level in resveratrol-treated cells was higher than that of the control cells was (Figure 3), suggesting that resveratrol induces oxidative stress in QGY-7701 cells.

Effect of Resveratrol on DNA Damage

The tailing rate and length values obtained in the comet assays reflected the degree of nuclear DNA damage. As shown in Figure 4, resveratrol dose-dependently increased the number of DNA-damaged cells.

Discussion

Toxicity tests at the cellular level are convenient and easy to perform and enable the avoidance of many ethical issues. For these reasons, they benefit studies of toxicity mechanisms[14]. In this study, we evaluated the toxic effect of resveratrol and explored the associated underlying mechanisms. The WST-1 colorimetry results showed that resveratrol significantly decreased QGY-7701 cell viability at concentrations exceeding 8 μmol·L-1.

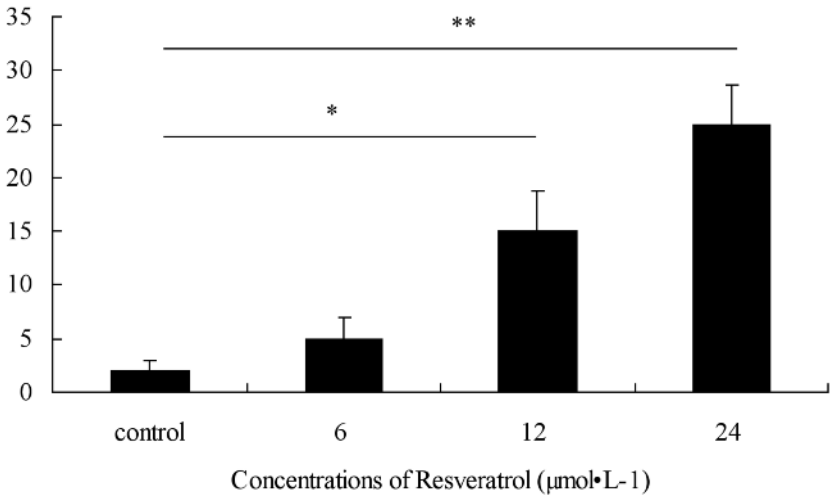

Figure 4. DNA damage of QGY-7701 cells exposed to Resveratrol. Data are shown as means ± SD from three independent experiments. Asterisk denotes a response that is significantly different from the control (*$p < 0.05$, **$p < 0.01$).

Apoptosis is a programmed cell death process characterized by cell shrinkage, nuclear condensation, DNA fragmentation, expression of apoptosis-related genes, and activation of the caspase cascade[15]. In this study, cellular morphological observation revealed that resveratrol exposure induced typical apoptosis of QGY-7701 cells.

Excessive intracellular ROS production induced by a toxicant within the mitochondria or cytoplasm can damage many types of biological macromolecules such as membrane lipids, DNA, and enzymes. Furthermore, ROS induce mitochondrial depolarization and permeability transition[16,17]. In this study, the DCF fluorescence assay showed that intracellular ROS level in resveratrol-treated cells was higher than that of the control cells was. This observation suggests that resveratrol exposure caused oxidative stress, mitochondrial permeability transition, and apoptosis of QGY-7701 cells. Therefore, excessive ROS induction by resveratrol exposure may be a key early factor in the cellular damage and apoptosis of QGY-7701 cells.

The SCGE showed that resveratrol caused severe DNA damage and inhibited DNA damage repair at concentrations exceeding 12 μmol·L-1. In our previous study, we found that at concentrations exceeding 6 μmol·L-1, resveratrol induces excessive generation of ROS in cells, causing cellular oxidative stress[18]. Therefore, the generation of ROS occurs before DNA damage. Many researchers believe that ROS can cause DNA damage[19,20] because intermediate products of ROS, free radicals, directly act on nucleic acids, resulting in base modification and DNA chain rupture. DNA rupture activates DNA-dependent protein kinase and ataxia telangiectasia mutated (ATM; the mutein of Louis-Bar syndrome). The activated protein kinase and ATM activate c-Ab1 (a tyrosine protein kinase), which binds P53 and increases its stability. Then, P53 binds to the genetic promoters of some apoptotic effectors and regulates their transcription. Consequently, the expression of some proteins such as Fas and FasL is upregulated while those of others such as Bcl-2 is downregulated[21,22]. B-cell lymphoma 2 (Bcl-2) is an anti-apoptosis protein that inhibits the release of cytochrome C induced by numerous apoptosis-inducing agents, as well as the subsequent apoptosis. In this study, the results showed that resveratrol caused excessive generation of ROS in the cells, which may directly act on and damage DNA.

Conclusions

Resveratrol at certain concentrations induces excessive ROS generation, which induces cellular DNA damage and subsequent apoptosis.

Acknowledgements

This research was supported by the Natural Science Foundation of Shandong Province, China (ZR2014YL034).

Conflict of Interest

The Authors declare that they have no conflict of interests.

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