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RESEARCH ARTICLE

Resveratrol Dose-Dependently Protects the Antioxidant Mechanism of Hydrogen Peroxide-Exposed Healthy Cells and Lung Cancer Cells

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ABSTRACT

Objective: The objective of this study was to investigate the protective effects and the underlying mechanisms of resveratrol against hydrogen peroxide (H_2O_2)-induced oxidative stress in healthy human and lung cancer cells.

Materials and Methods: The cytotoxic doses and IC_{50} values of resveratrol and hydrogen peroxide for cells were determined by the Cell Titer Blue-Viability Assay kit. The amount of malondialdehyde (MDA) was determined by fluorescence spectrophotometer. The amount of intracellular reduced glutathione level and antioxidant enzyme activities were analyzed by spectrophotometric methods.

Results: In both cells, H_2O_2 treatment alone (IC_{50} and IC_{50}) increased MDA, glutathione reductase, glutathione S-transferase, selenium-dependent glutathione peroxidase and non-selenium-dependent glutathione peroxidase activities, but glutathione levels decreased compared to the $H_2O_2^+$ resveratrol treatment. In addition, high doses of resveratrol alone (IC_{50} and IC_{70}) induced more oxidative stress in cancer cells than in healthy cells. High doses of resveratrol alone (IC_{50} and IC_{70}) also showed cytotoxic effects in cells and decreased cell viability. Resveratrol caused more cytotoxic effects in cancer cells compared to healthy cells.

Conclusion: The results of this study show that the increase in MDA level and antioxidant enzyme activity caused by high-dose resveratrol treatment reveals the prooxidant effect of resveratrol. Our results also showed an antioxidant effect by reducing oxidative stress in cells pre-incubated with low-dose resveratrol and then exposed to H_2O_2. Resveratrol has a dose-dependent biphasic (pro/antioxidant) effect on the antioxidant mechanism of cells. However, more research is needed to confirm this.

Keywords: Antioxidant enzymes, Resveratrol, Dose-dependent toxicity, Hydrogen peroxide

INTRODUCTION

Many factors, such as heat stress, transport stress, and nutrient restriction generated reactive oxygen species (ROS) cause oxidative stress. Increased ROS production causes high cellular damage when it exceeds the capacity of the cellular antioxidant system.[1-4] While low levels of ROS show beneficial effects, excessive accumulation of ROS causes various disorders, including carcinogenesis.[5] Since the lungs are exposed to oxidants of endogenous or exogenous origin (air pollutants, cigarette smoke, etc.) every day, the level of oxidative stress is very high.[6-8] Lung cancer risk can be reduced by consciously consuming antioxidant-containing foods and paying attention to nutrition. Resveratrol, also known as 3, 5, 4-trihydroxy-trans-stilbene, is a polyphenolic compound that occurs naturally in various dietary sources, including grapes and peanuts. It has been shown to possess several pharmacological properties, including anticancer and antioxidant activities.[9-13] As it is understood from the studies mentioned above, it is revealed that the antiproliferative, antioxidant, prooxidant, and anticancer properties of resveratrol vary depending on the doses, and therefore it is not safe for humans. The antioxidant effect of resveratrol can change depending on the dose. At lower doses, such as nM or 5-10 µM, resveratrol acts as a potent antioxidant, while at higher doses, it may function as a pro-oxidant.[14] Human studies suggest resveratrol can be supplemented at dosages up to 5 g daily, but the most common daily doses range from 50 to 500 mg. It is important to note that resveratrol supplements have not been well-studied in people, and more high-quality research is needed to determine the most effective and safe doses.[15] Therefore, before taking resveratrol, it is advisable to consult a healthcare professional to determine the appropriate dosage for specific health benefits and to minimize potential side effects.[16] Enzymes involved in the antioxidant mechanism of living organisms can neutralize oxidants by directly acting on oxidants exceeding a certain level. In our study, the dose-dependently

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effect of resveratrol on malondialdehyde (MDA) and reduced glutathione (GSH) levels, and selenium-dependent glutathione peroxidase (Se-GPx), non-selenium-dependent glutathione peroxidase (non-Se-GPx), glutathione S-transferase (GST), and glutathione reductase (GR) activities of human healthy (MRC-5) and lung cancer cells (H1299).

MATERIALS AND METHODS

Cell Culture and Chemical

H1299 and MRC-5 cell lines were purchased from ATCC (American Type Culture Collection) (Rockville, MD, USA). Resveratrol (≥99%) was purchased from Sigma-Aldrich (Germany). H1299 cell line were cultured in RPMI 1640 medium containing 10% fetal bovine serum and 1% antibiotic antimycotic solution at 37 °C within a humidity atmosphere that contained 5% CO_2. Healthy cells (MRC-5) were grown using the same conditions plus DMEM medium containing 1% amino acids.

Cell Viability Assay

10,000 cells were seeded in a monolayer in each well of a 96-well plate as part of the experiment. On the following day, the cells were treated with various doses of resveratrol (10, 15, 25, 50, 100, 150, 200, 300, 400, 500, 600, 700, 800 µM) in the medium for 24, 48, and 72 h. Since the most effective IC_{50} value was reached in 72 h resveratrol application, the hydrogen peroxide (H_2O_2) study was continued over 72 h application. The H_2O_2 concentrations applied were (25, 50, 75, 100, 150, 150, 200, 200, 250, 250, 300, 350, 400, 450, 500 µM). The resulting cytotoxicity of resveratrol and H_2O_2 on H1299, and MRC-5 cells was measured spectro-fluorometrically using the Cell Titer Blue- Viability Assay kit.[8] The reduction of cells from resozurin to resorufin was calculated by measuring the excitation at 560 nm and emission values at 590 nm in a fluorescent spectrophotometer (PerkinElmer LS55). The data obtained were the mean values derived from eight wells for each dose, and the IC10, IC_{20},IC_{30},IC_{50}, and IC_{70} values were calculated using linear functions. To measure the antioxidant effect of resveratrol against H_2O_2 (IC_{50} and IC_{70}) cytotoxicity, cells were pre-incubated with different concentrations of resveratrol (IC_5,IC_{10},IC_{20},IC_{30}) for 1 h before 72 h H_2O_2 treatments. Each concentration and control was performed five times. IC_{50} values were calculated from linear equations.

Supernatant Preparation for Chemical Parameters

For the supernatant to be used in the experiments, cells were seeded in flasks and treated with different concentrations of resveratrol (IC_{30},IC_{50},IC_{70}) for 72 h and a control group was seeded without resveratrol treatment. The cells in the resveratrol-treated and control flasks were trypsinized at the end of 72 h. The pellet obtained by centrifugation at 600 xg was washed 3 times with PBS (phosphate buffered saline, pH 7) solution. The homogenization buffer was prepared by mixing 300 µl buffer (100 mM K_2HPO_4 and 100 mM KH_2PO_4 solutions, pH 7), 1180 µL distilled water and 20 µL protease inhibitor cocktail (Sigma). The pellet remaining from the last wash was diluted with the homogenization buffer and transferred into Eppendorf tubes. The pellet was homogenized in a Branson Sonifier ultrasonic disintegrator for 3x15 sec on ice and centrifuged at 32,000 xg for 45 min at 4°C. The supernatant obtained was stored at -80°C until use. MDA, glutathione and enzyme measurements were performed using the supernatant obtained.

Determination of MDA Level

To evaluate the prooxidant (membrane damaging) effect of resveratrol, cells were subjected to varying doses of both resveratrol and hydrogen peroxide (at IC_{50} and IC_{70} concentrations) for a period of 72 h. To assess the antioxidant (membrane protective) effect of resveratrol, the cells were treated with the highest cytoprotective doses of resveratrol for a period of 1 h before exposure to H_2O_2 (at IC_{50} and IC_{70} concentrations) for a period of 72 h. The levels of MDA in the cells were determined using the fluorometric method.[17] The quantification of proteins was carried out using the Bradford method, with bovine serum albumin serving as a standard.[18]

Determination of Enzyme Activity

The enzymatic assays were conducted using the supernatant obtained from the samples. To determine GST activity, the method developed by Habig and Jakoby was used, with 1-chloro-2,4-dinitrophenol as a substrate.[19] Enzyme activity was quantified based on the binding of one µM of GSH per minute, corresponding to one unit of enzyme activity. GR activity was assessed by monitoring the oxidation of NADPH at 340 nm using a spectrophotometric method.[20] The reaction mixture comprised 100 mM potassium phosphate buffer (pH 7.4), 0.1 mM NADPH, oxidized glutathione (GSSG), and 1 mM EDTA. One unit of enzyme activity corresponded to the reduction of 1 µmol of GSSG per minute. Non-Se-GPx activity was measured according to the method described by Paglia and Valentine.[21] To determine Se-GPx activity, 0.25 mmol/L H_2O_2 was used as a substrate, while 0.25 mmol/L cumene H_2O_2 was used to measure total GPx.[22] By monitoring the reduction of 5,5'-dithiobis(2-nitrobenzoic) acid by NADPH in the presence of GR, spectrophotometry was utilized to determine the total GSH concentration. Then, the GSH content was calculated as nmol/mg protein.[23]

Statistical Analysis

The statistical analysis of the collected data was conducted using the IPM Spss Statistics 20 program. The intergroup eval-

uation of the results was carried out through ANOVA-Post-Hoc analysis to determine the significance level of the observed differences. Additionally, the Duncan Multiple Comparison Test was utilized to evaluate the significance of the differences.[24]

RESULTS

To demonstrate the dose-dependent prooxidant (cytotoxic) effect of resveratrol on H1299 and MRC-5 cells, the cells were treated with different doses of resveratrol at different incubation periods (Figure 1). After 24, 48, and 72-hour incubation periods, the cytotoxicity of resveratrol was found to be higher (IC_{50}; 200, 100, 50 µM and IC_{70}; 500, 300,125 µM) in H1299 cells than in MRC-5 cells (IC_{50}; 250, 150, 75 µM and IC_{70}; 700, 400, 200 µM). When H1299 and MRC-5 cells were treated with different doses of H_2O_2, a strong prooxidant, cytotoxicity increased in both cell lines due to increased H_2O_2 doses (Figure 2). As a result of cytotoxicity measurements, IC_{50} and IC_{70} values were determined for 72 h. These values were found to be 300 and 420 µM for H1299 cells and 200 and 300 µM for MRC-5 cells, respectively.

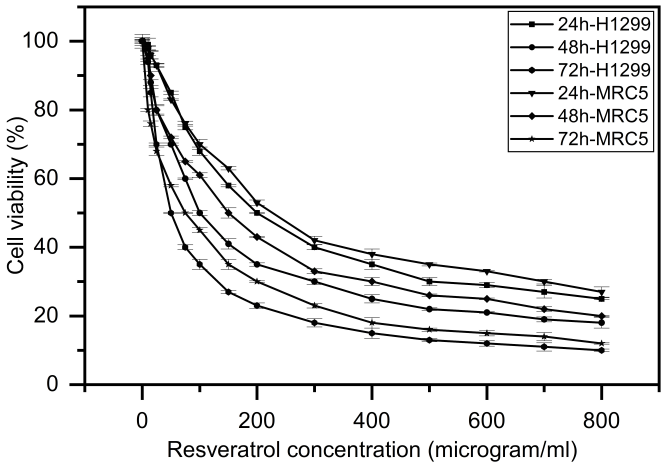

Figure 1. The dose-dependent cytotoxic effect of resveratrol in H1299 and MRC-5 cells at 24, 48 and 72 h. Values are expressed as the mean of three separate experiments. Error bars represent the standard deviation (SD) of the mean from five replications (ANOVA with Tukey's test, $p<0.05$).

The dose-dependent antioxidant (cytoprotective) effect of resveratrol was demonstrated by measuring the viability of cells treated with H_2O_2(IC_{50} and IC_{70}) for 72 h after pre-incubation with low doses of resveratrol (IC_5, IC_{10}, IC_{20}, and IC_{30}). Table 1 shows the levels of H_2O_2-induced cytotoxicity in H1299 and MRC-5 cells pre-incubated with different doses of resveratrol. Maximum cytoprotective doses of resveratrol were found to be 30 µg/mL for H1299 cells and 20 µg/mL for MRC-5 cells against H_2O_2 cytotoxicity. The cytotoxic effect of H_2O_2 application after pre-incubation with resveratrol was found to be lower than the cytotoxic effect of H_2O_2 alone, which showed us the cytoprotective effect of resveratrol. From these

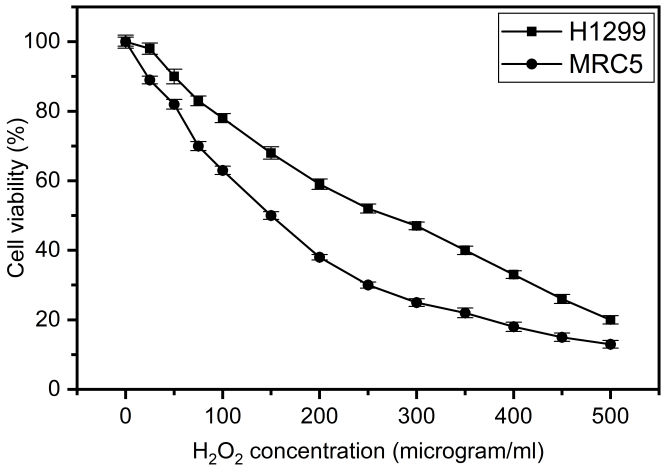

Figure 2. The dose-dependent cytotoxic effect of H_2O_2 in H1299 and MRC-5 cells. Values are expressed as the mean of three separate experiments. Error bars represent the standard deviation (SD) of the mean from five replications (ANOVA with Tukey's test, $p<0.05$).

results we obtained, it has been revealed that resveratrol has a dose-dependent biphasic (pro/antioxidant) effect on H1299 and MRC-5 cells.

At high concentrations, antioxidants can cause membrane damage because they act as pro-oxidants, while at lower concentrations they can protect the membrane against oxidants by showing an antioxidant effect. Oxidative stress created by pro-oxidants in the cell leads to membrane damage by lipid peroxidation and increases the MDA level. To evaluate the dose-dependent membrane-damaging and membrane-protective effects of resveratrol on H1299 and MRC-5 cells, MDA levels were assayed (Table 2). The amount of MDA obtained after exposure of H1299 cells to resveratrol at IC50 and IC70 doses was approximately 7 and 9 times higher than control cells. Similarly, MDA levels in H1299 cells exposed to IC_{50} and IC_{70} doses of H_2O_2, a strong oxidant, were 7 and 13 times higher, respectively, than in the control (Table 2). Here, resveratrol acts as a prooxidant depending on the dose. Resveratrol increases outer membrane permeability and membrane depolarization in cells.[25] Intracellular ROS accumulation caused significantly increased MDA levels confirmed, indicating resveratrol's prooxidant activity. On the other hand, the MDA level measured in H1299 cells exposed to low-dose resveratrol (IC_{30}) for one hour before 72 h of H_2O_2 (IC_{50} and IC_{70}) administration was found to be lower than those treated with only H_2O_2. Under the same experimental conditions, the low dose of resveratrol (IC_{20}) for MRC-5 cells showed membrane protective effect against H_2O_2-induced membrane damage. These results indicated that the significantly reduced MDA levels were due to the antioxidant effect of resveratrol. All the results we obtained from the measurement of MDA level revealed that resveratrol has a dose-dependent biphasic effect in H1299 and MRC-5 cells by showing both membrane protective effects against H_2O_2 and damaging membrane effects.

Table 1. Protective effect of resveratrol against H_2O_2 (<IC_{50} and IC_{70}) cytotoxicity on H1299 and MRC-5 cells.

Resveratrol Concentrations	**% Cell Viability ± S.H.**	**Resveratrol Concentrations**	**% Cell Viability ± S.H.**
H1299 cells		**MRC-5 cells**	
IC_5 Resveratrol + IC_{50} H_2O_2	45 ± 1.2	**IC_5 Resveratrol + IC_{50} H_2O_2**	40 ± 0.9
IC_5 Resveratrol + IC_{70} H_2O_2	39 ± 1.3	**IC_5 Resveratrol + IC_{70} H_2O_2**	33 ± 1.4
IC_{10} Resveratrol + IC_{50} H_2O_2	39 ± 1.3	**IC_{10} Resveratrol + IC_{50} H_2O_2**	45 ± 1.1
IC_{10} Resveratrol + IC_{70} H_2O_2	42 ± 1.4	**IC_{10} Resveratrol + IC_{70} H_2O_2**	35 ± 1.3
IC_{20} Resveratrol + IC_{50} H_2O_2	47 ± 1.2	**IC_{20} Resveratrol + IC_{50} H_2O_2**	57 ± 1.2
IC_{20} Resveratrol + IC_{70} H_2O_2	46 ± 1.2	**IC_{20} Resveratrol + IC_{70} H_2O_2**	45 ± 1.5
IC_{30} Resveratrol + IC_{50} H_2O_2	38 ± 1.4	**IC_{30} Resveratrol + IC_{50} H_2O_2**	34 ± 1.3
IC_{30} Resveratrol + IC_{70} H_2O_2	11 ± 1.1	**IC_{30} Resveratrol + IC_{70} H_2O_2**	3 ± 1.0
IC_{50} Resveratrol	50 ± 2.3	**IC_{50} Resveratrol**	75 ± 1.5
IC_{70} Resveratrol	125 ± 2.9	**IC_{70} Resveratrol**	200 ± 1.9
IC_{50} H_2O_2	51 ± 1.6	**IC_{50} H_2O_2**	50 ± 1.2
IC_{70} H_2O_2	31 ± 1.3	**IC_{70} H_2O_2**	30 ± 1.1
Control	100 ± 1.9	**Control**	100 ± 1.6

The glutathione system, which consists of GSH and enzymes like GPx and GR, which permit its reversible conversion (oxidation or reduction), serves as the fundamental building block for the antioxidant defense system. Reduced GSH serves as both a substrate for chemical reactions and an essential component for reducing selenolate groups found in the catalytic center of enzymes that become oxidized during the glutathione peroxidase reaction. GSH depletion in resveratrol (IC_{50} and IC_{70}) exposed cells was confirmed, which indicated the prooxidant activity of resveratrol. The changes in the GSH level in H1299 and MRC-5 cells exposed to different concentrations of resveratrol (IC_{30}/$_{20}$, IC_{50}, IC_{70}) and H_2O_2 (IC_{50} and IC_{70}) are given in Table 2. Resveratrol did not show any prooxidant effect at low doses applied to both cells. As a result of exposure of H1299 and MRC-5 cells to an IC_{50} resveratrol dose for 72 h, GSH levels decreased by 7% and 6%, compared to the control group, respectively. This is a clear sign of oxidative stress. As with resveratrol (IC_{50} and IC_{70}) administration, different doses of H_2O_2 (IC_{50} and IC_{70}), which is a strong oxidant, statistically significantly decreased the GSH level in both cells ($p<0.05$). However, as seen in Table 2, the amount of GSH in cells pre-incubated with low dose resveratrol and then exposed to H_2O_2 was higher than the amount of GSH in cells exposed to H_2O_2 alone. Here, resveratrol protected the cells by showing antioxidant effect against H_2O_2 which is a strong oxidant.

The antioxidant defense network relies on the coordinated actions of key enzymes, including GR, GST, Se-GPx, and non-Se-GPx. GR catalyzes the reduction of oxidized glutathione (GSSG) to its active form (GSH), utilizing nicotinamide adenine dinucleotide phosphate (NADPH) as an electron donor, ensuring a continuous supply of the essential cellular antioxidant. GST, on the other hand, plays a pivotal role in detoxification by conjugating electrophilic compounds with GSH, facilitating their elimination. Se-GPx and non-Se-Px function as crucial components in the neutralization of H_2O_2 and lipid peroxides, respectively, by utilizing GSH as a co-factor. These peroxidases protect cells from oxidative damage by converting harmful ROS into less harmful substances, emphasizing their integral roles in maintaining cellular redox homeostasis and preventing oxidative stress-induced damage. On the other hand, Se-GPx (1.2-folds in H1299, 1.4-folds in MRC-5), non-Se-GPx (1.1-folds in H1299, 1.2-folds in MRC-5), GST (1.6-folds in

Table 2. Prooxidant and antioxidant effect of resveratrol on MDA and GSH levels, and Se-GSH-Px, GSH-Px, GST, and GSH-Rx activities in H1299 and MRC-5 cells.

Resveratrol Concentrations	MDA Level nmol/mg X± SE	GSH Level nmol/mg X± SE	GR mU/mg X ± SE	GST mU/mg X ± SE	Se-GPx µU/mg X± SE	Non-Se-GPx µU/mg X±SE
IC_{30} Resveratrol (H1299)	1.3 ± 0.20 ^{b}	59 ± 2.10 ^{d}	16 ± 1.9 ^{b}	44 ± 4.3 ^{ab}	520 ± 12 ^{ab}	679 ± 11 ^{b}
IC_{50} Resveratrol (H1299)	2.3 ± 0.32 ^{c}	53 ± 2.34 ^{cd}	25 ± 2.5 ^{bc}	64 ± 2.3 ^{cd}	600 ± 11 ^{b}	750 ± 12 ^{c}
IC_{70} Resveratrol (H1299)	3.0 ± 0.45 ^{cd}	49 ± 2.21 ^{c}	35 ± 2.9 ^{cd}	79 ± 3.3 ^{e}	695 ± 11 ^{c}	795 ± 14 ^{d}
IC_{50} H_2O_2 (H1299)	2.5 ± 0.45 ^{c}	40 ± 2.33 ^{b}	44 ± 3.5 ^{de}	88 ± 3.2 ^{f}	714 ± 13 ^{c}	800 ± 14 ^{d}
IC_{70} H_2O_2 (H1299)	4.3 ± 0.34 ^{e}	33 ± 2.20 ^{a}	56 ± 3.6 ^{f}	95 ± 4.1 ^{fg}	773 ± 12 ^{d}	851 ± 15 ^{e}
IC_{30} Resveratrol + IC_{50} H_2O_2 (H1299)	1.8 ± 0.25 ^{bc}	50 ± 2.55 ^{cd}	20 ± 1.2 ^{b}	55 ± 2.5 ^{bc}	528 ± 14 ^{ab}	660 ± 13 ^{a}
IC_{30} Resveratrol + IC_{70} H_2O_2 (H1299)	3.3 ± 0.45 ^{d}	40 ± 2.45 ^{b}	25 ± 1.0 ^{bc}	60 ± 2.7 ^{c}	595 ± 12 ^{b}	750 ± 14 ^{c}
DMSO (H1299)	0.32 ± 0.13 ^{a}	58 ± 2.63 ^{d}	10 ± 0.9 ^{a}	38 ± 1.3 ^{a}	509 ± 12 ^{a}	658 ± 13 ^{a}
Control (H1299)	0.33 ± 0.15 ^{a}	57 ± 1.58 ^{d}	10 ± 0.8 ^{a}	39 ± 2.0 ^{a}	506 ± 13 ^{a}	660 ± 14 ^{a}
IC_{20} Resveratrol (MRC-5)	0.8 ± 0.03 ^{ab}	53 ± 2.32 ^{cd}	11 ± 1.0 ^{ab}	40 ± 3.0 ^{b}	311 ± 12 ^{ab}	431 ± 10 ^{a}
IC_{50} Resveratrol (MRC-5)	1.4 ± 0.03 ^{b}	49 ± 2.38 ^{c}	21 ± 1.5 ^{bc}	53 ± 4.3 ^{c}	363 ± 11 ^{b}	481 ± 11 ^{b}
IC_{70} Resveratrol (MRC-5)	1.8 ± 0.04 ^{bc}	45 ± 2.28 ^{bc}	29 ± 1.3 ^{c}	68 ± 2.2 ^{e}	484 ± 11 ^{c}	527 ± 12 ^{b}
IC_{50} H_2O_2 (MRC-5)	2.0 ± 0.06 $^{b c}$	37 ± 2.29 ^{b}	31 ± 2.6 ^{cd}	72 ± 3.4 ^{ef}	497 ± 13 ^{c}	548 ± 14 ^{bc}
IC_{70} H_2O_2 (MRC-5)	2.5 ± 0.08 ^{c}	30 ± 2.28 ^{a}	40 ± 3.1 ^{d}	84 ± 3.5 ^{fg}	553 ± 12 ^{cd}	601 ± 13 ^{c}
IC_{20} Resveratrol + IC_{50} H_2O_2 (MRC-5)	1.5 ± 0.03 ^{b}	45 ± 2.35 ^{bc}	14 ± 0.8 ^{ab}	48 ± 2.5 ^{c}	350 ± 14 ^{ab}	425 ± 12 ^{a}
IC_{20} Resveratrol + IC_{70} H_2O_2 (MRC-5)	2.0 ± 0.03 ^{bc}	38 ± 2.41 ^{b}	19 ± 0.9 ^{b}	52 ± 3.4 ^{cd}	375 ± 12 ^{ab}	470 ± 11 ^{b}
DMSO (MRC-5)	0.29 ± 0.02 ^{a}	51 ± 2.43 ^{cd}	6 ± 0.7 ^{a}	30 ± 1.0 ^{a}	260 ± 12 ^{a}	405 ± 13 ^{a}
Control (MRC-5)	0.26 ± 0.01 ^{a}	52 ± 1.40 ^{cd}	7 ± 0.4	29 ± 1.9 ^{a}	263 ± 13 ^{a}	400 ± 13 ^{a}

Results are means of eight different experiments. Values that are followed by different letters within each column are significantly different ($p \leq 0.05$). df1=2, df2=95, F=11.96. SE: Standard Error. MDA: Malondialdehyde, GSH: Reduced glutathione, Se-GPx: Selenium-dependent glutathione peroxidase, Non-Se-GPx: Non-selenium-dependent glutathione peroxidase GST: Glutathione S-transferase, GR: Glutathione reductase.

H1299, 1.8-folds in MRC-5), as well as increased GR activity (2.5-folds in H1299, 3-folds in MRC-5) was seen in IC_{50} resveratrol exposed H1299 and MRC-5 cells respectively. Also, the activity of these enzymes was found to be significantly higher in IC_{70} resveratrol-treated cells than in control cells (Table 2). As a result, high resveratrol doses like H_2O_2-induced oxidative stress in H1299 and MRC-5 cells showed an oxidative effect. Both resveratrol and H_2O_2 increased enzyme activities in cells.

On the other hand, H1299 cells, preincubated with IC_{30} resveratrol doses (the highest cytoprotective effect dose for H1299 cells against H_2O_2 cytotoxicity) for 1 h, before H_2O_2 treatment (IC_{50} and IC_{70}) for 72 h, had lower enzyme activity than non-preincubated cells (Table 2). Under the same experimental conditions, the IC_{20} dose of resveratrol for MRC-5 cells showed a protective effect against H_2O_2-induced oxidative stress. We assume that the antioxidant action of resveratrol can accompany decreasing H_2O_2 -induced oxidative stress with lower doses. These findings suggest that resveratrol exhibits anticancer/antioxidant effects depending on the dose in both cells.

DISCUSSION

Lung cancer is a major cause of cancer-related mortality worldwide, with rising incidence rates globally.[26] This may be due to increased oxidative stress due to daily exposure of the lungs to oxidants from both endogenous and exogenous sources (such as air pollution and cigarette smoke).[6-8] Therefore, lung cancer cells can be selected as a model for oxidative stress studies. ROS generation is increased under oxidative stress, and antioxidant defense mechanisms like GSH, GST, GPx and GR are significantly less effective. Specifically, ROS can impair the lipid membrane by increasing its fluidity and permeability. However, appropriate doses of herbal products with antioxidant properties such as resveratrol can decrease cellular damage caused by oxidative stress.[27] Resveratrol is a natural polyphenolic compound with a well-known capacity to modulate ROS, especially hydroxyl radicals (•OH). In recent years, the scientific community has expressed considerable interest in resveratrol due to its biphasic function, which is dose-dependent. For example, in one study, while ROS play a role in muscle repair, excessive amounts of ROS over long periods of time can lead to oxidative stress. Antioxidants such as resveratrol can reduce oxidative stress, restore mitochondrial function and promote myogenesis and hypertrophy. However, resveratrol dose efficacy for muscle plasticity is unclear. Therefore, we investigated the dose-response of resveratrol on C2C12 myoblast and myotube plasticity in the presence and absence of different degrees of oxidative stress. Low resveratrol concentration (10 μM) stimulated myoblast cell cycle arrest, migration and sprouting, which were inhibited by higher doses (40-60 μM). Resveratrol did not increase oxidative capacity. In contrast, resveratrol caused loss of mitochondria, reduced cell viability and ROS production, and activated stress response pathways [Hsp70 and pSer36-p66(ShcA) proteins]. However, the deleterious effects of H_2O_2 (1000 μM) on cell migration were attenuated after preconditioning with 10 μM-resveratrol. This dose also enhanced cell motility mediated by 100 μM-H_2O_2, while higher doses of resveratrol increased H_2O_2-induced impaired myoblast regeneration and mitochondrial dehydrogenase activity. In conclusion, low doses of resveratrol promoted in vitro muscle regeneration and attenuated the effect of ROS, whereas high doses enhanced oxidative stress-induced decreased plasticity and metabolism. Thus, the effects of resveratrol depend on the dose and the degree of oxidative stress.[28] Clinical studies have reported that resveratrol can confer health benefits (both in animal and human studies) at moderate doses, while higher doses may trigger a pro-apoptotic tumoricidal effect.[14,29,30] For instance, a study showed that the resveratrol derivative produced by high-pressure treatment exhibited a proliferative inhibitory effect on cervical cancer cells.[10] It has been revealed that resveratrol exhibits many biological activities with different drug combinations at different concentrations in colorectal cancer cells.[9] When the antioxidant interaction between resveratrol (8 μg/ml) and eugenol EUG (8 mg/mL) in the carboxymethyl cellulose biodegradable film was evaluated, the combination of the two showed a synergistic antioxidant effect.[11] As noted in the studies above, scientists today focus on the dose-dependent biological effects of resveratrol, resveratrol derivatives, combinations of resveratrol with other ingredients, and encapsulated resveratrol in drug development studies. Our study explains the biphasic function (anti-/pro-oxidant) of resveratrol in a dose-dependent manner. The data we obtained showed that resveratrol has a prooxidant effect at high cell concentrations, while it protects cells pre-incubated with low concentrations of resveratrol from H_2O_2-induced oxidative stress.

In a study supporting our findings, it was observed that micromolar concentrations of trans-resveratrol reduced the MDA levels caused by t-BHP-induced oxidative stress in erythrocytes.[31] Similarly, another study found that resveratrol played a protective role in regulating oxidative damage by modulating GSH homeostasis against the environmental carcinogen $NaAsO_2$.[32] In human endothelial cells, resveratrol modulated the expression of both pro-oxidative and antioxidative enzymes, increasing mRNA expression of SOD1 and GPx1. Furthermore, pretreatment of cells with resveratrol completely prevented DMNQ-induced oxidative stress.[33] Resveratrol also demonstrated no cytotoxicity to human lymphocytes at concentrations of 10-100 μM, while inhibiting DNA damage in these cells induced by H_2O_2. Resveratrol increased the activity of GR, GST, and GPX, indicating that the modulation of antioxidant enzymes (GPX, GR, and GST) and an increase in GSH levels were responsible for resveratrol's inhibitory effect on oxidative DNA damage in human lymphocytes caused by H_2O_2.[34] In a study, the protective effects of resveratrol against H_2O_2-induced oxidative stress in bovine skeletal muscle cells (BMCs) were investigated. Pretreatment of BMCs with resveratrol before H_2O_2

exposure increased cell viability, decreased ROS and stabilized redox status compared to H_2O_2 treatment alone. H_2O_2 exposure activated sirtuin type 1 (SIRT1) and nuclear factor E2-related factor 2 (NRF2) mediated signaling pathways. Pretreatment with resveratrol did not alter SIRT1-regulated genes, but increased heme oxygenase 1 (HO-1) expression while inhibiting the up-regulation of NRF2. These results suggest that resveratrol has beneficial effects against oxidative stress.[35] In another study, the neuroprotective and antioxidant effects of resveratrol against H_2O_2 in embryonic neural stem cells were investigated. H_2O_2 treatment alone increased catalase and GPx activities, but did not change superoxide dismutase levels compared to $H_2O_2^+$ resveratrol treatment. Nitric oxide synthase activity and concomitant nitric oxide levels increased in response to H_2O_2 treatment. Conversely, nitric oxide synthase activity and nitric oxide levels were decreased in cells exposed to H_2O_2 after preincubation with resveratrol. Resveratrol also reduced H_2O_2-induced nuclear or mitochondrial DNA damage. Resveratrol decreased nitric oxide production and nitric oxide synthase activity by inducing the activity of antioxidant enzymes and reduced the potential for oxidative stress by reducing both nuclear and mitochondrial DNA damage. Therefore, it has been suggested that it may be a promising agent to protect embryonic neural stem cells.[36] In this study, the effects of resveratrol on glutamate-induced oxidative cell death were investigated. Cultured HT22 cells, an immortalized mouse hippocampal neuronal cell line, were used as an *in vitro* model. Oxidative stress and neurotoxicity in these neuronal cells were induced by exposure to high concentrations of glutamate. Resveratrol potently protected HT22 cells from glutamate-induced oxidative cell death. The neuroprotective effect of resveratrol was independent of its direct radical scavenging property, but instead depended on its ability to selectively induce mitochondrial superoxide dismutase (SOD2) expression and subsequently reduce mitochondrial oxidative stress and damage. The induction of mitochondrial SOD2 by resveratrol was mediated by activation of PI3K/Akt and GSK-3β/β-catenin signaling pathways. Taken together, the results of this study suggest that up-regulation of mitochondrial SOD2 by resveratrol represents an important mechanism for the protection of neuronal cells against oxidative cytotoxicity resulting from mitochondrial oxidative stress.[37] In our study, the involvement of antioxidant enzyme systems such as Se-dependent GRx, non-Se-GPx, GST, and GR was explored in the detoxification of ROS in H1299 and MRC-5 cells.

CONCLUSION

Based on the findings of our study, it can be suggested that high-dose resveratrol has the potential as a natural source for the production of new anticancer drugs. Our results demonstrated that high-dose resveratrol had cytotoxic effects on H1299 cells and increased membrane damage and antioxidant enzyme levels. In addition, using low-dose resveratrol to develop antioxidant drugs for healthy cells against strong oxidants will also contribute to protecting human health. Thus, dose- and time-dependent use of resveratrol may contribute to identifying new strategies for treating lung carcinoma and protecting healthy cells against prooxidants. A better understanding of the intracellular mechanisms of resveratrol will bring new strategies for producing anticancer and antioxidant drugs. More detailed studies should be conducted on phenolic compounds such as resveratrol, which show biphasic effects such as antioxidant/prooxidant in cells depending on dose and time.

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