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Dose-dependent effect of resveratrol on proliferation and apoptosis in endothelial and tumor cell cultures

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Abbreviations: LDL, low density lipoprotein

Abstract

Experimental data suggest that Resveratrol, a compound found in grapes and other fruits may influence cell proliferation and apoptosis. The aim of our experiments was to study the effect of Resveratrol on tumor cell cultures and an endothelial cell culture in order to examine the effect of various doses of this compound on active cell death and cell proliferation. Human tumor (HT-29, SW-620, HT-1080) and endothelial (HUV-EC-C) cells were treated with various doses of (0.1 to 100.0 μg/ml) Resveratrol in vitro. Cell number, apoptotic and mitotic index was measured 24, 48 and 72 h after treatment. Low doses (0.1-1.0 μg/ml) of Resveratrol enhance cell proliferation, higher doses (10.0-100.0 μg/ml) induce apoptosis and decrease mitotic activity, which is reflected in changes of cell number. Resveratrol influences dose dependently the proliferative and apoptotic activity of human tumor and endothelial cells. The possible role of formaldehyde in the mechanism of action of Resveratrol is discussed.

Keywords: Resveratrol, apoptosis, mitosis

Introduction

Resveratrol, 3,5,4'-trihydroxystilbene, which exists in both cis and trans configurations, occurs naturally in grapes (Langcake *et al*., 1976) and grape products (Siemann and Creasy 1992), as well as in other plant species [*e.g.* Arachis hypogea (Ingham *et al*., 1976), Polygonum cuspidatum (Arichi *et al*., 1982), Yucca schidigera (Uenobe *et al*., 1997)]. The biological function of trans-Resveratrol is thought to be the protection of plants from environmental stress and fungal attack (Soleas *et al*., 1997).

Resveratrol intake has been associated with a moderate consumption of red wine and has been suggested to play a role in the reduction of risk of coronary heart disease (Pace-Asciak *et al*., 1995). This stylbene derivative has been shown to protect human (low density lipoprotein) LDL against copper-catalyzed oxidation *in vitro* (Franke *et al*., 1993) and to inhibit human platelet aggregation and thromboxane B2 synthesis (Bertelli *et al*., 1995; Pace-Asciak *et al*., 1995). An *in vivo* study demonstrated that daily consumption of red wine for 2 weeks reduced the susceptibility of LDL to lipid peroxidation, whereas white wine consumption showed the opposite effect (Fuhrman *et al*., 1995; Nigdikar *et al*., 1998). Although Resveratrol is a powerful antioxidant *in vitro*, it doesn't prevent the oxidation of plasma lipids in healthy rats (Turrens *et al*., 1997). These controversial results demand a dose-dependent study of the action of Resveratrol using endothelial cells.

Resveratrol has been shown to have a cancer preventive activity in tumor initiation, promotion and progression (Jang *et al*., 1997). Resveratrol has a direct antiproliferative effect on human breast epithelial cells (Mgbonyevi *et al*., 1998). Synthetic Resveratrol in concentrations equivalent to those in red wine inhibit proliferation of oral cancer cells (Goldberg *et al*., 1995; Romeroperez *et al*., 1996; ElAttor *et al*., 1999). Resveratrol induced apoptotic cell death in HL60 leukemia cells as well as in T470 breast carcinoma cells (Clement *et al*., 1998). According to recent investigations, Resveratrol is an effective inhibitor of cell growth in general, triggers partial arrest of G1/S transition in prostate cancer cell lines and induces apoptosis (Hsieh *et al*., 1999).

The present paper was designed to study the dose-dependent effect of synthetic Resveratrol on proliferation and apoptosis in endothelial and tumor cells in culture.

Materials and Methods

Various human cell lines HT-29 human colon carcinoma (ATCC HTB-38), SW-620 human colon carcinoma (ATCC CCL-227), HT-1080 human fibrosarcoma (ATCC CLL-121), HUV-EC-C human endothelial (ATCC CRL-1730)

cells were cultured in 6 well and 24 well Grainer plates (Kremsmünster, Austria) using RPMI supplemented with 10% fetal calf serum (Protein Biochemical Ltd., Gödöllõ, Hungary), in a humidified CO_2 incubator. The number of cells at plating was 10^5/ml. Triplicate samples of cultured cells were treated with 1.0-10.0 100.0 μg/ml Resveratrol (Sigma), 24 h after plating. In case of HUV-EC-C cells, 0.1 μg/ml Resveratrol treatment was also performed. Treatment was performed in serum-free RPMI and medium was not changed further during the experiment. Three samples of cells, which were cultured in 24-well plates, were used for cell count. Cells were detached by rinsing after trypsinisation (0.25%) and counted in a Buerker chambre. Three samples of cultures, which were maintained and treated in 6 well plates, were used for morphological studies. For this purpose, cover slips were posited on the bottom of the wells. The cultured cells growing on the cover slips were fixed with methanol and stained with hematoxylin and eosin (HE). Samples were taken for cell count (all types of cultures) and for counting apoptosis and mitosis (HT-29 HT-1080 and HUV-EC-C cultures) 24 and 48 h after Resveratrol treatment.

According to the criteria of Wyllie (1987) mitotic and apoptotic figures were counted in 2000 cells, the mitotic and apoptotic index were given as percent. Statistical analysis was performed using Student's t-test.

Results

Our results show dose-dependent effect on normal endothelial cells in culture. Figure 1 illustrates that the cell number of HUV-EC-C cells in culture is decreased drastically at 10 and maroly 100 μg/ml concentration of trans-Resveratrol for 24 to 48 h. One μg/ml of resveratrol exerted a slight antiproliferative effect. However, a significant, well-measurable proliferation promoting effect on HUV-EC-C cells in culture for 24 to 48 h was detected if the amount of Resveratrol applied was very small (0.1 μg/ml). Table 1 shows the effect of resveratrol on the mitotic and apoptotic index of HUV-EC-C endothelial cell culture. Mitotic index is practically zero at high doses of Resveratrol and at the small dose (0.1 μg/ml) the mitotic index exceeded the control value for 24 to 48 h. The apoptotic index decreased with the decrease of the concentration of resveratrol and this change was near to zero at the 0.1 μg/ml dose.

Figure 2, 3 and 4 demonstrate the response of various human tumor cells as SW-620, HT-29 and HT-1080 to the administration of various doses of resveratrol. Hundred μg/ml treatment caused marked decrease in

Table 1. Effect of resveratrol on the apoptotic and mitotic index of HUV-EC-C Endothelial cell culture

	Apoptosis		Mitosis	
	24 h	48 h	24 h	48 h
Control	3	2	5	6
0.1 μg/ml	0	1	6	7
1.0 μg/ml	1	2	6	7
10 μg/ml	4	5	0	0
100 μg/ml	100	100	0	0

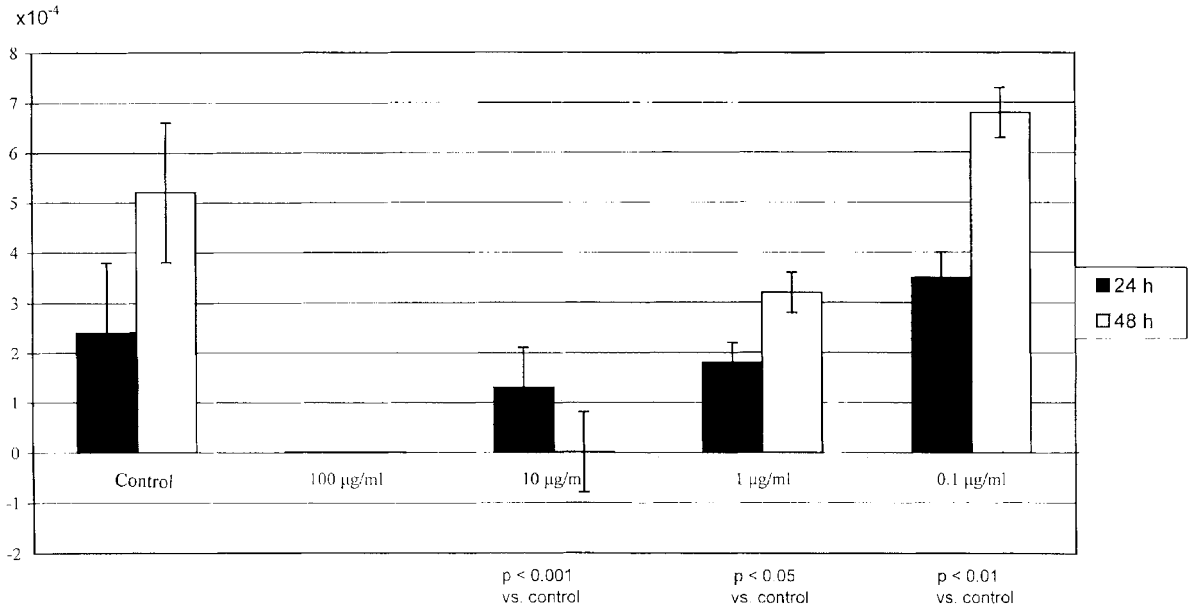

Figure 1. Effect of reserveratol on the proliferation of HUV-EC-C cells. HUV-EC-C human endothelial cells (10^5 cells/ml) were plated and cultured in 24 well Grainer plates as described in the Methods. Triplicate samples of cells were treated with 0.1, 1, 10, 100 μg/ml reserveratol for 24 (open bar) to 48 (solid bar) h in a serum free RPMI. The Control was without reserveratol. Cells were counted in Buerker chamber after trypsinization.

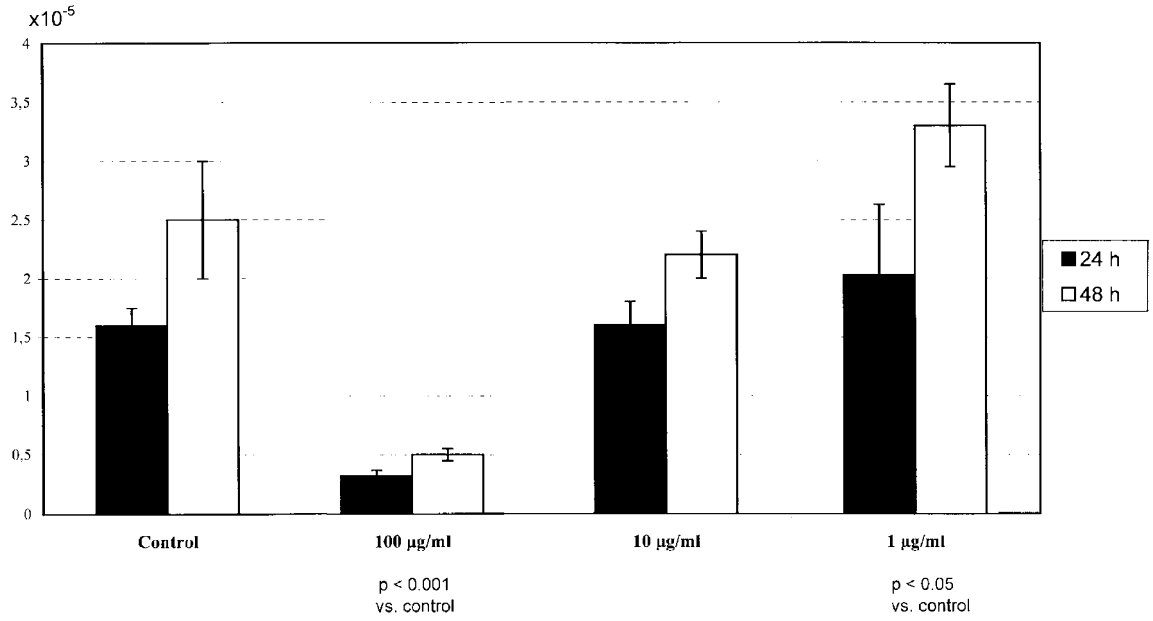

Figure 2. Effect of reseveratol on the proliferation of SW-620 cells. SW-620 human colon carcinoma cells (10^5 cells/ml) were plated and cultured in 24 well Grainer plates as described in the Methods. Triplicate samples of cells were treated with 1, 10, 100 μg/ml Reserveratol for 24 (open bar) to 48 (solid bar) h in a serum free RPMI. The control was without reserveratol. Cells were counted in Buerker chamber after trypsinization.

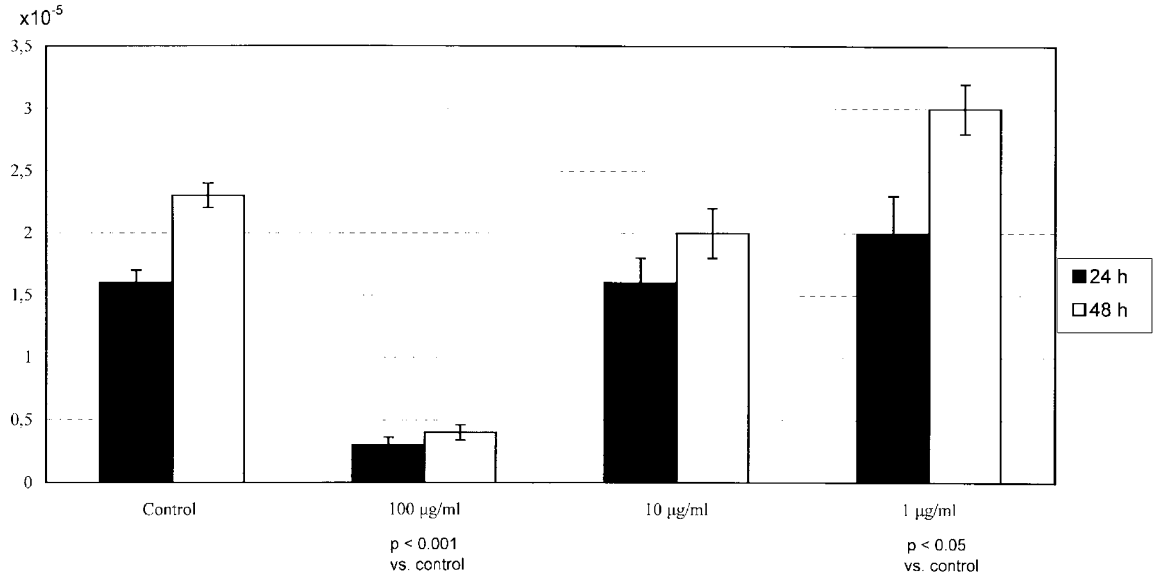

Figure 3. Effect of Reserveratol on the proliferation of HT-29 cells. HT-29 human colon carcinoma cells (10^5 cells/ml) were plated and cultured in 24 well Grainer plates as described in the Methods. Triplicate samples of cells were treated with 1, 10, 100 μg/ml Reserveratol for 24 (open bar) to 48 (solid bar) h in a serum free RPMI. The Control was without Reserveratol. Cells were counted in Buerker chamber after trypsinization.

cell number for 24 and 48 h. Administration of 10 μg/ml was less effective in SW-620, HT-29 and HT-1080 cultures (at 48 h the normal HUV-EC-C cells were totally eradicated Figure 1). Proliferation of the tumor cell lines (SW-620, HT-29 and HT-1080) were stimulated by 1.0 μg/ml in various degrees. Table 2, 3 show apoptotic and mitotic activity of HT-29 and HT-1080 tumor cell cultures, respectively. Mitotic activity was blocked by 100 μg/ml resveratrol in both cultures. Decrease or blockade of mitotic activity was observed after 10 μg/ml resveratrol and slight or no change in mitotic activity was found after 1.0 μg/ml resveratrol. Apoptotic activity was strongly enhanced by 100 μg/ml of resveratrol in all tumor cell cultures. Ten μg/ml of resveratrol caused a slight increase of apoptotic activity and 1 μg/ml did not change or decrease the apoptotic index.

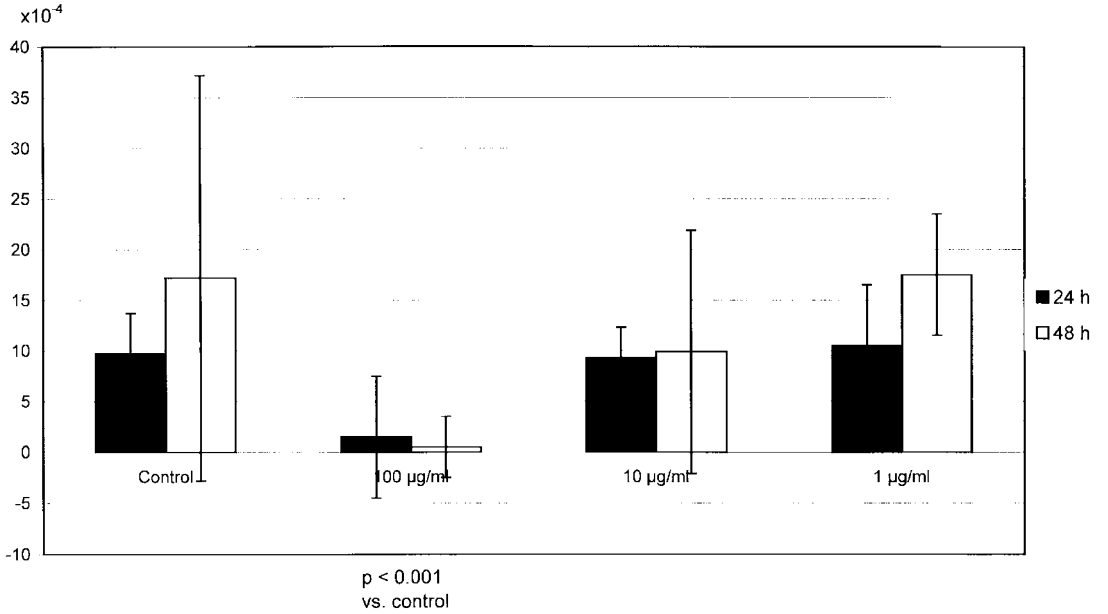

Figure 4. Effect of reserveratol on the proliferation of HT-1080 cells. HT-1080 human fibrosarcoma cells (10^5 cells/ml) were plated and cultured in 24 well Grainer plates as described in the Methods. Triplicate samples of cells were treated with 1, 10, 100 µg/ml Reserveratol for 24 (open bar) to 48 (solid bar) h in a serum free RPMI. The control was without Reserveratol. Cells were counted in Buerker chamber after trypsinization.

Table 2. Effect of resveratrol on the apoptotic and mitotic index of HT-29 cell culture

	Apoptosis		Mitosis	
	24 h	48 h	24 h	48 h
Control	3	2	3	3
1 µg/ml	2	2	4	5
10 µg/ml	22	30	2	1
100 µg/ml	75	75	0	0

Table 3. Effect of resveratrol on the apoptotic and mitotic index of HT-1080 cell culture

	Apoptosis		Mitosis	
	24 h	48 h	24 h	48 h
Control	1	1	4	5
1 µg/ml	2	2	2	5
10 µg/ml	2	8	0	1
100 µg/ml	20	80	0	0

Discussion

A growing body of literature indicates that Resveratrol, a plant constituent enriched in the skin of blue grapes (Kiraly-Veghely *et al.*, 1998), is one of the promising agents for the prevention of heart disease (Frankel *et al.*, 1993; Pendurthi *et al.*, 1999), cancer (Jang *et al.*, 1997; Clement *et al.*, 1998; ElAttor *et al.*, 1999) and inflammatory (Goldberg *et al.*, 1997) diseases.

We demonstrated that Resveratrol has a dose-dependent effect on mitotic and apoptotic activity of endothelial and tumor cell lines. These results partly support earlier similar observations regarding inhibition of cell-proliferation. It seems, however, that the cell-proliferation promoting activity of Resveratrol at small concentrations (0.1 to 1.0 µg/ml) is not negligeble.

It is important to notice that diverse activities of resveratrol are dose-dependent. For instance we demonstrated that resveratrol induced apoptosis in the same concentration range that inhibited cell proliferation. The induction of apoptosis is a possible explanation for the antiproliferative effect of resveratrol (Huang *et al.*, 1999).

Some conceptions are known about the mechanism of action of resveratrol. Similarly to other polyphenols Resveratrol exerts an antioxidant effect (Frankel *et al.*, 1993; Miller *et al.*, 1995). This stylbene derivative has a characteristic double bond and the phenolic hydroxyl groups generate electron withdrawal. In addition Resveratrol as an antioxidant is a H-donor molecule. These characteristics of Resveratrol favor its reactivity with formaldehyde (HCHO) (Tyihak *et al.*, 1998). Our preliminary observations suggest that trans-resveratrol is a natural concentration-dependent HCHO-capturing molecule. Several hydroxymethylated derivatives from the reaction mixture of resveratrol and diluted formaldehyde solution were isolated and identified (Tyihak *et al.*, 1998). It seems that resveratrol facilitates the elimination (collection) of uncontrolled HCHO from tissues. The first step in the elimination of HCHO with resveratrol may lead to the formation of reaction products (hydroxymethyl derivatives) between resveratrol and HCHO

which may promote apoptotic activity (Szende *et al*., 1999). HCHO mobilization from hydroxymethyl groups due to the H-donor potential of resveratrol may be the basis of the diverse biological effects of trans-Resveratrol.

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