Biomedicine & Pharmacotherapy 67 (2013) 43–47

Available online at
SciVerse ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

Resveratrol affects the cross talk between immune and colon cancer cells

Michael Bergman [a,*,c], Gal Sahav Levin [a,c], Hanna Bessler [b,c], Meir Djaldetti [b,c], Hertzel Salman [a,c]

[a] Department of Internal Medicine C, Rabin Medical Center, Hasharon Hospital, 7, Keren Kayemet Street, Petah-Tiqva, Israel
[b] Laboratory for Immunology and Hematology Research, Rabin Medical Center, Hasharon Hospital, Petah-Tiqva, Israel
[c] Sackler School of Medicine, Tel-Aviv University, Ramat-Aviv, Israel

ARTICLE INFO

Article history:
Received 21 October 2012
Accepted 23 October 2012

Keywords:
Resveratrol
Cytokines
Mononuclear cells
Colon cancer cells

ABSTRACT

Resveratrol, a natural polyphenolic compound found mainly in grapes and their seeds, is gaining a widespread appreciation as a therapeutic adjuvant in a variety of diseases including cancer prevention. We examined the effect of resveratrol as a modulator of the immune dialog between peripheral blood mononuclear cells and those from two human colon carcinoma lines, expressed by a possible alteration of cytokine production. Resveratrol, incubated with non-stimulated mononuclear cells, caused a certain reduction of IL-6, IL-1ra and IL-10, and a moderate increase of TNFα release. On the other hand, resveratrol did not affect cytokine production by cancer cells from both lines. When resveratrol was added to immune and cancer cells jointly, an altered dose-dependent decreased production of the examined cytokines was obtained. These results favor the existence of a mechanism, additional to those already described, that may explain the preventive effect of resveratrol on tumorigenesis.

1. Introduction

The effect of nutrients on human health has been firmly established. The majority of them poses several biological activities, such as inhibition of oxidative processes, acting as anti-inflammatory factors, and even exerting anti-cancer properties, observed mainly in vitro. Recently, following reports on its beneficial effect on a wide range of diseases thoroughly reviewed by Yadav et al. [1], the application of resveratrol as a therapeutic adjuvant gains a rising interest. Studies have shown that the French Paradox could be explained by moderate red wine consumption that induces a cardio-protective effect due to the angiogenic, antihypercholesterolemic and antidiabetic properties of resveratrol, a polyphenolic compound presented in the skin of red grapes and red wine [2–4]. The biological effects of resveratrol occur through various systemic, cellular and even sub-cellular pathways that have been reviewed in a number of papers [5–8]. Considering the close relationship between inflammation and cancer development [9], studies have been carried out to connect the carcino-preventing effect of resveratrol with its anti-inflammatory activities. Apparently, the anti-inflammatory properties of resveratrol occur by a variety of mechanisms, such as inhibited synthesis of pro-inflammatory mediators, inhibition of immune cells and suppression of cyclooxygenase-2 production [10]. In addition, it has been shown that the inhibitory activity of resveratrol on monocyte respiratory burst plays an important role in inflammatory suppression [11,12]. Previous in vitro studies in our laboratory have implied that the inflammatory response linked with colon cancer development may proceed through a cross talk between immune and cancer cells [13]. This process may be modulated by a number of drugs, for example aspirin and statins [14,15], as well as by nutrients, such as caffeine and curcumin [16,17]. The purpose of the present study was to address the possibility that resveratrol may affect the cross talk between human peripheral blood mononuclear cells (PBMC) and those from two human colon cancer lines i.e. HT-29 and RKO. The results may enlighten the way by which resveratrol affects cancer development.

2. Materials and methods

2.1. Cell preparation

PBMC were separated from venous blood obtained from adult blood bank donors by gradient centrifugation using Lymphoprep-1077 (Axis-Shield PoC AS, Oslo, Norway). The cells were washed twice in phosphate buffered saline (PBS) and suspended in RPMI-1640 medium (Biological Industries, Beith Haemek, Israel) containing 1% penicillin, streptomycin and nystatin, 10% fetal calf serum (FCS), and designated as complete medium (CM).

2.2. Cell lines

HT-29 and RKO human colon cancer cell lines were obtained from the American Type Cultural Collection (ATCC), Rockville, MD. The cells were maintained in CM containing MacCoy's 5A

* Corresponding author. Tel.: +972 3 9372306; fax: +972 3 9372432.
E-mail address: bermanm@clalit.org.il (M. Bergman).

0753-3322/$ – see front matter © 2012 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.biopha.2012.10.008

(Biological Industries Co, Beth-Haemek, Israel), or modified eagle medium (MEM- Biological Industries Co., Beth-Haemek, Israel) respectively, supplemented with 10% FCS, 2 mM L-glutamine and antibiotics (penicillin, streptomycin and nystatin-Biological Industries Co, Beth-Haemek, Israel). The cells were grown in T-75 culture flasks (Nunc, Roskidle, Denmark) at 37 °C in a humidified atmosphere containing 5% CO_2.

2.3. Resveratrol

Resveratrol (Sigma, Israel) was dissolved in dimethyl sulfoxide (DMSO- Sigma, Israel) at a concentration of 100 mM and kept at –20 °C. Further dilutions were prepared in DMSO before use. Resveratrol was added at final concentrations of 10, 25 and 60 μM. The concentration of DMSO in the cultures with and without reservatrol (controls) was 0.1%.

2.4. Effect of resveratrol on cytokine production by peripheral blood mononuclear cells induced by cancer cells

0.5 ml of PBMC (4×10^6/ml of CM) was incubated with equal volume of CM or with each type of cancer cells (4×10^5/ml of CM) suspended in the appropriate CM. Resveratrol was added at the onset of cultures at concentrations as described above. The cultures were incubated for 24 hours at 37 °C in a humidified atmosphere containing 5% CO_2. At the end of the incubation period, the cells were removed by centrifugation at 1500 rpm for 10 minutes, the supernatants were collected and kept at –75 °C until assayed for cytokine content.

2.5. Effect of resveratrol on the secretion of colon cancer cells' conditioned medium

Colon cancer cells suspended at 4×10^5/ml in the appropriate CM were incubated for 24 hours at 37 °C in a humidified atmosphere containing 5% CO_2 with or without resveratrol at concentrations as indicated. At the end of the incubation period, the cells were removed by centrifugation at 1500 rpm for 10 minutes and the conditioned media were assayed for their effect on cytokine secretion by PBMC as follows: 0.5 ml of PBMC (4×10^6/ml of CM) were incubated for 24 hours at 37 °C with equal volume of the various conditioned media. At the end of the incubation period, the cells were removed by centrifugation at 1500 rpm for 10 minutes, the supernatants were collected and kept at –75 °C until assayed for cytokine content.

2.6. Effect of resveratrol on malignant cell proliferation (XTT test)

0.1 ml aliquots (4×10^5/ml) of HT-29 or RKO cells suspended in CM were placed in each one of 96-well flat-bottomed plates (Nunc, Roskidle, Denmark). Resveratrol was added at the onset of the culture at final concentrations of 10, 25 and 60 μM. The cultures, set up in triplicates, were incubated for 24 hours at 37 °C in a humidified atmosphere supplemented with 5% CO_2. At the end of the incubation period XTT test (Biological Industries, CO Beth-Haemek, Israel) was performed according to the instructions provided by the manufacturer.

2.7. Cytokine content in the supernatants

The concentration of the following cytokines: TNFα, IL-1β, IL-1ra, IL-6, IL-10 and IFNγ in the supernatants was tested using ELISA kits specific for human cytokines (Biosource International, Camarillo, CA) as detailed in the guide-line provided by the manufacturer. The detection levels of these kits were: 15 pg/ml for IL-6, and 30 pg/ml for the others.

2.8. Statistics

Data was analyzed using ANOVA with repeated measures for each cytokine, and paired *t*-test to compare the difference between the levels of cytokine obtained with or without various concentrations of resveratrol. P values of < 0.05 were considered as statistically significant. The results are expressed as mean ± SEM.

3. Results

3.1. Effect of resveratrol on malignant cell proliferation

A dose-dependent inhibition of the proliferation of both HT-29 and RKO cell lines was found following 24 h of incubation with increasing doses of resveratrol ($F3,35 = 3.4$, $P = 0.034$ and $F3,35 = 7.12$, $P = 0.00136$, respectively) (Fig. 1). At 10 μM of resveratrol, the proliferation of both cell lines was not significantly affected. At resveratrol concentration of 25 and 60 μM, the proliferation of HT-29 cells was reduced by 18 and 27%, respectively ($P = 0.008$ and $P = 0.002$, respectively) and that of RKO cells by 17 and 17% respectively ($P = 0.0037$and $P = 0.0008$, respectively).

3.2. Effect of resveratrol on cytokine production by cancer cells

Incubation of malignant cells with resveratrol at doses as indicated had no effect on the production of any of the cytokines examined in this study. Conditioned media from HT-29 or RKO cells cultured for 24 hours did not contain detectable levels of the above- tested cytokines.

3.3. Effect of resveratrol on cytokine production by peripheral blood mononuclear cells

The amount of cytokines secreted by non-stimulated PBMC was significantly lower than that produced by PBMC incubated for 24 hours with cancer cells (Table 1). Incubation of PBMC with resveratrol caused a significant inhibition of the secretion of IL-6 ($F3,23 = 10.75$, $P = 0.0005$) and IL-10 ($F3,23 = 6.97$, $P = 0.0036$), whereas that of TNFα was slightly stimulated ($F3,23 = 4.13$,

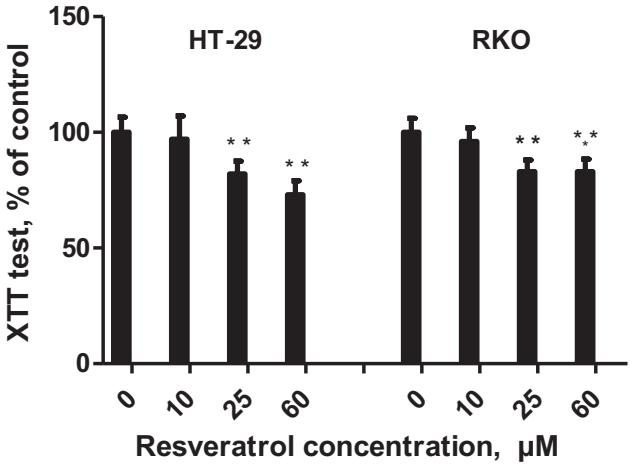

Fig. 1. Effect of resveratrol on the proliferation rate of HT-29 and RKO colon cancer cells tested by XTT-proliferation test. 4×10^4 cells/well were incubated for 24 hours with or without resveratrol at doses as indicated. At the end of incubation period XTT test was performed. The results were calculated as percent of the control (cells incubated without Resveratrol). Each column represents the mean of 7 experiments. Bars represent SEM. Asterisks represent statistically significant difference from peripheral blood mononuclear cells incubated without resveratrol ($^{**}P < 0.01$, $^{***}P < 0.001$).