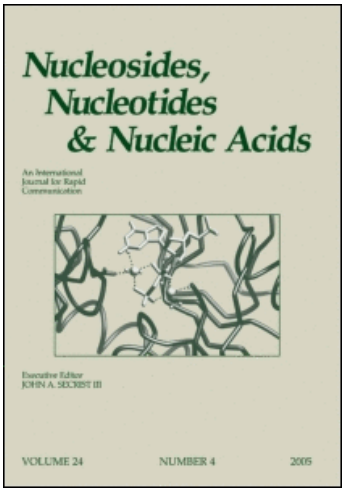

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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Z. Horvath[a]; P. Saiko[a]; C. Illmer[a]; S. Madlener[a]; T. Hoechtl[a]; W. Bauer[a]; T. Erker[b]; W. Jaeger[b]; M. Fritzer-Szekeres[a]; T. Szekeres[a]
[a] Clinical Institute of Medical and Chemical Laboratory Diagnostics, General Hospital of Vienna, Medical University of Vienna, Vienna, Austria [b] Department of Pharmaceutical Chemistry, University of Vienna, Vienna, Austria

To cite this Article Horvath, Z. , Saiko, P. , Illmer, C. , Madlener, S. , Hoechtl, T. , Bauer, W. , Erker, T. , Jaeger, W. , Fritzer-Szekeres, M. and Szekeres, T.(2006) 'Resveratrol, an Ingredient of Wine, Acts Synergistically with Ara-C and Tiazofurin in HL-60 Human Promyelocytic Leukemia Cells', Nucleosides, Nucleotides and Nucleic Acids, 25: 9, 1019 — 1024
To link to this Article: DOI: 10.1080/15257770600890574
URL: http://dx.doi.org/10.1080/15257770600890574

Nucleosides, Nucleotides, and Nucleic Acids, 25:1019–1024, 2006

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770600890574

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RESVERATROL, AN INGREDIENT OF WINE, ACTS SYNERGISTICALLY WITH ARA-C AND TIAZOFURIN IN HL-60 HUMAN PROMYELOCYTIC LEUKEMIA CELLS

Z. Horvath, P. Saiko, C. Illmer, S. Madlener, T. Hoechtl, and W. Bauer □ *Clinical Institute of Medical and Chemical Laboratory Diagnostics, General Hospital of Vienna, Medical University of Vienna, Vienna, Austria*

T. Erker and W. Jaeger □ *Department of Pharmaceutical Chemistry, University of Vienna, Vienna, Austria*

M. Fritzer-Szekeres and T. Szekeres □ *Clinical Institute of Medical and Chemical Laboratory Diagnostics, General Hospital of Vienna, Medical University of Vienna, Vienna, Austria*

□ *Resveratrol (RV), a naturally occurring stilbene derivative, is a potent free radical scavenger causing a number of biochemical and antineoplastic effects. It was shown to induce differentiation and apoptosis in leukemia cells and was also identified as an inhibitor of ribonucleotide reductase (RR), a key enzyme of DNA synthesis.*

In this study, we report about the biochemical effects of RV in HL-60 human promyelocytic leukemia cells. RV effectively inhibited in situ *RR activity. Furthermore, incubation of HL-60 cells with RV significantly decreased intracellular dCTP, dTTP, dATP and dGTP concentrations. In growth inhibition and clonogenic assays, RV acted synergistically with both Ara-C and tiazofurin in HL-60 cells. We conclude that RV could become a viable candidate as one compound in the combination chemotherapy of leukemia and therefore deserves further* in vitro *and* in vivo *testing.*

Keywords Resveratrol; Leukemia; Ara-C; Tiazofurin; Synergistic combination effects

INTRODUCTION

The biochemical effects of RV have been subject to intense studies, and it is understood that its antitumor activity has multifactorial causes. It has been shown that RV is an effective inhibitor of the enzyme ribonucleotide reductase (RR, EC1.17.4.1),[1] which catalyzes the rate-limiting step of de

This study was supported by the "Medizinisch Wissenschaftlicher Fonds des Buergermeisters der Bundeshauptstadt Wien #2296" to T. S.

Address correspondence to Zsuzsanna Horvath, Clinical Institute of Clinical Pathology, Medical University of Vienna, 18-20 Währinger Gürtel, Vienna, Austria. E-mail: zsuzsanna.Horvath@gmx.at

novo DNA synthesis. The enzyme is significantly upregulated in malignant cells, which makes RR an excellent target for cancer chemotherapy.

We now revealed that RV depletes dNTP concentrations including dCTP and dGTP pools and that it is able to effectively inhibit the incorporation of ^{14}C-labelled cytidine into DNA. Furthermore, it was tested whether a combination of RV and Ara-C or RV and tiazofurin also could yield synergistic cytotoxic and apoptotic effects in human HL-60 promyelocytic leukemia cells. The results of these experiments could lead to additional options for chemotherapy of human leukemia.

MATERIALS AND METHODS

Chemicals and Cell Culture

All chemicals used were obtained from Sigma-Aldrich, Vienna, Austria. Tiazofurin was kindly provided by the National Cancer Institute (NCI Bethesda, MD).

The HL-60 human promyelocytic leukemia cell line was purchased from ATCC (American Type Culture Collection, Rockville, MD, USA).

Growth Inhibition Assays

HL-60 cells were incubated with various concentrations of RV for 72 hours. To determine the IC_{50} value of RV, cells were then counted. To analyze the combination effects of RV with Ara-C, HL-60 cells were simultaneously incubated with various concentrations of RV and Ara-C or tiazofurin, while keeping the combination ratio constant.

Determination of Deoxynucleoside-Triphosphates

Logarithmically growing HL-60 cells were incubated with RV for 24 hours and dNTPs were extracted as described by Garrett and Santi.[2] dNTPs were measured using a Merck "La Chrom" HPLC system equipped with an L-7200 autosampler, an L-7100 pump, an L-7400 UV detector, and a D-7000 interface. The concentration of dNTPs was calculated as percent of control.

Incorporation of ^{14}C-Labelled Cytidine into DNA

To analyze the effect of RV incubation on the *in situ* activity of ribonucleotide reductase, an assay was performed as described previously.[3]

Hoechst Dye 33258 and Propidium Iodide Double Staining

After incubation for 48 hours with various concentrations of RV and Ara-C or tiazofurin, cells were stained with Hoechst dye 33258 (5 μg/ml)

and propidium iodide (2 μg/ml), examined by fluorescence microscopy using DAPI filters and judged according to their morphology, cell membrane integrity and presence of apoptotic bodies. Results are presented as percentage of total cell count exhibiting apoptotic features.

Statistical Calculations and Calculation of Synergism

The calculations of combination effects were performed using the "Calcusyn" software designed by Chou and Talalay[4] (Biosoft, Ferguson, MO, USA). Synergistic combination effects are indicated by a combination index <1. Significant differences were calculated by t-test using the Prism 3.0 software (GraphPad Software, San Diego, CA, USA).

RESULTS

Changes in Intracellular Concentrations of dNTPs after Treatment with Resveratrol

All dNTP pools were significantly decreased compared with untreated controls after incubation with 12.5 μM RV for 24 hours (69%, 61%, 26%, and 30% of control for dCTP, dTTP, dATP, and dGTP concentrations, respectively). Incubation with 6.25 μM RV significantly decreased intracellular dTTP and dGTP pools to 56% and 24% of control values, respectively.

Inhibition of Incorporation of ^{14}C-cytidine into DNA

After treatment of HL-60 cells with 12.5, 25, and 50 μM RV for 24 hours, ^{14}C-cytidine incorporation into DNA was significantly decreased to 45%, 31.5%, and 2.5% of control values, respectively. Results are shown in Figure 1.

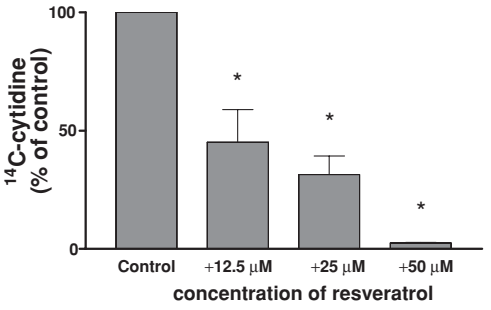

FIGURE 1 Incorporation of ^{14}C-cytidine into DNA of HL-60 cells after treatment with resveratrol. Data are means ± SEM of two measurements. Values significantly ($p < 0.05$) different from control are marked with an asterisk (*).

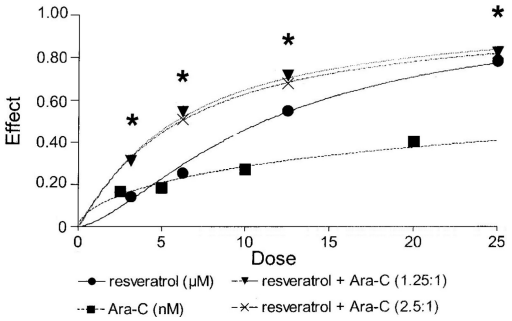

FIGURE 2 Simultaneous growth inhibition assay using resveratrol and Ara-C. Standard deviations were <5%. Synergistic combination indices <1 are marked with an asterisk (∗).

Simultaneous Growth Inhibition Assay Using Resveratrol and Ara-C or Tiazofurin

Synergism between Ara-C and RV was apparent in all concentrations tested. Growth curves are depicted in Figure 2. RV also was able to synergistically or additively enhance the cytotoxic effects of tiazofurin, depending on the concentrations applied. Combination of RV and tiazofurin doses below

TABLE 1 Growth Inhibitory Effects of Resveratrol and Tiazofurin Applied as Single Drugs and in Combination in HL-60 Human Promyelocytic Leukemia Cells

Agent/Combination	Concentration	Cell count (% of control)	Predicted value[a]	Combination Index
Resveratrol, μM	3.125	79.4		
	6.25	71.9		
	12.5	42.4		
Tiazofurin, nM	2.5	98.2		
	5	89.0		
	10	70.6		
	20	24.0		
Resveratrol + Tiazofurin	3.125 + 2.5	87.9	77.9	1.83
Resveratrol + Tiazofurin	3.125 + 5	70.7	70.7	0.89[b]
Resveratrol + Tiazofurin	3.125 + 10	43.5	56.1	0.95[b]
Resveratrol + Tiazofurin	6.25 + 2.5	73.7	70.6	1.67
Resveratrol + Tiazofurin	6.25 + 5	46.0	63.9	0.92[b]
Resveratrol + Tiazofurin	6.25 + 10	30.1	50.8	0.87[b]

Cells were simultaneously incubated with resveratrol and tiazofurin for 72 hours. Then cell count was determined.

Data are means of two determinations, and standard deviations were within 5%.

[a]Predicted values are: $\frac{\text{resveratrol} \times \text{tiazofurin}(\%)}{100}$.

[b]Values indicate synergistic combination effects according to Chou and Talalay.[4]

or near IC_{50} values yielded combination indices <1 as shown in Figure 2 and Table 1.

Induction of Apoptosis

All three compounds, RV, Ara-C and tiazofurin induced apoptosis in a dose-dependent manner. However, RV was capable to synergistically enhance the number of apoptotic cells in combination with either Ara-C or tiazofurin (Table 2).

TABLE 2 Induction of Apoptosis by Resveratrol, Ara-C and Tiazofurin Applied as Single Drugs and in Combination in HL-60 Human Promyelocytic Leukemia Cells

Agent/Combination	Concentration	Apoptosis (% of cell count)	Predicted value[b]	Combination Index
Control		0.8		
Resveratrol, μM	3.125	1.3		
	6.25	2.8		
	12.5	4.1		
	25	7.2		
Ara-C, nM	2.5	1.6		
	5	2.2		
	10	1.7		
	20	2.8		
Tiazofurin, μM	2.5	2.4		
	5	3.1		
	10	16.9		
	20	29.6		
Resveratrol	3.125			
+ Ara-C	2.5	56.0	2.9	0.17[c]
Resveratrol	6.25			
+ Ara-C	5	6.8[a]	5.0	0.29[c]
Resveratrol	12.5			
+ Ara-C	10	15.6[a]	5.7	0.21[c]
Resveratrol	25			
+ Ara-C	20	50.3[a]	9.9	0.07[c]
Resveratrol	3.125			
+ Tiazofurin	2.5	5.8[a]	3.7	0.60[c]
Resveratrol	6.25			
+ Tiazofurin	5	7.7[a]	5.9	0.93[c]
Resveratrol	12.5			
+ Tiazofurin	10	26.7[a]	21.0	0.61[c]
Resveratrol	25			
+ Tiazofurin	20	65.9[a]	36.8	0.38[c]

Cells were simultaneously incubated with resveratrol + Ara-C or resveratrol + tiazofurin for 48 hours. Then cells were double stained with Hoechst 33258 and propidium iodide and visualized as described in the methods section. Data are means of two determinations, and standard deviations were within 5%.

[a]Values are significantly ($p < 0.05$) higher than calculated for additive apoptosis induction.

[b]Predicted values are: $\frac{\text{resveratrol} + \text{Ara}-\text{C/tiazofurin}(\%)}{100}$.

[c]Values indicate synergistic combination effects according to Chou and Talalay.

DISCUSSION

In the present study, we could show that RV inhibits in situ RR activity in human promyelocytic HL-60 leukemia cells.[5,6] In addition, we revealed that RV decreased all four intracellular dNTP concentrations, the products of RR. By inhibition of RR it is possible to specifically target rapidly proliferating tumor cells without affecting normal cells. In combinations with Ara-C and tiazofurin, RV could synergistically enhance the cytotoxic effects of these compounds. Being an ingredient of grapes and wine, RV, therefore, might have limited toxic side effects.

The use of RV might be a promising additional option for combination treatment of human leukemia. Based on our data, in vivo studies using RV in combination with compounds like Ara-C or tiazofurin, for chemotherapy of leukemia should be reasonable.

REFERENCES

1. Fontecave, M.; Lepoivre, M.; Elleingand, E.; Gerez, C.; Guittet, O. Resveratrol, a remarkable inhibitor of ribonucleotide reductase. *FEBS Lett.* **1998**, 421, 77–279.
2. Garrett, C.; Santi, D.V. A rapid and sensitive high pressure liquid chromatography assay for deoxyribonucleoside triphosphates in cell extracts. *Anal. Biochem.* **1979**, 99, 268–273.
3. Szekeres, T.; Gharehbaghi, K.; Fritzer, M. et al. Biochemical and antitumor activity of trimidox, a new inhibitor of ribonucleotide reductase. *Cancer Chemother. Pharmacol* **1994**, 34, 63–66.
4. Chou, T.C.; Talalay, P. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Adv. Enzyme Regul.* **1984**, 22, 27–55.
5. Nicander, B.; Reichard, P. Relations between synthesis of deoxyribonucleotides and DNA replication in 3T6 fibroblasts. *J. Biol. Chem.* **1985**, 260(9), 5376–5381.
6. Weckbecker, G.; Cory, J.G. Ribonucleotide reductase activity and growth of glutathione-depleted mouse leukemia L1210 cells in vitro. *Cancer Lett.* **1988**, 40(3), 257–264.