# Antiproliferative activities of resveratrol and related compounds in human hepatocyte derived HepG2 cells are associated with biochemical cell disturbance revealed by fluorescence analyses.

Didier Colin, Allan Lancon, Dominique Delmas, Gerard Lizard, Jessica Abrossinow, Edmond Kahn, Brigitte Jannin, Norbert Latruffe

**Fig. 1**

**Chemical structures of compounds**

Chemical structures of trans-resveratrol (A, R=OH), trans-resveratrol triacetate (A, R=$CH_3$-COO), ε-viniferin (B, R=OH), and ε-viniferin pentaacetate (B, R=$CH_3$-COO).

**Fig. 2**

**Compared antiproliferative effects of resveratrol, ε-viniferin and their acetate derivatives, and vineatrol on HepG2 cells.**

**A**, Antiproliferative effects of ε-viniferin and resveratrol-containing extract (vineatrol) compared to resveratrol.

HepG2 cells were incubated with either 0.1% Ethanol (control), 30 µM resveratrol (R 30 µM), ε-viniferin (εV 30 µM), 60 µM resveratrol (R 60 µM), ε-viniferin (εV 60 µM) or with vineatrol in an equivalent amount of 10 µM resveratrol (Vinea 10) or of 30 µM resveratrol (Vinea 30). Treated cells were harvested daily and cell growth was estimated by counting viable cells with a haemocytometer. The number of viable cells was estimated by trypan blue dye exclusion test. Values represent the means of viable cells in three wells per time point from one representative experiment repeated three time ±SD. Means are significantly different by Mann-Whitney test with $p<0.01$: (*) *versus* control; (#) *versus* R 30 µM.

**B**, Effects of acetylation on antiproliferative potential of resveratrol and ε-viniferin.

HepG2 cells were incubated with 0.1% Ethanol (control), 30 µM resveratrol (R 30 µM), resveratrol triacetate (R3A 30 µM), ε-viniferin (εV 30 µM), or ε-viniferin pentaacetate (εV5A 30 µM). Cell growth was estimated and represented as described in A.

**C**, Polyphenols IC50 determinations.

HepG2 cells were incubated with either 0.1% Ethanol (control), 1, 5, 10, 30, 60 and 100 µM of each polyphenols and also with vineatrol expressed as 1, 5, 10, 30, 60, and 100 µM of final resveratrol concentrations. Treated cells were harvested after 48h of treatments and cell growth was estimated as described above. Values are ratios between average numbers of viable cells in treated and control.

**Fig. 3**

**Competitive effect of resveratrol or resveratrol triacetate excess on tritiated resveratrol uptake by HepG2 cells.**

HepG2 cells were incubated at 37°C or at 4°C for 10 minutes with [$^3$H] resveratrol alone in

complete medium (R*) and with a 20-fold excess of resveratrol triacetate (R3A) or unlabeled resveratrol (R). After cell lysis, the cell-associated radioactivity was counted to determine tritiated resveratrol uptake. Presented data are from a representative experiment among three and each point represent the mean of three determinations on separate wells ±SD. Values are significantly different by Mann-Whitney test from the control tritiated resveratrol uptake at 37°C with $p<0.01$ (##).

**Fig. 4**

**Excitation (A) and emission (B) spectra of resveratrol (R), resveratrol triacetate (R3A), ε-viniferin (εV) and ε-viniferin pentaacetate (εV5A).**

Polyphenols stock solutions (10 µM) were prepared in ethanol and scanned in quartz microcuves for fluorescence emission and excitation in a Kontron SFM25 spectrofluorimeter. Excitation spectra were obtained by all emitted wavelengths acquisition after excitation. Emission spectra were obtained by scanning emitted wavelengths at 330 nm light excitation. Ethanol spectra were used for baseline subtraction.

**Fig. 5**

**Time course of HepG2 cells polyphenols uptake evaluated by fluorescence emission.**

HepG2 cells were treated with 0.1% ethanol (control), 10, 30 and 60 µM resveratrol (R), resveratrol triacetate (R3A), ε-viniferin (εV) or ε-viniferin pentaacetate (εV5A) for 1, 2, 5, 10, 30 minutes, 3h, 6h, 24h and 48h. After treatment, cells were harvested, quickly washed in cold PBS and maintained at 4°C during flow cytometry analyses. Cells were excited by using a 350 nm lamp and the emitted fluorescence (>420 nm) was acquired in a FL4 channel. Values are means of the cell fluorescence fold increase from three different wells against controls of one representative experiment among three independent ones ±SD. All kinetics were significantly different from the control after ANOVA tests with $p<0.05$.

**Fig. 6**

**Polyphenols induced NADPH accumulation**

HepG2 cells were treated with 0.1% ethanol (control), 30 and 60 µM of resveratrol, resveratrol triacetate, ε-viniferin and ε-viniferin penataacetate for 48h. After 3 and 6h, cells were lysed and NADPH amount in extracts were quantified. Values are means of NADPH levels in three different wells of one representative experiment among three independent ones ±SD after 3h ▒ or 6h treatments ■. All NADPH levels in treated cells are significantly

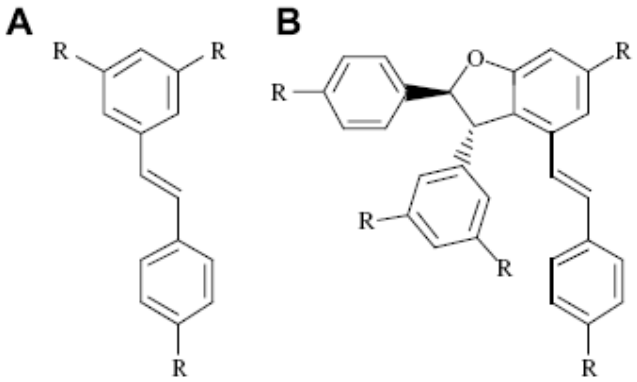
different from control cells and between 3 and 6h treatments by Mann-Whitney test with $p<0.05$.

**Fig. 7**

**Polyphenols induced cell responses revealed by autofluorescence time course record.**

HepG2 cells were treated with 0.1% ethanol (control), resveratrol (R) at 10, 30 and 50 µM, resveratrol triacetate (R3A), ε-viniferin (εV) or ε-viniferin pentaacetate (εV5A) altogether at 30 µM for 24 and 48h. After treatment, cells were harvested, quickly washed in cold PBS and maintained at 4°C during flow cytometric analysis. (**A)**, cells were excited by a 488 nm lamp and the emitted fluorescence (520±10 nm) was acquired in a FL1 channel. Values are means of the cells fluorescence fold increase from three different wells against controls of one representative experiment among three independent, ±SD. Means are significantly different by Mann-Whitney test *versus* control with $p<0.05$ (*) and $p<0.01$ (**). (**B)**, cells were then cytospined and mounted using a fluorescent mounting medium. Spectral analyses of the obtained slides were performed using the FAMIS process on confocal laser scanning microscopic sequences of images, showing in situ fluorescence emissions after 488 nm laser excitation. (**C)**, Side-Scatter parameter changes on cells after 48h treatments.

## FIGURES :

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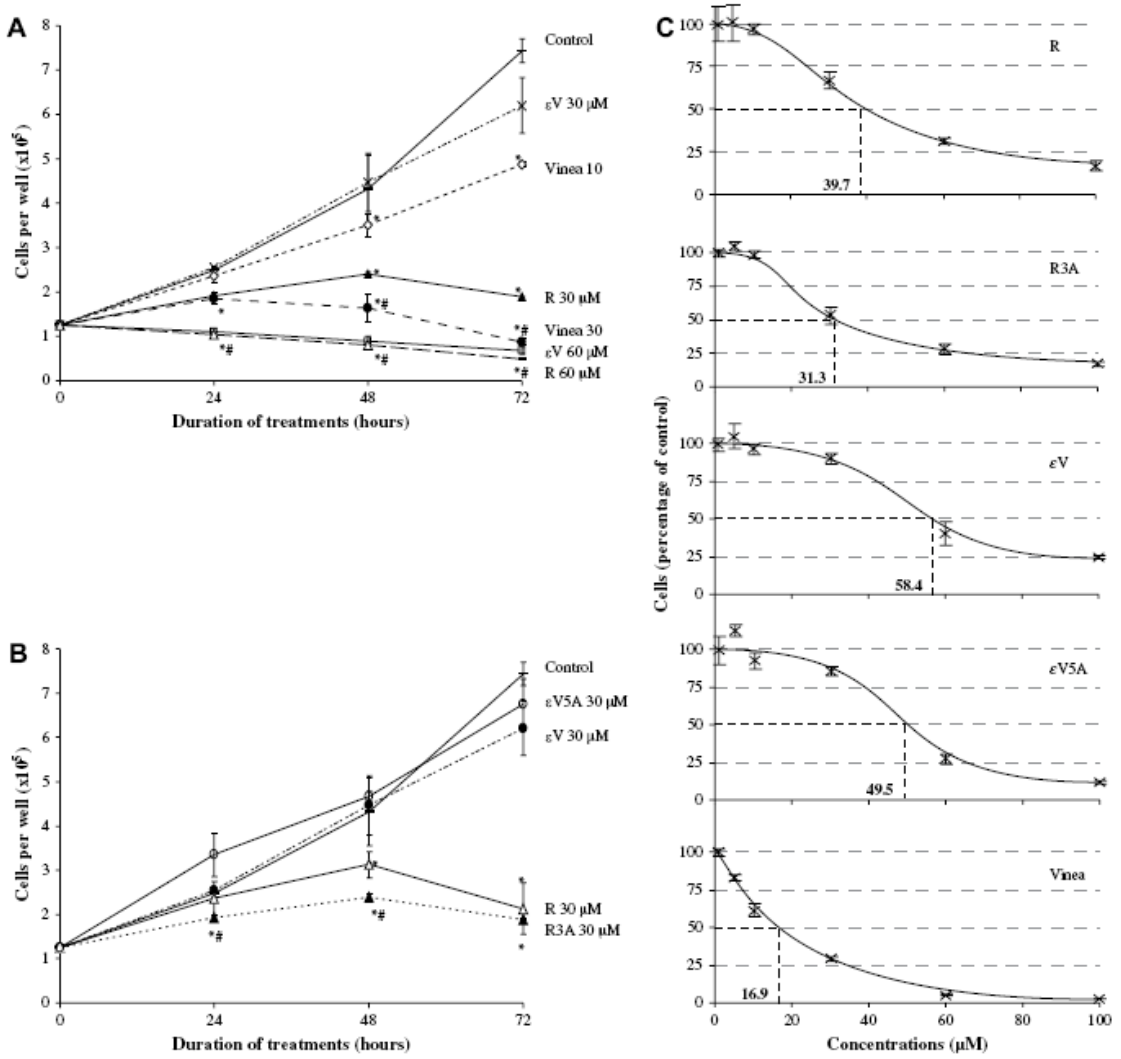


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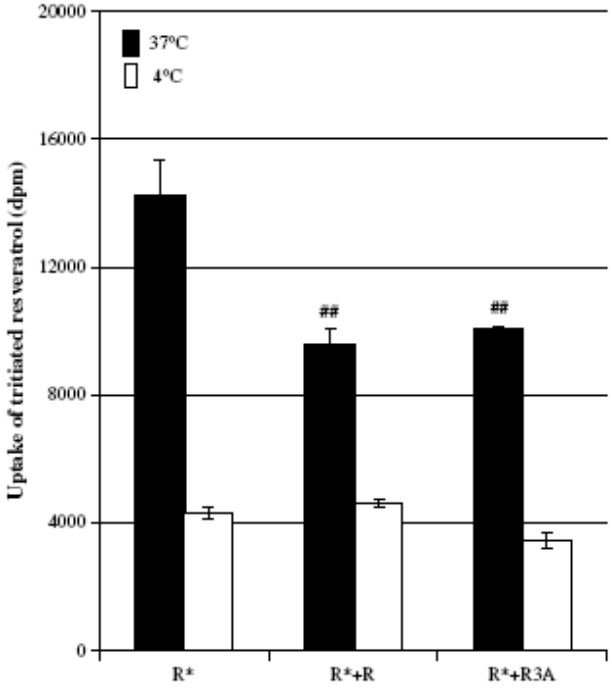


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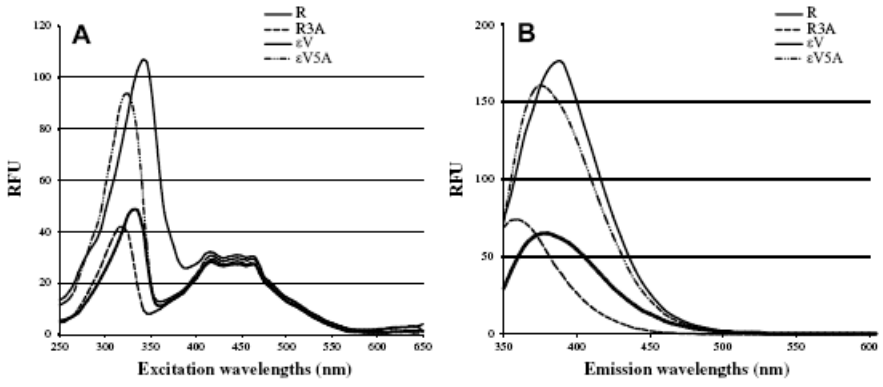


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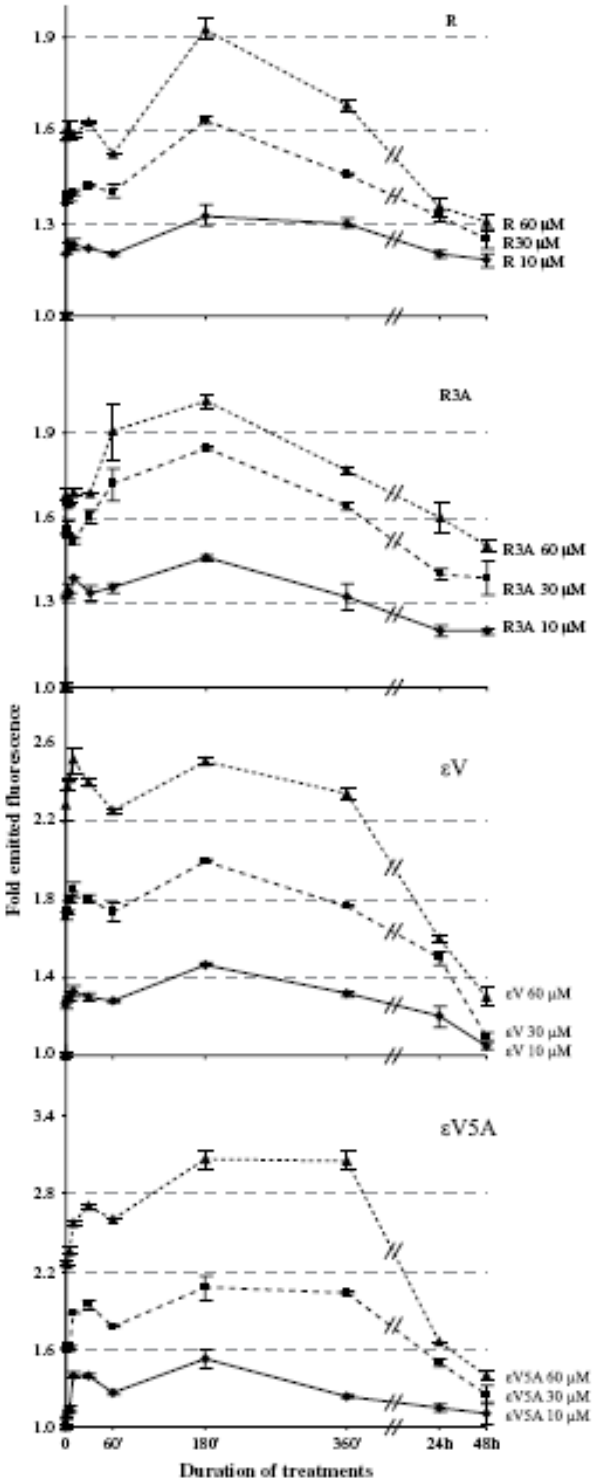


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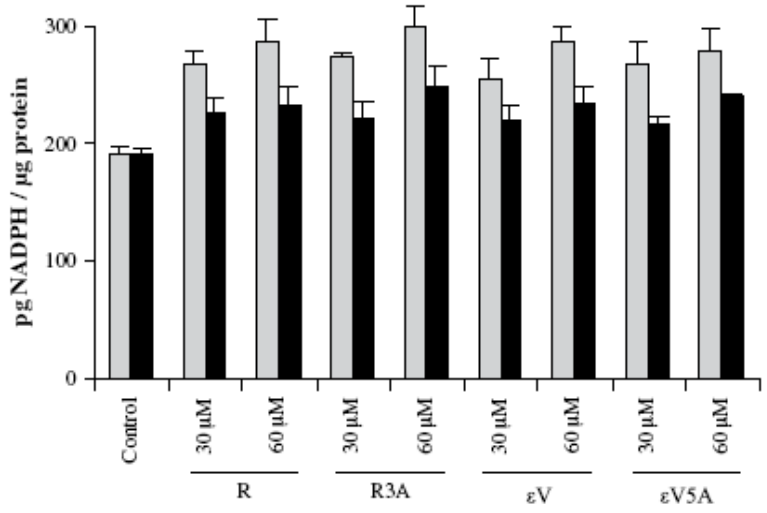


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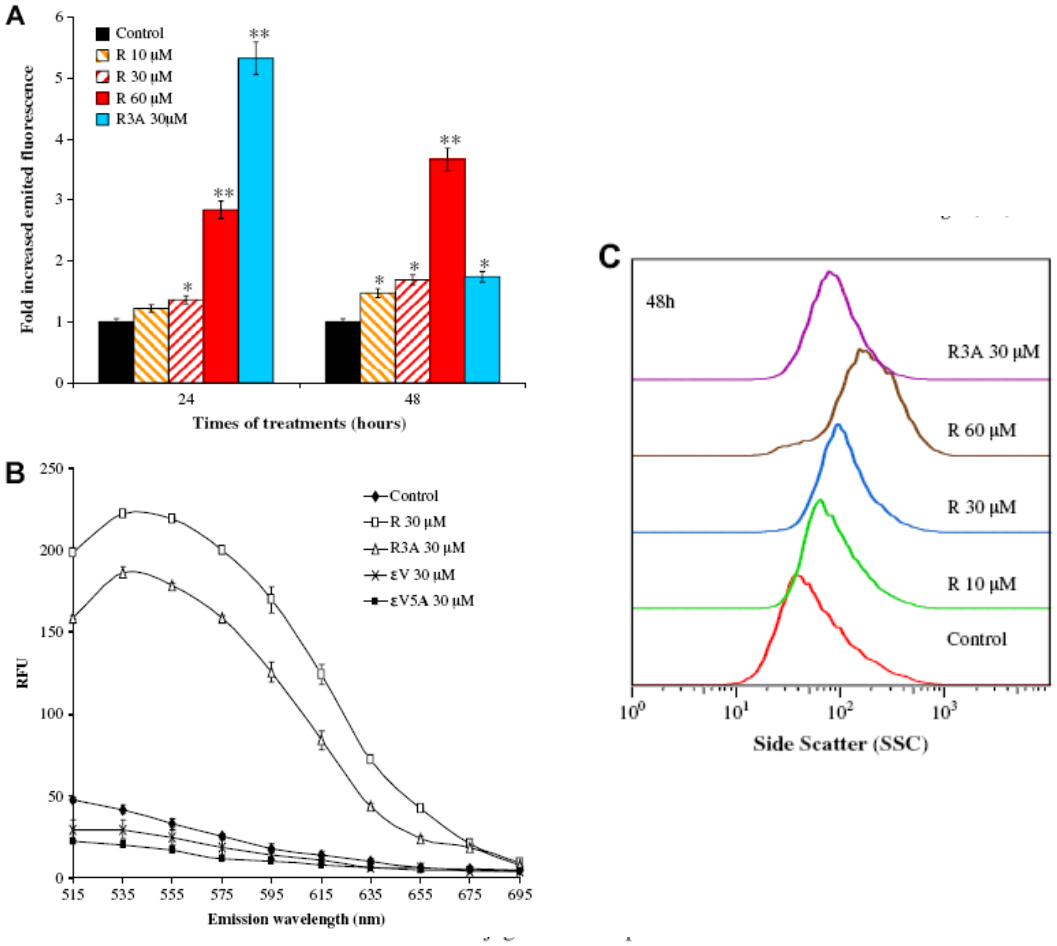


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