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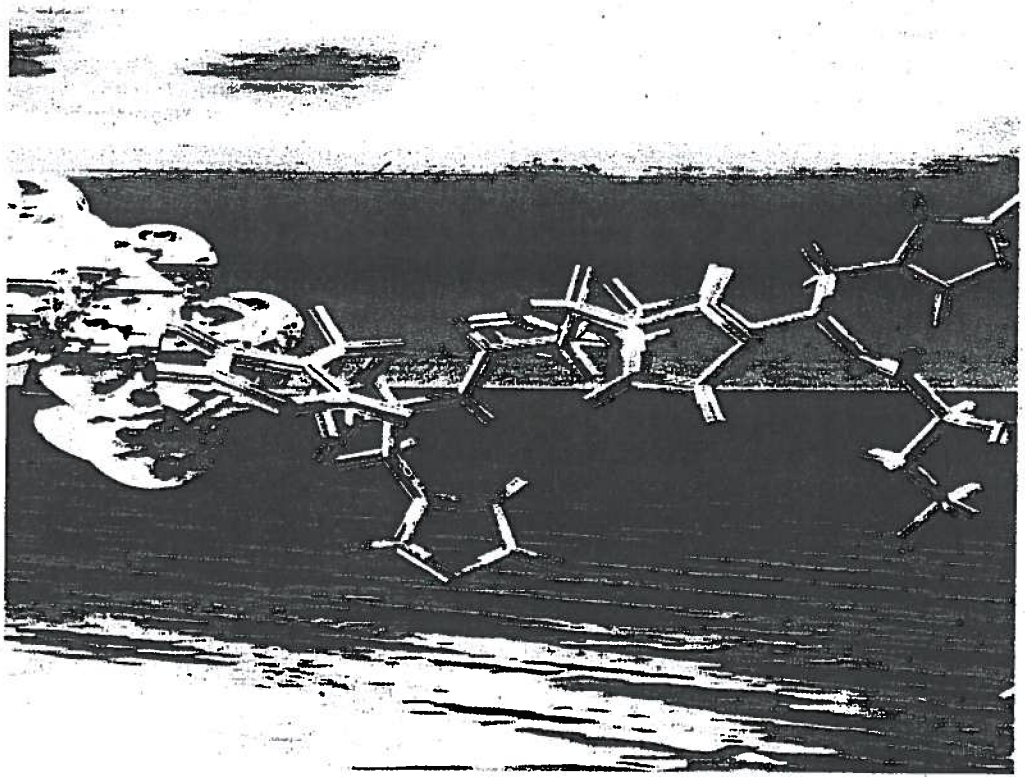

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EFFECTS OF *trans*-RESVERATROL ON PACLITAXEL-INDUCED CELL CYCLE ARREST AND ITS REGOLATORY ELEMENTS IN HUMAN NEUROBLASTOMA SH-SY5Y CELL LINE

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INTRODUCTION: Resveratrol is a polyphenol found in grape and black wine. *trans*-resveratrol is the biologically active form of the polyphenolic compound. In different models it has been shown to have antioxidant, anti-inflammatory, antiplatelet aggregation activity. It has been also shown to have anticancer activity, to inhibit cell cycle progression and DNA synthesis

Paclitaxel is an antineoplastic drug which is active against metastatic tumor of lung and breast but it causes peripheral neuropathy, accumulating in dorsal root ganglia (1). Paclitaxel is able to alter the microtubules polymerization rate and inhibits their depolimeryzation. On tumoral cells, paclitaxel is active during the mitosis phase of the cell cycle because it interferes with mitotic spindle formation.

In previous studies we have demonstrated that *trans*-resveratrol was able to inhibit paclitaxel-induced apoptosis in SH-SY5Y acting on paclitaxel-induced apoptosis pathway, while it did not alter paclitaxel-induced microtubules polymerization (2,3). In the present study we have investigated the hypotesis that the antiapoptotic effect of *trans*-resveratrol was due to its action on cell cycle progression preventing SH-SY5Y cells enter into mitosis and be killed by the antineoplastic drug.

MATERIAL AND METHODS: SH-SY5Y cells, cultured in Dulbecco's Modified Eagle's Medium additioned with 10% Fetal Bovine Serum, were incubated for different times in the presence of paclitaxel 1μM, of resveratrol 50μM or with both the substances simultaneously. SH-SY5Y cell cycle distribution was determined by Propidium Iodide staining and FACS analysis while mitotic index was evaluated through Giemsa stainig.

To assess cyclin E, cyclin A, cyclin B1 level and cdk 1 phosphorylation state total cellular protein extracts were prepared at different time points and analyzed by immunoblotting.

RESULTS: Paclitaxel 1μM blocked SH-SY5Y treated cells in mitosis while the addition of 50μM resveratrol pratically reverted this situation. The mitotic index [(cells in mitosis/total cell count) x 100] of paclitaxel-treated cultures was 59.4 $\pm$ 4 while it was reduced to 11.9 $\pm$ 2.5 in co-treated ones. Flow cytometry analysis confirmed that paclitaxel alone arrested cell in G2/M while resveratrol was able to prevent this arrest and cause a slowing down in early S phase.

Resveratrol 50μM had effect on cell cycle control proteins such as cyclin E, increasing its level starting from 4/6 hours. The level of cyclin A increased after 14 hours both in cultures exposed to 50μM resveratrol alone or in cultures co-treated with 1μM paclitaxel and 50μM resveratrol. Furthermore, 50μM resveratrol was able to inhibit both 1μM paclitaxel-induced cyclin B1 accumulation as early as at 4-6 hours and cdk1 dephosphorylation, i.e. activation, that began after 14 hours of treatment.

Together with the previous results the present ones suggest that the effect of resveratrol on paclitaxel-induced apoptosis was partly due to its effect on apoptotic transduction pathways, and partly to its effects on cell cycle progression, preventing the cells to reach the mitosis phase during which paclitaxel is able to induce apoptosis.

1. Cavaletti G. at al. (2000) Neurotox. 21, 389-93.
2. Nicolini G. et al. (2001) Neurosci Lett. 302, 41-4.
3. Nicolini G. et al. (2003) Neurochem Int. 42,419-29.