CURCUMIN AND PLATINUM DRUGS COMBINATION IN HUMAN OVARIAN CARCINOMA CELLS: CELL-CYCLE INHIBITION AND APOPTOSIS

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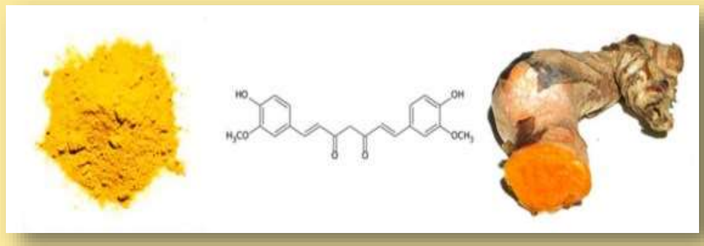
INTRODUCTION

New strategies to improve clinical response and prevent side effects of cancer therapy focused on substances than can be used with usual treatment. In particular new tumor-targeted agents focus on cell-cycle arrest, apoptosis or premature cell-cycle checkpoint exit. This study evaluates the effect of a combined treatment with cisplatin or oxaliplatin and curcumin, on cell-viability, cell cycle parameters and apoptosis in human ovarian cancer cell line (2008) and in cisplatin-resistant variant (C13).
Principal drugs used in epithelial, testicular and ovarian cancer treatment are cisplatin and oxaliplatin . They act at nuclear level of cells, after non-enzymatic conversion to active derivatives, forming platinum-DNA adducts and DNA-protein cross links.
Moreover they indirectly cause apoptosis and cell-cycle perturbation, by blocking DNA replication and trascription .
For this, they are characterized by many side effects, such as severe toxicity and an intrinsic and acquired resistance. To reduce side effect we decided to combine platinum drugs with a cytostatic agent: curcumin.
Curcumin (diferuloylmethane) isolated from rhizome of *Curcuma longa*, is a naturally occurring antioxidant and anti-inflammatory agent. Preclinical and clinical evidence support curcumin chemopreventive and antitumoral effects, because it acts into intercellular signaling pathways related to cell proliferation and apoptosis by affecting various molecular targets, such as up-regulating cdk inhibitors and p53 and down-regulating cyclin D1, cdk-1, cdk-2 and NFkB.

The aim of the study was to evaluate the effect of a combined treatment with platinum drugs and curcumin, on cell-viability, ROS generation, cell-cycle and apoptosis in human ovarian cancer cell line (2008) and in cisplatin-resistant variant (C13).

CURCUMIN: is the most active polyphenol derived from the plant Curcuma longa, commonly called turmeric.
lit is not only a naturally occurring antioxidant and anti-inflammatory agent but also it has chemopreventive and antitumoral effects, because it acts into intercellular signaling pathways related to cell proliferation and apoptosis In this study we used a solution of curcumin at different concentrations, prepared in DMSO and used in cultered medium.



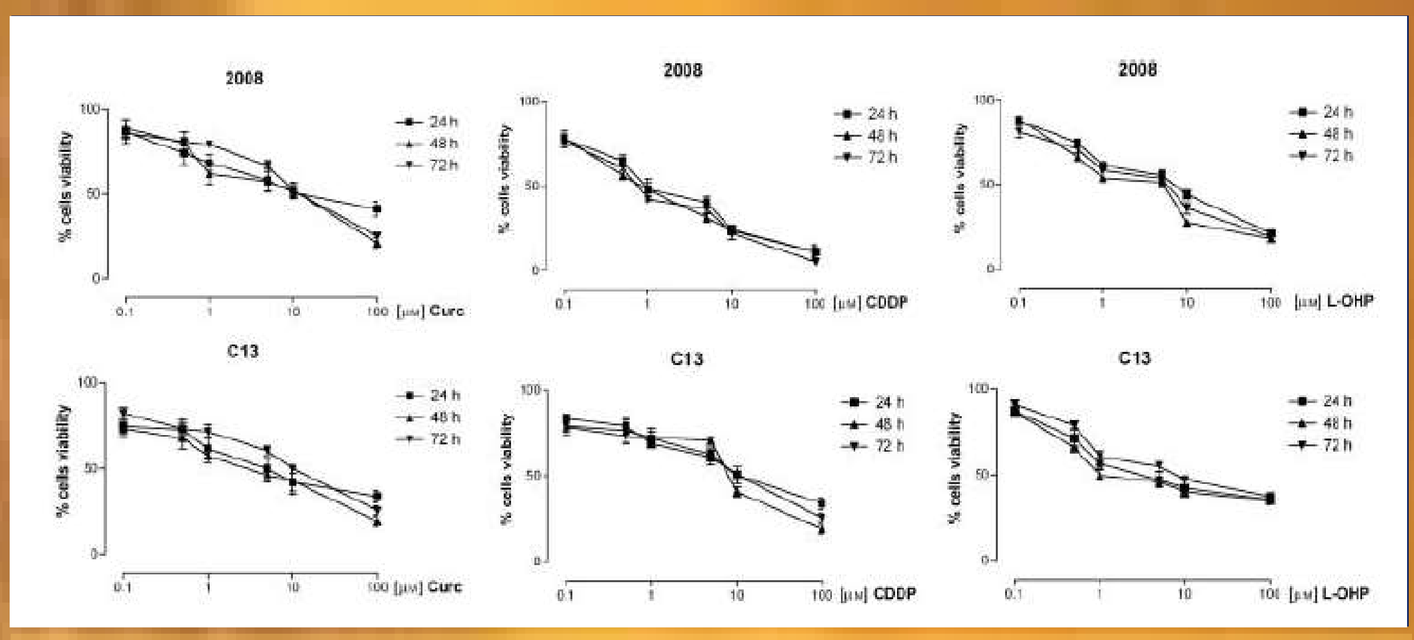


Fig. 1 Cell viability in 2008 and C13 cells after treatment with curcumin, CDDP and oxaliplatin. Effect of treatment was measure by MTT assay. Results are Mean ± ES from at least three independent experiments in quadruplicate.

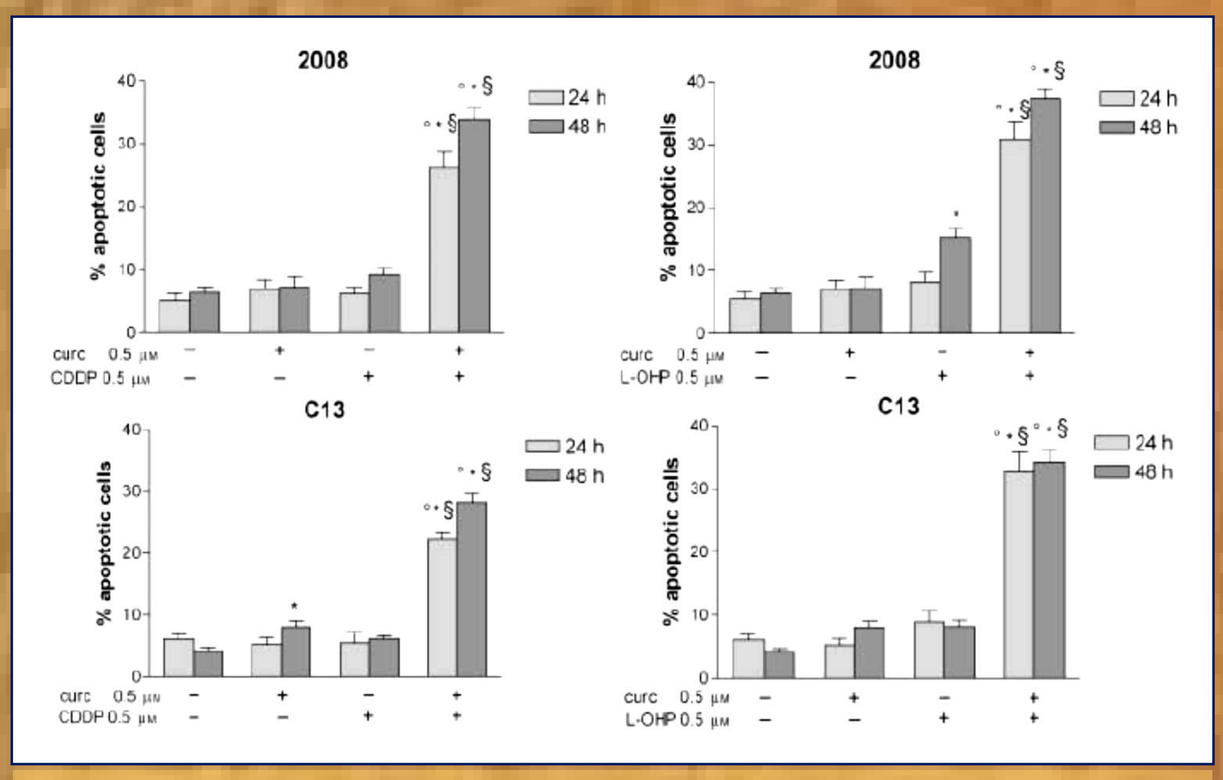


Fig.2 Apoptotic cells, exposed to curcumin, cisplatin, oxaliplatin or drug combination, were detected by flow citometry with annexin V/propidium iodide assay.

METHODS

Cell lines and reagents: 2008 and c13 cells derive from human ovary carcinoma. The cisplatin resistan variant were obtained by in vitro selection following treatment with increasing concentrations of cisplatin. Cells were grown in RPMI 1640 medium , 10% fetal bovine serum, 5% l-glutamine and 1% pen-strep, in humidified condition at 5% CO2 and 37°C.
All reagents were from Lonza (Basel, Switzerland).
Cell viability assay: was determined after 24h, by MTT (3-(4,5-?) reduction assay at λ=570 nm (Mossmann T. 1983) and by trypan blue exclusion assay.
Cell-cycle analysis by flow cytometry: after 24h of treatment, cells were fixed and resuspended in a solutions of propidium iodide (Invitrogen, Paisley, UK) and RNAase-DNAase free (sigma) in PBS for 30 min, at room temperature in the dark. Cells were analyzed in an Epics XL flow cytometer and a photomultiplier PMT2.
Annexin V/propidium iodide staining for apoptosis: cells were stained withAlexa 488/Annexin V/ propidium iodide (PI) and analyzed on an Epics XL flow cytometer Cells staining negative for both annexin V and PI are viable, cells annexin V+/PI – are in early apoptosis, cells annexin V+/PI + are necrotic or in late apoptosis.
Ros intercellular level: detection of intercellular steady state levels of Reactive oxygen species (ROS) was carried out on living cells using 2',7'-dichlorofluorescein-diacetate (Molecular Probes), after 2h of treatment. Cells were analyzed on an Epics XL flow cytometer.
Intracellular glutathione assay: Intracellular glutathione concentrations were measured by addition of 2 U glutathione reductase on coulter cells. Product formation was recorded continuously with a spectrophotometer. Total amount of glutathione in samples was determined from a standard curve obtained by plotting known amounts (0.05–0.4 µg/ml) of glutathione against rate of change in absorbance at 412nm.

RESULTS

Results showed that curcumin and platinum drugs *per se* caused time (24-72h) and concentration-dependant (0.1-100µM) reduction in cell viability, which persisted after 72h (Fig. 1). Moreover, we noticed that curcumin is more active in cisplatin-resistant cells than in wild type ones.
Combination of curcumin and platinum drugs had a synergic effect both in wild type 2008 and c13 cells (results not shown).
As regards to the cell-cycle, this is not significantly different in untreated wild type and resistant cells, but curcumin reduced the percentage of cells in G2/M phase and caused apoptosis (increase sub-G0), both in 2008 and c13 cells.
Moreover, curcumin increased number of c13 cells in G0/G1 phase and reduced cells percentage in S (Fig.2). In wild type cells and resistant ones, after a combine treatment curcumin (0.5-1-5 µM) and platinum drugs, we observed an increased apoptosis and G0/G1 phase (not in resistant cells), and a G2/M decreased in comparison with drugs alone (Fig. 2).
We decided to test also reactive oxygen species (ROS) generation and glutathione levels on cancer cells after treatment with curcumin, to test the antioxidant action of this one.
ROS generation was test after treatment with curcumin (0.5-1-5 µM). We measured a lowest basal ROS level in cisplatin-resistant cells than in wild type cells, but curcumin induced an increase of ROS level in both cells lines (Fig. 3). Intercellular glutathione (GSH) was measured after 2h and 24h of treatment with curcumin (0.5-1.5 µM). Basal level of GSH were higher in resistant cells than in wild type. In both lines, curcumin decreased level of GSH after 2h, whereas it increased GSH after 24h (Fig. 4).

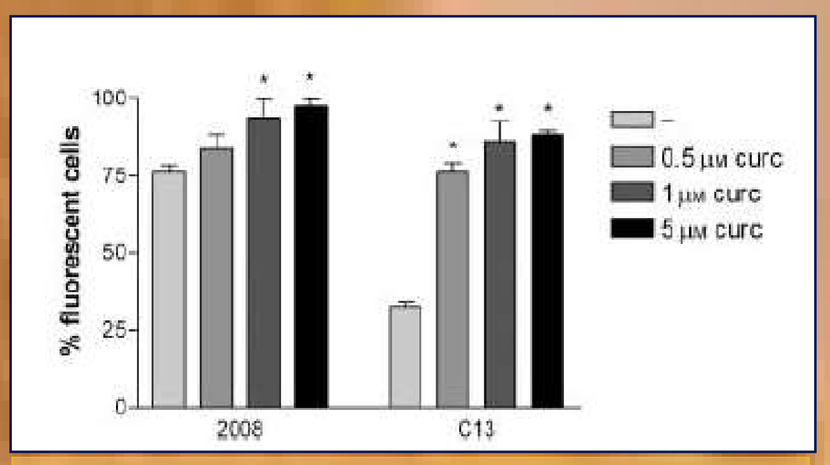


Fig.3 Effect of curcumin on ROS generation in 2008 and C13 cells. The cells were exposed for 2 hours to (0.5, 1.0 and 5.0 µm) curcumin and ROS detected by H2DCF staining and flow cytometry.

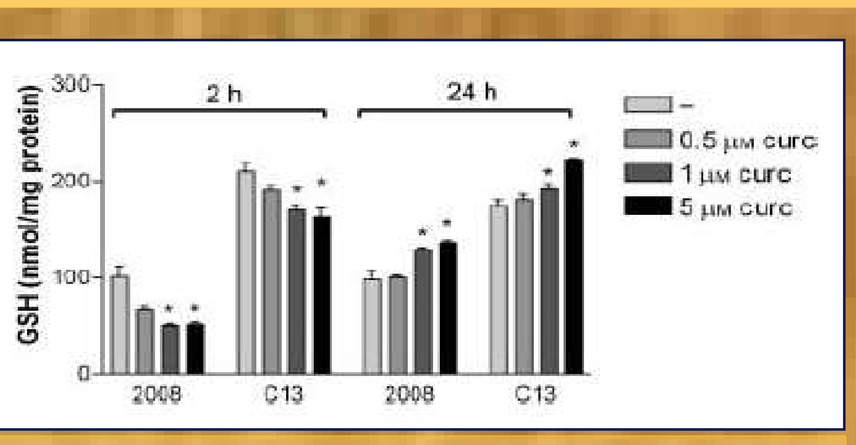


Fig.4 Effect of curcumin on glutathione (GSH) content in 2008 and C13 cells. Cells were exposed for 2 and 24 hours to (0.5, 1.0 and 5.0 µm) curcumin.

CONCLUSIONS

Our results suggest that curcumin inhibits cell viability of ovarian cancer cells. Furthermore, comparated to single-drugs treatment , curcumin combined with cisplatin and oxaliplatinum drugs, at concentration lower than IC50, causes best dose- and time-dependent increases in cell-cycle arrest and apoptosis. The combination makes cisplatin-resistant ovarian cancer cells more susceptible to the action of platinum drugs.
A further suggested mechanism for drug resistance is increased in intercellular thiols in the redox pathway, which may inactivate and remove platinum compound.
Our experiments show that in cisplatin-resistant cells (compared to cisplatin-sensitive ones) ,higher GSH content is parallel to lower Ros level, and that curcumin causes an increas of ROS after 2h incubation, but increase in GSH afetr 24h. So curcumin has early pro-oxidative effect and late antioxidant effect.
Curcumin increased gluthation level both in C13 and 2008 cells, suggesting that the increased susceptibility of ovarian cancel cell to combined tratment is not related to a glutathion depletion.
In conclusion, our study demonstrate that curcumin is cytotoxic in ovarian cancer cells and that it increases effect of platinum drugs. Our future purpose will be investigate into chemopreventive and chemoprotective propertise of curcumin and its synergism with platinum drugs.

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