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The effects of quercetin vs cisplatin on proliferation and the apoptotic process in A549 and SW1271 cell lines in *in vitro* conditions

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Experience over several years has indicated that chemotherapy, even if widely used, does not always remain effective in the therapy of lung tumours and, in addition, is linked to serious side effects. In parallel, some plant polyphenols are known to exert a proapoptotic action on tumour cells while, in contrast, representing anti-cancerogenic anti-oxidants in living organisms. Our studies were aimed at comparing the effects of a polyphenol, quercetin, and cisplatin on cells of various types of lung cancer in in vitro *conditions. In these studies we also attempted to define the relationship between the dose and the duration of the activity of the compounds. Cisplatin alone was found to induce only a small reaction in the cells, while in combination with quercetin its anti-proliferative and pro-apoptotic effects were amplified, depending upon the type of tumour, the dose and the duration of the drug's action.*

Key words: quercetin, cisplatin, cell culture, apoptosis, lung tumours

INTRODUCTION

Cisplatin or *cis*-diaminedichloroplatinum (DDP) represents a widely used drug in several types of tumour, including those of the lungs. Regrettably, it is not always effective and its application is linked to serious side affects. First of all, the drug is both nephrotoxic and ototoxic and damages the bone marrow [6]. Plant polyphenols, including quercetin, are described as agents of anti-cancerogenic properties due to their anti-proliferative and pro-apoptotic activity on tumour cells and represent very strong anti-oxidants [7]. This suggests a potential for a combined chemotherapy with polyphenol supplementation, which might amplify the chemotherapeutic effects but which might be accompanied by fewer side effects. The preliminary *in vivo* and *in vitro* studies seem to confirm these expectations [1, 2, 5]. Since cisplatin is frequently used to treat lung tumours, our experiments have been performed on cell cultures involving two cell lines of lung cancer.

MATERIAL AND METHODS

The studies were performed *in vitro* on two cell lines: SW1271 (small cell lung cancer, SCLC) and A549 (pulmonary adenocarcinoma). The cultures were established in 25 cm^2 flasks and maintained at 37°C in 5% CO_2. MEM medium was used, supplemented with foetal calf serum and antibiotics (Biokom). The cells were harvested using trypsin and subcultured in 24-well plates at 2×10^4 per ml MEM. After 24 h culture the cells were scored in a Bürker haemocytometer and the cultures supplemented with quer-

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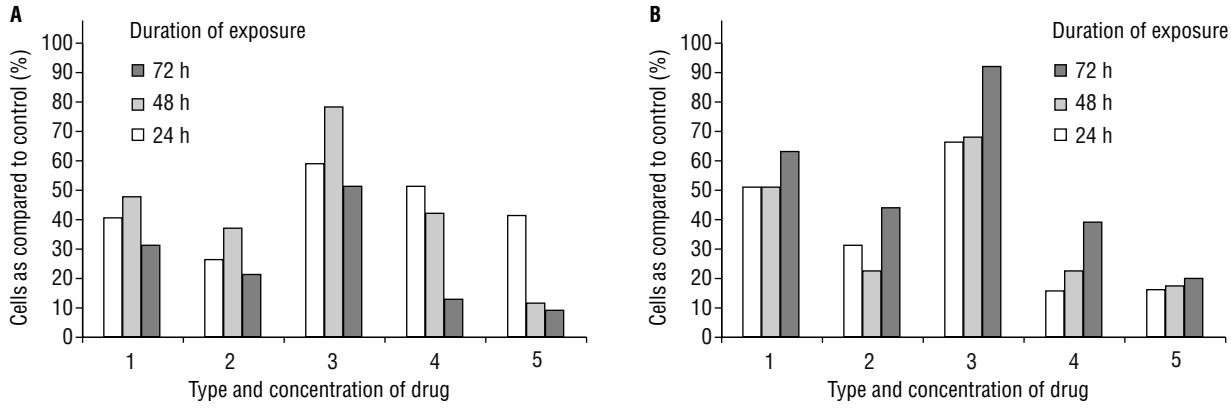

Figure 1. Cell content in cultures in relation to type, concentration and duration of exposure to the drug. Abscissa: 1) 5 μM quercetin, 2) 10 μM quercetin, 3) DDP 500 ng/ml, 4) 5 μM quercetin + DDP 500 ng/ml, 5), 10 μM quercetin +DDP 500 ng/ml; **A**. A549 cell line, **B.** SW1271 cell line; DDP, cisplatin — *cis*-diaminedichloroplatinum, SCLC — small cell lung cancer

cetin (Sigma) and/or cisplatin in appropriate concentrations and in 5 combinations: 1) 5 μM quercetin; 2) 10 μM quercetin; 3) 500 ng/ml (1.6×10^{-3} μM) DDP [3]; 4) 5 μM quercetin plus 500 ng/ml DDP; 5) 10 μM quercetin plus 500 ng/ml DDP. Some wells of the plate were left unsupplemented, serving as a control. In the cultures the cells were scored following 24, 48 and 72 h. The percentage of apoptotic cells, necrotic cells and cells with intermediate lesions was tested using the comet technique [4].

RESULTS AND DISCUSSION

The results of our studies indicated that quercetin reduced cell number in the cell culture both in the case of A549 and that of SW1271 and that the process depended upon the dose of drugs and the duration of incubation (Fig. 1). The most pronounced effect was noted in the case of SW1271 following 48 h incubation and in the case of A549 following 72 h incubation. The reaction of cells to DDP was relatively mild in the cell lines studied and was most pronounced in A549 following 72 h exposure to the drug. SW1271 cells were less sensitive to DDP, particularly following longer incubations, which suggested the potential for development of cell clones resistant to the cytostatic drug. The application of both drugs, quercetin and DDP, in parallel was followed by a clearly amplified anti-proliferative effect, particularly in the case of SW1271 after 24 h (Fig. 1B). The effects were not related to the concentration of quercetin. In the case of A549 the reaction developed after a longer incubation time and was dose-dependent (Fig. 2A). Moreover, the studies demonstrated that DDP alone was very inefficient in the induction of apoptosis in the cell lines studied and, after 48 h, the lesions were mainly of an intermediate type (Fig. 2). Quercetin administered without DDP exerted a pro-apoptotic action, particularly in the case of SCLC cells. On the other hand, the combination of quercetin and DDP applied to SW1271 cultures augmented both the number of apoptotic cell nuclei and the intermediate type comets, which might also reflect the presence of apoptotic cells (Fig. 2B). In A549 cells the amplified effect was observed only after 48 h (Fig. 2A).

The studies performed suggest that quercetin may amplify the action of cisplatin, particularly in the case of SCLC, in which the drug is most frequently used, although it seldom results in a complete remission [6]. Studies performed *in vitro* have confirmed both a more pronounced reduction in cell number and more evident apoptosis following the addition of DDP in combination with quercetin. To sum up, our results may prove suitable for augmenting the sensitivity of tumour cells to DDP in some lung tumours, particularly in cases which are resistant to cisplatin.

REFERENCES

1. Chan MM, Fong D, Soprano KJ, Holmes WF, Heverling H (2003) Inhibition of growth and sensitization to cisplatin-mediated killing of ovarian cancer cells by polyphenolic chemopreventive agents. J Cell Physiol, 194: 63–70.
2. Conklin KA (2000) Dietary antioxidants during cancer chemotherapy: impact on chemotherapeutic effectiveness and development of side effects. Nutr Cancer, 37: 1–18.
3. Dietel M, Bals U, Schaefer B, Herzig I, Arps H, Zabel M (1993) *In vitro* prediction of cytostatic drug resistance in primary cell cultures of solid malignant tumours. Eur J Cancer, 29A: 416–420.

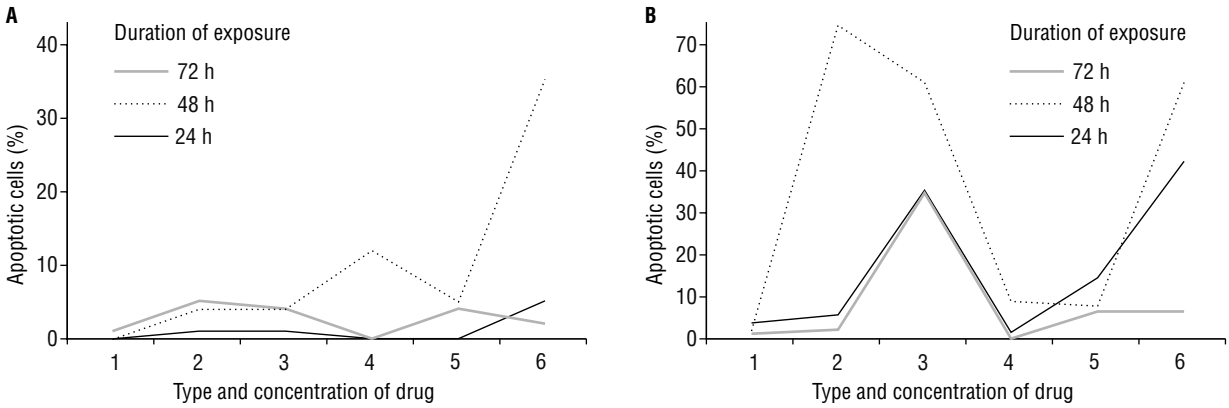

Figure 2. Percentage of apoptotic cell nuclei in relation to type, concentration and duration of exposure to the drug. Abscissa: 1) 5 μM quercetin, 2) 10 μM quercetin, 3) DDP 500 ng/ml, 4) 5 μM quercetin + DDP 500 ng/ml, 5), 10 μM quercetin +DDP 500 ng/ml; **A.** A549 cell line, **B.** SW1271 cell line; DDP, cisplatin — *cis*-diaminedichloroplatinum, SCLC — small cell lung cancer

4. Drąg-Zalesińska M, Wysocka T, Dumańska M, Jagoda E, Zabel M (2002) Comparison of techniques permitting to detect apoptosis *in situ*. Folia Histochem Cytobiol, 40: 125–126.
5. Giacomelli S, Gallo D, Apollonio P, Ferlini C, Distefano M, Morazzoni P, Riva A, Bombardelli E, Mancuso S, Scambia G (2001) Silybin and its bioavailable phospholid complex (IdB 1016) potentiate *in vitro* and *in vivo* the activity of cisplatin. Life Sci, 70: 1447–1459.
6. Orzechowska-Juzwenko K (2000) Outline of chemotherapy of organ and systemic tumours (in Polish). Volumed, Wrocław.
7. Yang CS, Landau JM, Huang M-T, Newmark HL (2001) Inhibition of carcinogenesis by dietary polyphenolic compounds. Annu Rev Nutr, 21: 381–406.