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Synergistic antiproliferative activity of quercetin and cisplatin on ovarian cancer cell growth

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It has been demonstrated that the flavonoid quercetin (3,3′,4′,5-7-pentahydroxyflavone) (Q) inhibits the growth of several cancer cell lines and that the antiproliferative activity of this substance is mediated by a so-called type II estrogen binding site (type II EBS). We investigated the effects of quercetin and cisplatin (CDDP) alone and in combination on the proliferation of the ovarian cancer cell line OVCA 433. Both drugs exhibited a dose-related growth inhibition in a range of concentrations between 0.01 and 2.5 μM and 0.01 and 2.5 μg/ml for Q and CDDP respectively. The combination of the two drugs resulted in a synergistic antiproliferative activity. Two other flavonoids tested, i.e., rutin (3-rhamnosylglucoside of quercetin) and hesperidin [7-b rutinoside of hesperetin (3′-5-3-hydroxy-4-methoxyflavone)] were ineffective both alone and in combination with CDDP. Since both rutin and hesperidin do not bind to type II EBS it can be hypothesized that Q synergizes with CDDP by acting through an interaction with these binding sites.

Key words: Flavonoids, quercetin, cisplatin, ovarian cancer.

Introduction

Flavonoids are a large class of natural substances with a wide variety of biological actions.[1] In particular, the flavonoid quercetin (Q) has a powerful growth inhibitory activity on human breast[2] and leukemic[3,4] cancer cells. Moreover, recent data from our laboratory suggest that Q may also be very effective against ovarian cancer cells.[5] Therefore it has been suggested that this substance might have some therapeutic potential. An additional interesting feature of Q results from an *in vitro* study showing that Q might have a synergistic antiproliferative effect with cisplatin (CDDP) on rat carcinoma cells.[6] Despite the fact that CDDP is the most active agent against ovarian cancer, the development of resistant clones as well as the kidney toxicity of the drug represent the major limitations of CDDP treatment. Therefore the identification of agents able to act synergistically with CDDP is of utmost importance in clinical oncology.

We report here that Q acts synergistically with CDDP on the inhibition of proliferation of the human ovarian cancer cell line OVCA 433.

Materials and methods

Cell culture

OVCA 433 ovarian cancer cells were kindly provided by Dr B Littlefield (Department of Gynecology, Yale University, USA). Cells were grown in monolayer culture in minimum essential medium (MEM) supplemented with 10% fetal calf serum (FCS) and 200 U/ml penicillin. Cells were trypsinized weekly and plated at a density of 8×10^4 cells/ml. They were incubated at 37°C

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under 5% CO_2–95% air in a high humidity atmosphere.

Growth experiments

Cells were plated in 6-well flat bottom plates (Falcon 3046, Becton Dickinson, Lincoln Park, NJ) at a concentration of 1×10^5 cells/ml in MEM supplemented as above. After 24 h, the medium was replaced with fresh medium and Q (3,3′,4′,5-7-pentahydroxyflavone), rutin (3-rhamnosylglucoside of Q), hesperidin [7-b rutinoside of hesperetin (3′-5-3-hydroxy-4-methoxyflavone)], CDDP (cisplatin) or else a combination of drugs at the indicated concentrations were added. Control cells were treated with the same amount of vehicle alone (ethanol or DMSO). The final ethanol and DMSO concentrations never exceeded 1% (v/v) and 0.5% (v/v) in either control or treated samples, respectively.

Quadruplicate hemocytometer counts of triplicate culture dishes were performed after 3 days of exposure to the substances.

Statistical analysis

Data were analyzed by two-way analysis of variance.[7]

Results

Table 1 shows the inhibitory effects of Q and CDDP alone or in combination on OVCA 433 cell growth. Both drugs exhibited a dose-related growth inhibition in a range of concentrations between 0.01 and 2.5 μg/ml and 0.01 and 2.5 μM for CDDP and Q, respectively. It should be noted that in these ranges the dose intervals were the same for both drugs.

When the two drugs were used in combination at a fixed dose ratio there was a significantly greater inhibitory effect on cell growth than that observed at the corresponding dose of the single drug (Figure 1). Two other flavonoids tested, i.e. rutin and hesperidin, which were ineffective in inhibiting OVCA 433 cell growth, did not produce any potentiating effect with CDDP (Table 2).

In order to verify whether the combined effect of Q and CDDP was due to an additive or synergistic action, data were analyzed by two-way analysis of variance. As reported in Table 3, the inhibitory effect of Q and CDDP alone is highly significant. The interaction between the effects of the two drugs is also significant, supporting the possibility that Q and CDDP act synergistically.

Discussion

Our data indicate that Q inhibits the growth of human ovarian cancer cells and that the simultaneous treatment of these cells with Q and CDDP results in a synergistic antiproliferative activity.

The mechanism of the synergistic activity between Q and CDDP remains to be clarified. Hofmann *et al.*[6] hypothesized that enhancement of CDDP activity by Q in rat Walker cancer cells is due to an inhibition of protein kinase C activity. However, such an explanation does not fit with our data since we observed a synergizing activity even at a Q concentration of 0.5 μM. This last concentration is at least one logarithm lower than that minimally inhibitory on protein kinase C. Alternatively, it is possible that Q acts through the interaction with type II estrogen binding sites (type II EBS), originally described by Clark *et al.*,[8] which we have demonstrated in OVCA 433 cells.[5] Recent

Table 1. Effect of quercetin and cisplatin alone or in combination on OVCA 433 cell growth

CDDP (μg/ml)	Q (μM)					
	0	0.01	0.10	0.50	1.0	2.5
0	600000 ± 20000[a]	570000 ± 37470	534000 ± 12000	516000 ± 12000	444000 ± 12000	350000 ± 29800
0.01	558000 ± 36496	528000 ± 6000	516000 ± 6000	502000 ± 13464	406000 ± 12490	919000 ± 26300
0.10	454000 ± 15100	432000 ± 26153	416000 ± 19287	402000 ± 10392	360000 ± 33406	279000 ± 18129
0.50	404000 ± 48497	384000 ± 24000	318000 ± 6000	300000 ± 26153	270000 ± 15874	213000 ± 17559
1.0	328000 ± 24249	248000 ± 24980	232000 ± 24249	214000 ± 12490	194000 ± 18330	153000 ± 10689
2.5	198000 ± 20200	153000 ± 17100	142000 ± 11905	123000 ± 11105	121000 ± 8691	118000 ± 9280

[a] cells/plate; mean ± SD of three different experiments performed in triplicate.

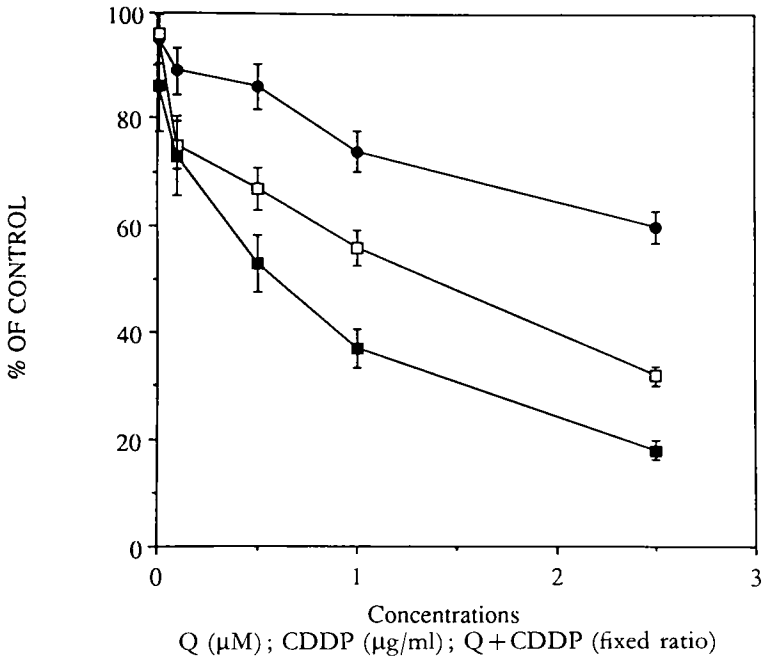

Figure 1. Effect of quercetin, CDDP and quercetin–CDDP (at a fixed ratio) on OVCA 433 cell proliferation. Cell counts were performed after 3 days of exposure to quercetin (●), CDDP (□) or quercetin–CDDP (■). Results are expressed as the mean ± SD of three different experiments performed in triplicate.

Table 2. Effect of quercetin, rutin, hesperidin and CDDP alone or in combination on OVCA 433 cell growth

	Experiment 1	Experiment 2
Controls	620000 ± 32000[a]	580000 ± 42000
Quercetin (1 μM)	454000 ± 48000	410000 ± 37000
Rutin (1 μM)	643000 ± 37000	570000 ± 53000
Hesperidin (1 μM)	616000 ± 40000	602000 ± 29000
CDDP (1 μg/ml)	359000 ± 28000	342000 ± 34000
Q (1 μM) + CDDP (1 μg/ml)	229000 ± 18000	203000 ± 16000
R (1 μM) + CDDP (1 μg/ml)	360000 ± 30000	336000 ± 28000
H (1 μM) + CDDP (1 μg/ml)	340000 ± 23000	360000 ± 28000

[a] cells/plate; mean ± SD of triplicate determinations from each single experiment.

data from our and other laboratories indicate that these type II EBS are present in several human primary tumors[3,5] and in cancer cell lines[2,9] and that Q binds to these sites with an affinity consistent with the concentration effective in synergizing CDDP activity. Furthermore, rutin and hesperidin, which do not bind to type II EBS,[3–5] are ineffective in synergizing with CDDP.

Table 3. Two-way analysis of variance of data in Table 1

	d.f.	Variance	*F*-ratio	*P*
Quercetin	4	2.13×10^{11}	426.13	0.0001
Cisplatin	4	3.78×10^{10}	75.58	0.0001
Interaction	16	1.00×10^{9}	2.01	0.0305
Residual	50	5.00×10^{8}		
Total	74	1.41×10^{10}		

The synergism between Q and CDDP could also be explained on the basis of cytokinetic considerations. We demonstrated that Q exerts a blocking action of cell transition from the G0–G1 to the S phase of the cell cycle.[4,5] Data obtained by Fraval *et al.*[10] indicate that cells are more sensitive to CDDP when exposed during the G1 (intermitotic) phase of the cell cycle.

Our observations further support the potential therapeutic application of Q in cancer therapy.

Interestingly, plasma concentrations of Q similar to those effective *in vitro* both in inhibiting ovarian cancer cell growth and synergizing CDDP action can be achieved following an intravenous injection of 100 mg without any apparent side effects.[11]

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