Quercetin inhibits proliferation and increases sensitivity of ovarian cancer cells to cisplatin and paclitaxel

Kwercetyna hamuje proliferację i zwiększa wrażliwość komórek raka jajnika na cisplatynę i paklitaksel

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Abstract

Introduction: *Due to frequent diagnosis of ovarian cancer at an advanced clinical stage, in most cases surgical debulking is followed by chemotherapy. The principal cause of therapeutic failure involves incomplete surgery and resistance of neoplastic cells to chemotherapy. A search continues for substances which would overcome resistance to treatment and, as a result, would increase efficacy of the applied treatment. Quercetin represents one of more interesting compounds, which at present in subjected to several tests.*

Material and methods: *Studies were performed on in vitro sensitivity of human ovarian cancer cell lines, SKOV-3, EFO27, OVCAR-3 and A2780P to low doses of quercetin and on the effect exerted by quercetin on sensitivity of the cell lines to cisplatin and pactitaxel.*

Results: *The experiments proved that the studied cells of ovarian cancer manifest a similar sensitivity to quercetin. Following incubation of the cells with two distinct concentrations of quercetin and the studied cytostatic agents all the cells lines were found to significantly increase their sensitivity to pactitaxel In cases of two cell lines, OVCAR-2 and A2780P, they also significantly increased their sensitivity to cisplatin.*

Discussion: *Our results demonstrated suitability of low quercetin doses (achievable using oral administration) as a substance which increases sensitivity of ovarian cancer cells to cisplatin and paclitaxel. The value of quercetin include its wide accessibility, efficacy and a broad range of activity but also its low toxicity, as compared to other examined compounds.*

Conclusions: *Used in low doses, quercetin increases chemosensitivity of ovarian cancer cells*

Key words: **quercetin** / **ovarian cancer** / **cisplatin** / **paclitaxel** / **chemoresistance** /

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Streszczenie

Wstęp: *Ze względu na częste rozpoznawanie raków jajnika w zaawansowanym stadium klinicznym w większości przypadków po przeprowadzeniu zabiegu operacyjnego stosowana jest chemioterapia. Podstawową przyczyną niepowodzeń stosowanej terapii jest nieradykalność leczenia operacyjnego oraz oporność komórek nowotworowych na chemioterapię. Poszukuje się substancji, które pozwolą zwalczyć oporność na leczenie i w efekcie zwiększyć skuteczność stosowanej terapii. Jednym z ciekawszych związków poddawanych obecnie szeregowi badań jest kwercetyna.*

Materiał i metody: *Przeprowadzono badania in vitro wrażliwości linii komórkowych ludzkiego raka jajnika SKOV-3, EFO27, OVCAR-3 i A2780P na niskie dawki kwercetyny oraz ocenę wpływu kwercetyny na wrażliwość linii komórkowych na cisplatynę i paklitaksel.*

Wyniki: *Przeprowadzone doświadczenia wykazały, że badane komórki raka jajnika wykazują zbliżoną wrażliwość na kwercetynę. W wyniku inkubacji badanych komórek z dwoma różnymi stężeniami kwercetyny i z badanymi cytostatykami wykazaliśmy, że wszystkie linie istotnie zwiększyły swoją wrażliwość na paklitaksel. W przypadku dwóch linii – OVCAR-2 i A2780P uzyskaliśmy również istotny wzrost wrażliwości na cisplatynę.*

Dyskusja: *Nasze badania wykazały przydatność niskich dawek kwercetyny (osiągalnych przy podaży doustnej), jako substancji zwiększającej wrażliwość komórek raka jajnika na cisplatynę i paklitaksel. Jej walory podkreśla nie tylko łatwa dostępność, skuteczność i szeroki zakres działania, ale również mała, w porównaniu z innymi badanymi substancjami, toksyczność.*

Wnioski: *Kwercetyna zastosowana w niskich dawkach powoduje wzrost chemiowrażliwości komórek raka jajnika*

Słowa kluczowe: **kwercetyna** / **rak jajnika** / **cisplatyna** / **paklitaksel** / **chemiooporność** /

Introduction

Tumours, next to circulatory diseases, provide the second in frequency cause of death. Detection of a tumour at its low stage of advancement provides at present high chances for a cure, due to achievements of contemporary surgery, chemo- and radiotherapy. The most pronounced problem is posed by tumours, which due to their location are detected at an advanced clinical stage. Ovarian cancer represents just an example of such a tumour.

In women, all over the world ovarian cancer represents the fifth in frequency cause of death due to malignant tumours. In countries of the European Union the respective incidence amounts to 12-17 new cases per 100,000 inhabitants per year, which corresponds to around 30,000 new cases per year. In addition, all over the world incidence of the tumour continues to grow. The first stage of treatment involves surgery and execution of an optimum cytoreduction represents the most important prognostic variable. In a prevailing majority of patients chemotherapy represents an indispensable next step of the therapy and it is used in most cases of ovarian cancer following surgical treatment. Most frequently it involves the scheme of cisplatin or carboplatin plus paclitaxel. Despite a significant primary chemosensitivity (60-80% patients develop a remission following the first line standard cytotoxic treatment) 5-year survival is reached by just around 30% of the patients. This reflects relapse of the neoplastic disease within two years in most patients while chemotherapy of the second and subsequent attacks of the disease is ineffective. An incomplete surgery and the phenomenon of chemoresistance (multi-drug resistance; MDR) represent principal causes responsible for poor results of treatment in ovarian cancer [1-3].

At present in several research centres the search continues for substances which might overcome tumour cell resistance to chemotherapy and which, in effect, would increase efficacy of the applied therapy. Quercetin involves one of more interesting compounds subjected to such studies. Quercetin is a flavonoid contained in several plant food products and, thus, it is a permanent component of human diet (green tea, onion, etc.). The compound was found to play a significant role in protection against action of free radicals. An important mechanism of its action involves prevention against oxidation of lipids in cell membranes, the phenomenon significant for body senescence and development of autoimmune diseases [4,5]. Nevertheless, high concentrations of quercetin were demonstrated to exert a mutagenic action. A distinct manner of its activity was noted in its lower concentrations. This manner of its action seems to be particularly interesting not only due to its anti-neoplastic effects but also because just in lower concentrations flavonoids are naturally present in human body. Studies on bioaccessibility showed that following oral administration of flavonoids their level in blood reaches just levels of 0-4 μmol/L [4]. Therefore, evaluation of effects exerted by low quercetin levels on tumours seems to be particularly interesting.

Aim of study

The study aimed at evaluation of effects exerted by low quercetin doses on sensitivity of ovarian cancer cells to cisplatin and paclitaxel.

Material and methods

Cell lines and cell cultures

Cell lines of human ovarian cancer, SKOV-3, EFO27, OVCAR-3 and A2780P were cultured in L15 medium (Biowhittaker, Walkersville, MD, USA) supplemented with 10% foetal calf serum (FCS)(GIBCO/BRL, Grand Island, NY, USA),

1mM L-glutamine, 6.25 mg/l fetuin, 80 IE/l insulin, 2.5 mg/ml transferrin, 0.5 g/l glucose, 1.1 g/l $NaHCO_3$, 1% solution of basic vitamins and 20,000 kIE/l trasylol in a humid atmosphere containing 5% CO_2, at the temperature of 37°C, as described earlier [6].

Evaluation of quercetin toxicity

In order to evaluate quercetin toxicity for the studied cells and to define the highest non-toxic concentrations 800 cells were plated into each well of 96 well plates. Following 24 hour incubation the cultures were supplemented with quercetin solutions in the culture medium to reach the final quercetin concentrations of 0, 1, 0.5, 1, 5, 10, 50, 100 and 500 μM, respectively. Medium alone was used as a control. Following four days of incubation the studied cells were fixed in trichloracetic acid for one hour at the temperature of 4⁰C. Subsequently, the plates were rinsed five times in a distilled water, dried and stained, using 0.4% sulphorhodamine B solution (SRB)(Sigma, St. Louis, MO, USA) in 1% acetic acid for 10 min at room temperature. The stained plates were dried and, then, the dye was dissolved in 10 mM Tris-HCl (pH=8.0). Absorbance was read at the wavelength of 562 nm using ELISA reader (EL340 Microplate Bio Kinetics Reader, BIO-TEK Instruments, Winooski, VT, USA). Each experiment was repeated three times [7].

Evaluation of quercetin effect on cell sensitivity to cisplatin and paclitaxel

In order to examine effect of quercetin on sensitivity of studied ovarian cancer cells to cisplatin and paclitaxel the proliferation tests were repeated with use of SRB staining. Again 800 of studied cells were plated per well in 96 well plates. Following 48 h incubation the cultures were supplemented with cisplatinin and paclitaxel without quercetin or with 1 μM or 5 μM quercetin.

The cytostatic agents were used in three concentrations (K1, K2 and K3). K1 = 10^{-1} x K2 and K3 = 10 x K2. The concentration of K2 corresponded to the level of a cytostatic agent reached during chemotherapy in a tumour tissue. For cisplatin the concentration amounted to 16.66 x 10^{-5} μM, and for paclitaxel it was 0.29 x 10^{-5} μM [6]. Following four days of incubation with the tested substances the cells were fixed and stained using SRB technique, which was followed by readout of the absorbance. The experiments were repeated three times.

Statistical analysis

Statistical analysis of the obtained results was conducted using Statistica 98PL software. The obtained values were compared to each other using the test U of Mann-Whitney [8].

Results

Toxicity of quercetin toward ovarian cancer cells

The performed experiments demonstrated a similar sensitivity of ovarian cancer cells to quercetin (Figure 1). IC_{50} for OVCAR-3 cells amounted to 42 μM, for EFO27 cells to 59 μM, for A2780P cells to 70 μM, and for SKOV3 cells to 90 μM. For the studies on effect of quercetin on sensitivity of ovarian cancer cells to cisplatin and paclitaxel the two highest quercetin concentrations were selected (1 μM and 5 μM quercetin), in the presence of which relative cell numbers manifested no significant differences as compared to the control (U test of Mann-Whitney, $p>0.05$).

Evaluation of quercetin effect on cell sensitivity to cisplatin and paclitaxel

The performed cytotoxicity tests demonstrated resistance of studied ovarian cancer cells to paclitaxel and a relatively high sensitivity of the cells to cisplatin. (Table I).

Table I. Sensitivity of various ovarian cancer cell lines to paclitaxel and cisplatin in therapeutic concentrations (K2) expressed in percentages of control cell numbers.

	Paclitaxel (0.29 x 10^{-5} μM) [% control]	Cisplatin (16.66 x 10^{-5} μM) [% control]
SKOV-3	100	21
EFO27	98	34
OVCAR-3	76	38
A2780P	61	12

Table II. Coefficients of statistical significance for comparisons of quercetin effects in concentration of 1 or 5 μM on cell line sensitivity to paclitaxel and cisplatin as compared to the control (test U of Mann-Whitney).

	5μM quercetin	1μM quercetin	5μM quercetin	1μM quercetin
SKOV-3	<0.001	0.04	>0.05	>0.05
EFO27	>0.05	0.002	>0.05	>0.05
OVCAR-3	0.007	>0.05	<0.001	>0.05
A2780P	<0.001	<0.001	0.03	>0.05

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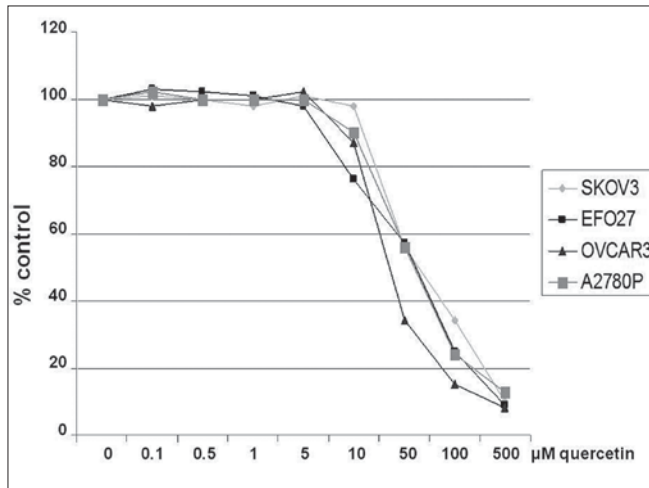

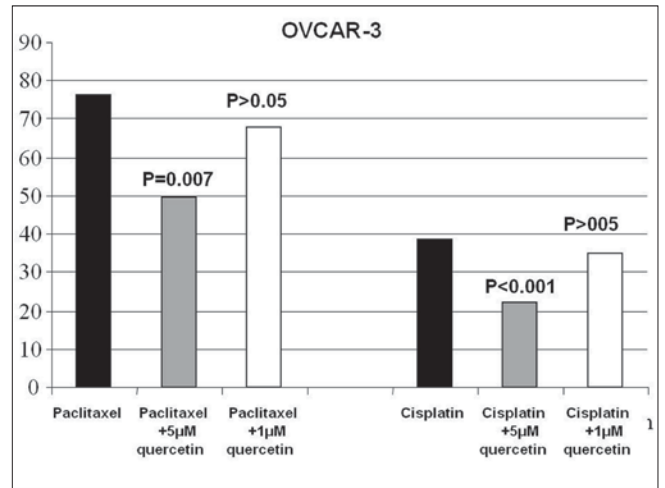**Figure 1.** Effect of quercetin on survival of studied ovarian cancer cells.

Figure 4. Comparison of OVCAR-3 cell line sensitivity to paclitaxel and cisplatin supplemented without or with 1 or 5 µM quercetin. Results are presented in percentages of the control with medium alone.
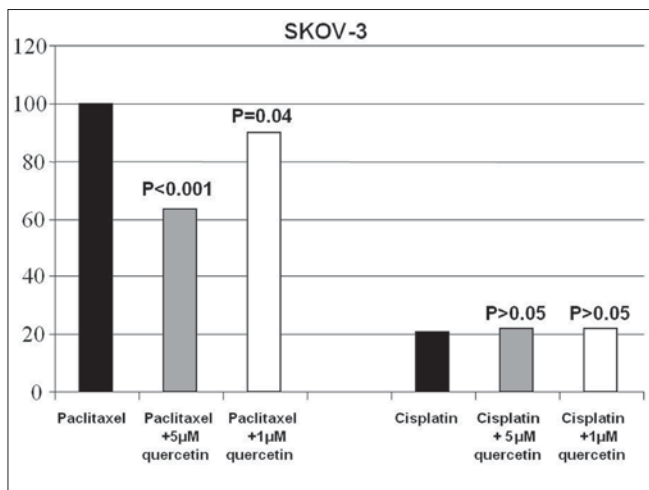

Figure 2. Comparison of SKOV-3 cell line sensitivity to paclitaxel and cisplatin used without or with 1 or 5 µM quercetin. Results represent percentages of the control with medium alone.
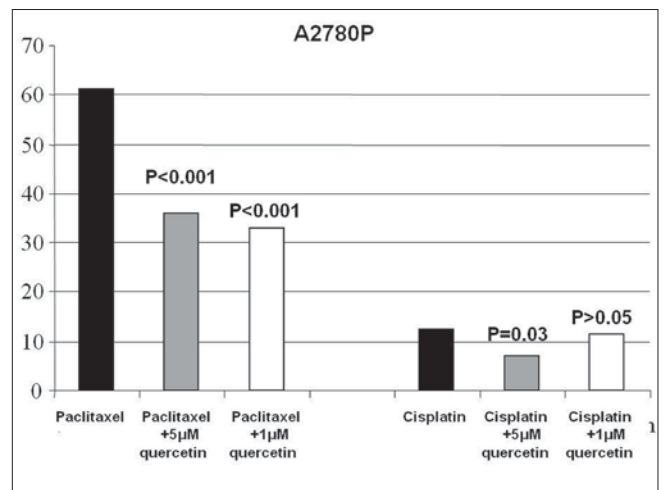

Figure 5. Comparison of A2780P cell line sensitivity to paclitaxel and cisplatin supplemented without or with 1 or 5 µM quercetin. Results are presented in percentages of the control with medium alone.
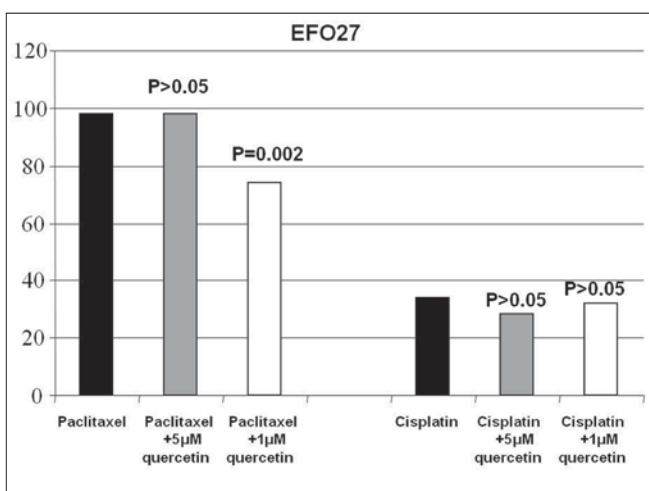

Figure 3. Comparison of EFO27 cell line sensitivity to paclitaxel and cisplatin supplemented without or with 1 or 5 µM quercetin. Results presented in percentages of the control with medium alone.

Following supplementation of culture medium with paclitaxel the relative cell number amounted to 61-100% of the control cell number while supplementation with cisplatin reduced the number to 12-38% of the control. Incubation of the studied cells with two different concentrations of quercetin and the studied cytostatic agents demonstrated a significant increase in sensitivity of all the cell lines to paclitaxel. This was particularly evident in the case of SKOV-3 cell line which before supplementation with quercetin was fully resistant to paclitaxel. In cases of two cell lines, OVCAR-2 and A2780P, also a significant increase in sensitivity to cisplatin was obtained.

The increased sensitivity to cisplatin was most clearly noted following quercetin supplementation of OVCAR-3 cultures, which were found to be most resistant to the chemotherapeutic agent before the supplementation. (Table II, Figs. 2-5).

Discussion

Incomplete surgery and resistant to chemotherapy are the main factors which determine the lack of efficacy in treatment of ovarian cancer patients. Causes of the observed resistance to chemotherapy are complex. It should be mentioned that in the cases of ovarian cancer the mechanism of primary resistance to multiple drugs plays an important rolebut the principal clinical problem involves appearance of a secondary resistance. Currently, several investigations continue on drugs which are supposed to reduce resistance to the applied chemotherapy. In the case of ovarian cancer the molecular target involves proteins linked to resistance to the therapy, first of all P-glycoprotein, the protein of the multi-drug resistance proteins (MRP) and breast cancer resistance proteins (BRCP) [7, 8, 10].

Beginning at the time when several biological properties of quercetin were demonstrated, numerous investigators attempt to describe the mechanism of its activity. The manner in which the flavonoid affects human cells was shown to depend on its concentration. While in high concentrations quercetin may exhibit mutagenic activity, in low concentrations, attainable upon oral administration, quercetin was demonstrated to be fully safe. In addition, it was shown that its low concentrations may promote induction of apoptosis in neoplastic cells. A proportion of authors suggest that quercetin inhibits expression of Hsp72 proteins in tumour cells, which manifests correlation with intensity of apoptosis [11, 12]. For example, Hu et al. [11] reported that quercetin supplementation of ovarian cancer cells treated with manumycin decreased the level of Hsp proteins. An intensified apoptosis was also demonstrated. Nevertheless, this was not the only mechanism in which flavonoids induced apoptosis: the pro-apoptotic effect of quercetin was demonstrated in cells with Hsp72 expression blocked earlier with anti-sense oligonucleotides. Jakubowicz-Gil et al. in their study evaluating effect of quercetin, administered with cisplatin, on HeLa cell line detected a decreased expression of procaspase-3 [12]. The lowered expression of procaspase-3 following supplementation with quercetin was probably linked to an increase in active form of the enzyme in the apoptotic process [13,14]. Similar observations were reported by Hu et al., and, therefore, the proapoptotic activity of quercetin may be expected to be linked mainly to caspases-linked mechanism of action [11, 13, 14].

An important effect of quercetin, on which we have focused our attention, involves an increase in chemosensitivity of neoplastic cells to action of cytostatic agents. The increase in tumour chemosensitivity under effect of flavonoids was documented in several studies. The publications described various mechanisms in which quercetin affected chemosensitivity. The relatively most numerous reports described its effect taking place due to a decrease in expression of P-glycoprotein [15]. Also van Zenden et al. described a decrease in MRP1- and MRP2-dependent chemoresistance under effect of incubation with quercetin [16]. The study of Jakubowicz-Gil et al. described lowered expression of MRP-2 under effect of quercetin and, consequently, an increased sensitivity of the cells to cisplatin [12]. An important mechanism in which quercetin acts involved blocking of glutathione transferase (GST) expression This was particularly significant in the context of modulation of ovarian cancer chemosensitivity, the cancer in which high activity of GST represented a poor prognostic index which correlated with resistance to cisplatin [17]. The demonstrated relationship between intensity of glutathione transferase expression and resistance to cisplatin both in ovarian cancer cell lines and in studies on ovarian cancer biopsies pointed to a significant role played by this chemoresistance mechanism in failures of the applied therapy [17, 18].

Our results have documented high value of quercetin as a substance modifying resistance to treatment with cisplatin and paclitaxel. Sensitivity of ovarian cancer cells to both drugs has been found to increase following supplementation with low quercetin doses. The increased chemosensitivity has been most clearly noted in paclitaxel-resistant cell lines which seems to be particularly interesting from the clinical point of view. It should be stressed that in our experiments low doses of quercetin have been supplemented, which have mimicked natural concentration of flavonoids in human blood following oral administration of the preparation. The observed in our studies action of quercetin has corroborated therapeutic suitability of the substance, applied in association with chemotherapy. Nevertheless, further studies are indispensable, which will allow to precisely determine conditions and mechanisms in which quercetin interacts with cytostatic drugs and will define optimum doses of the modifier. Resistance to chemotherapy involves a very complex phenomenon and it is difficult to assume that application of a single modulator will fully abolish the resistance. However, quercetin in this respect is particularly interesting and its value is accentuated not only by its accessibility, efficacy and broad spectrum of action but also by its low toxicity, as compared to other examined substance. It is also significant that application of quercetin does not preclude use of other modulators of resistance to cytostatic agents.

Conclusions

When used in low doses, attainable after oral administration, quercetin increases chemosensitivity of ovarian cancer cells.

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