

Basic Studies on Chemopreventive Properties of Quercetin and Curcumin and Other Plant-origin Compounds in Ovarian Cancer Cells – A Mini-review

Radosław Kujawski^{1*}, Justyna Baraniak², Marcin Ożarowski^{2,3}, Małgorzata Kujawska⁴, Magdalena Borowska¹, Małgorzata Kania², Joanna Bartkowiak-Wieczorek⁵, Agnieszka Sobczak⁶, Agnieszka Gryszczyńska², Katarzyna Manikowska¹, Joanna Liberska¹, Joanna Cichońska⁷, Michał Szulc¹ and Piotr Ruszkowski¹

¹Department of Pharmacology, Poznan University of Medical Sciences, Poland

²Department of Pharmacology and Phytochemistry, Institute of Natural Fibers and Medicinal Plants, Poland

³Department of Pharmaceutical Botany and Plant Biotechnology, Poznan University of Medical Sciences, Poland

⁴Department of Toxicology, Poznan University of Medical Sciences, Poland

⁵Department of Clinical Pharmacy and Biopharmacy, Poznan University of Medical Sciences, Poland

⁶Department of Pharmaceutical Chemistry, Poznan University of Medical Sciences, Poland

⁷Wielkopolska Centre for Advanced Technologies (WCAT), Adam Mickiewicz University in Poznan, Poland

*Corresponding author: Radosław Kujawski, Department of Pharmacology, Poznan University of Medical Science, Rokietnicka 5a Str., 60-806 Poznań, Poland, Tel: +480618547262; E-mail: radkuj@ump.edu.pl

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Abstract

Despite the increasing number of studies on the molecular actions of quercetin and curcumin, their anticancer efficacy, safety and molecular aspects of chemopreventive action in the ovarian cancer prophylaxis or treatment and also their potential in the sensitization to cytostatics used in the clinical practice remains still not clearly understood.

Based on basic studies we have summarized evidences for inhibitory activities of several often studied plant-origin bio-active compounds (mostly quercetin and curcumin) against ovarian cancer cells proliferation, their mechanisms of action as well as their strong potential to sensitization of ovarian cancer cells to the presence of several platinum-based cytostatics - cisplatin and oxaliplatin. Up to date only several dietary, clinical (cohort and case-control) studies evaluating the association of some flavonoids (mostly nonisoflavones) and its subgroup components consumption and ovarian cancer risk were already performed. According to the researchers, there has been no association between ovarian cancer risk and total nonisoflavone flavonoids intake. There is a still an insufficient amount of data designed to explain the effect of quercetin or curcumin (alone or together) on ovarian cancer development and/or its chemotherapy. Obtained results provide limited support for an association between nonisoflavone flavonoids intake and ovarian cancer risk, therefore there is a need for further and more accurate studies to be confirmed. We are of the opinion that this paper will contribute to a better understanding of the molecular basis for positive interactions between concomitant usage of quercetin or curcumin with above-mentioned cytostatics and other bio-active agents. This work may also contribute to an increase in the number of preclinical studies or other clinical, dietary trials using these or other phenolic / alkaloid, plant-origin constituents in order to investigate efficiency and safety of pharmacotherapy of ovarian cancer patients.

Keywords: Ovarian cancer; Chemoprevention; Flavonoids; Quercetin; Curcumin; Cisplatin

Abbreviations: A2780 (wild - type): A2780 (cisR) (cisplatin-resistant) and A2780 (ZD0473R) (ZD0473-resistant)-Ovarian Cancer Cells; CAT: Catalase; CDDP: Cis-diammine Dichloro Platinum; CHOP: CCAAT/Enhancer-binding protein Homologous Protein; cyto-c: Cytochrome c; DNA-PK: DNA-dependent Protein Kinase; DR5: Cell Death Receptor; EFO-27: Ovarian Cancer Cell Line; ENDOG: Endonuclease G; ERS: Endoplasmic Reticulum-Stress; GPx: Glutathione Peroxidase; IP3: Inositol, 1,4,5-Trisphosphate; MMP-2 and MMP-9: Two Metallo Proteinases; MPEP-PCL: Nanoformulated Quercetin; OVCAR: Human Ovarian Carcinoma Cell Line; PI: 1-Phosphatidylinositol; PIP Kinase: 1-Phosphatidylinositol 4-Phosphate 5-Kinase; Q: Quercetin, 3,3',4',5'-7-Pentahydroxyflavone; RF: Resistance Factor; ROS: Reactive Oxygen Species; SCOV-3-Human Ovary Cancer Cell Line; SOD 1: Superoxide Dismutase; TQ:

Thymoquinone; TRAIL: Apoptosis-Inducing Ligand; Type II EBS: Type II Estrogen Binding Sites; UCP2: Uncoupling Protein 2

Introduction

Ovarian cancer is the most leading cause of death from gynecological cancer in the Western world [1-3]. This may be due to the absence of early symptoms which causes the disease. In approximately two-thirds of patients the disease spreads beyond the ovaries [4] resulting in poor prognosis of ovarian cancer stems mainly from the high percentage of cases diagnosed at an advanced stage. Although most patients with advanced ovarian cancer respond to first-line chemotherapy, 80% of the patients ultimately succumb to death due to recurrence [5].

New strategies to improve clinical response and to reduce toxicity of cancer therapy focus nowadays on chemoprevention, which hopes to

identify substances that can suppress cancer transformation [6]. According to the current state of knowledge chemoprevention includes using agents that affect cell-cycle progression and apoptosis, signal transduction, oncogene activation, polyamine metabolism, angiogenesis, gap junctional intercellular communication, and more [6,7]. According to guidelines for cancer prevention published by the American Cancer Society Guidelines, one of the key lifestyle factors thought to modify cancer risk is a diet, especially that rich in plant-origin compounds [8-11]. Although nowadays natural therapies are being more and more often considered by physicians to be used even in cancer patients, together with targeted therapies, still little is known about their interaction with conventional chemotherapeutic agents. Numerous studies indicate that chemopreventive phytochemicals with phenolic structure, for example flavonoids or curcuminoids, possessing antiproliferative and antioxidant properties that can overcome problems of drug resistance and nonspecific toxicity towards normal cells, by reducing the side-effects that are associated with chemotherapy [10,12,13].

Flavonoids with several functions in pathological processes of cancer are polyphenolic compounds with a basic benzo- γ -pyrone structure being widely distributed in all foods of plant origin such as fruit, vegetable, tea and wine [9,14,15]. Some studies indicate that the observed pharmacological activities of flavonoids on ovarian cancer may depend on their structure [9,12]. Based on the range and structural complexity, flavonoids can be categorized into six major subclasses: flavones, isoflavones, flavonols, flavanones, anthocyanidins and flavan-3-ols [11,16], among which flavones, isoflavones and flavonols are reported in the highest amounts of consumption in the human diet and have biological activity in ovarian cancer [8,9,17,18].

One of the most abundant flavonol, identified in various types of vegetable [19-21], caper fruits [22], fruits [23], herbs (dill), some types of tea [24] and wine [25], being of the most extensively studied in this field is the quercetin [11,26-28]. Numerous studies have demonstrated that another plant-origin constituent-curcumin (a yellow substance belonging to the polyphenols superfamily; active component of turmeric, a common Indian spice, derived i.e. from the dried *Curcuma longa* rhizome) and its derivatives possess anti-oxidant, anti-inflammatory and anti-cancerous activities [29,30].

In this manuscript we aimed to summarize, based on basic studies, evidences of inhibitory activities against ovarian cancer cells proliferation of several plant-origin bio-active compounds, mainly quercetin and curcumin, described their cytotoxic potential when treated alone or with other phenolic or alkaloid substances, as well as their strong potential to sensitization of ovarian cancer cells to the presence of several cytostatics, mostly platinum-based ones. Only few experiments with quercetin and curcumin applied together on ovarian cancer cells were carried out so far.

Already published literature presents a number of experimental, pre-clinical results of *in-vitro*, *in-vivo* studies concerning the mechanism of action of quercetin and curcumin and their bio-active metabolites against ovarian cancer cells proliferation and/or *in vivo* tumor development. Although there have been published some reviews summarizing knowledge in this subject [31-34], in this paper we have presented more precise of dose-dependent *in vitro*, *in vivo* aspects of action of above mentioned plant origin bio-active compounds in relation to platinum-based cytostatics towards ovarian cancer.

Methods

We have done a review of *in vitro* and *in vivo* studies evaluating the correlation between the ability to inhibit ovarian cancer cells proliferation, defining the mechanisms of action of these bio-active compounds, administered alone, in combine treatment, in combination with other metabolites or two platinum-based cytostatics (cisplatin, oxaliplatin). For this purpose a systematic search for relevant publications was made using PubMed, Medline, Google Scholar databases. English and nonEnglish papers published up to February 2017 were screened using the following keywords “flavonoid”, “quercetin”, “curcumin”, “ovarian cancer”, “molecular”, “platinum-based cytostatics”, “cisplatin”. Clinical (cohort or case-control studies) studies scanned based on their title, abstract, and their major aims, their full texts were excluded.

Results

The knowledge gained as a result of the above methodology is presented below.

Chemoprotective activities in ovarian cancer cells of quercetin, alone or with cytostatics or other plant compounds

Early *in vitro* study on OVCAR 433 ovarian cancer cell line demonstrated synergistic antiproliferative activity of quercetin (Q) with cisplatin (CDDP) acting through an interaction with type II estrogen binding sites (type II EBS). A dose-dependent growth inhibition (range of concentrations was 0.01 μ M to 2.5 μ M and 0.01 μ g/ml to 2.5 μ g/ml for Q and CDDP, respectively) when administered separately, while their combination resulted in a synergistic action. Two other studied flavonoids as rutin (3-rhamnosyl-glucoside of quercetin) and hesperidin (3'-5-3-hydroxy-4-methoxyflavone) were ineffective, both alone and in combination with CDDP [35]. Another experiment showed the capability of quercetin to reversible dose-dependent inhibition of cancer cells proliferation in the range of concentrations between 10 nM to 10 μ M, without such effect of rutin and hesperidin [35]. Moreover, a synergistic, dose-dependent inhibition of colony formation of cells from 4 primary ovarian tumors expressing type II EBS and reduction of hyperthermia (42°C) effect in three out of four tumors analyzed was also observed in the case of quercetin (0.1 μ M to 10 μ M; max 10 μ M), while rutin and hesperidin were ineffective in synergizing with hyperthermia [36]. Quercetin inhibited proliferation of human ovarian carcinoma OVCAR-5 cells (IC₅₀ for growth inhibition=63 μ M; LC₅₀ for cytotoxicity=17 μ M) via suppression of signal transduction activity by concentration-dependent declining in 1-phosphatidylinositol (PI) 4-kinase leading to an 80% decrease in PI kinase activity and a 65% decrease in the concentration of the second messenger, inositol 1,4,5-trisphosphate (IP₃), closely correlated with its concentration change. PI-4 kinase is the first committed enzyme in the PI phosphorylation pathway of signal transduction leading to the production of the second messengers [37].

Studied flavonol acted synergistically also with one of the major soy isoflavones genistein, is a promising reagent for cancer chemoprevention and/or treatment [38-44] with minimal or no toxicity to nonmalignant human cells [45,46] in ovarian carcinoma OVCAR-5 cells causing i.e. arrests the cell cycle at G1 and S phase [37]. As the authors assumed quercetin blocked the cell cycle at G1 and S phase boundary, while the second one attacked the cell cycle at G2

and/or early M phase. In another experiment [47] both metabolites, as in the case by Prajda et al. [37], blocked the phosphatidylinositol conversion to IP₃ signal transduction pathway, mainly by the inhibition of PI kinase (EC 2.7.1.67) and another member of the signaling route-1-phosphatidylinositol 4-phosphate 5-kinase (PIP kinase, EC 2.7.1.68), respectively. For these experiment conditions the IC₅₀ values for quercetin and genistein (mean ± SE) were as follows: 66 ± 3.0 and 32 ± 2.5 μM; in clonogenic assays they were 15 ± 1.2 and 5 ± 0.5 μM, respectively. When such flavone was added to the cultures of OVCAR-5 cells followed 8 h later by genistein, synergism was still observed in growth inhibition [47]. This flavonoid acted also synergistically with antiviral ribavirin in OVCAR-5 cells (as well as in human myeloma 8226 cell line). Additionally, quercetin inhibited of PI-4 kinase activity as well as the activity of another studied enzyme-PIP-5 kinase (EC 2.7.1.68) in cells. The observed IC₅₀ and LC₅₀ values in growth inhibition for ribavirin were of 35 and 23 μM, respectively. A synergistic antiproliferative action was observed when quercetin was added 24 h after ribavirin and in combined treatment of ribavirin with studied flavonol [48]. An inhibition of activity of PI kinase by quercetin in ovarian cancer cells was also observed by Weber et al. [49], as well as a dose-dependent inhibition of PIP kinase in the case of genistein, while tiazofurin lowered the guanosine tri-phosphorane (GTP) concentration required for phosphoinositide-specific phospholipase C (PLC) protein. Combinations of tiazofurin with quercetin, tiazofurin with genistein, and quercetin with genistein yielded a synergistic kill of ovarian cancer cells [49].

An *in vitro* (A2780 and A2780cisR ovarian cancer cell lines) time (sequence)-dependent synergistic mechanism of action of quercetin and other bio-active metabolite-thymoquinone (TQ) with cytostatics such as cisplatin and oxaliplatin, was observed by Nessa et al. [50]. TQ is known antioxidant from a variety of biological activities including chemoprevention and inhibition of tumor growth [51]. It was demonstrated that although the oxaliplatin was the most active from all used compounds, among plant-derived metabolites, thymoquinone was the most active, with higher activity in the resistant cell line than in the parent cell line. Although these two phytochemicals were less active than both platinum cytostatics, they had resistance factor (RF) higher than the platinum drugs. RF is defined as the ratio of the concentration of the drug required for 50% cell kill in the resistant cell line to that in the parent cell line. The greatest synergism was observed when the phytochemicals were added first followed by platinum drug 2 h later and the least synergism was observed when the two compounds were administered as a bolus. Authors hypothesized that the addition of the plant-origin compounds 2 h before platinum drug may sensitize cancer cells to platinum action, thus offering a means of overcoming drug resistance [51].

Results by Gao et al. [52] with a novel form of nano-formulation of quercetin yielded a promising *in vitro* and *in vivo* antiproliferative effects. Such form of quercetin inhibited the growth of A2780S ovarian cancer cells on a dose dependent manner. In this experiment quercetin treatment induced the apoptosis of A2780S cells associated with activation of proteases essential in programmed cell death development-caspase-3 and caspase-9. The profile of other molecular markers, Mcl-1 and Bcl-2 proteins, has also changed causing their down regulation, also Bax upregulation and mitochondrial transmembrane potential change, suggesting an induction of apoptosis through the mitochondrial apoptotic pathway. Furthermore, an intravenous administration of such nanoformulated quercetin (MPEG-PCL micelles) significantly suppressed the growth of established xenograft A2780S ovarian tumors through causing cancer cell

apoptosis and inhibiting angiogenesis *in vivo* [52]. In another study [53] an analysis of cytotoxic potential of quercetin (with final concentrations: 0.1; 0.5; 1, 5, 10, 50, 100, 500 μM) in a set of ovarian cancer cells: SCOV-3, EFO27, OVCAR-3, A2780P revealed a significantly increase of sensitivity of some of them to cisplatin and paclitaxel. In this study a similar sensitivity of studied ovarian cancer cells for quercetin was obtained (with IC₅₀ values for OVCAR-3 cells=42 μM; for EFO27 cells=59 μM; for A2780P cells=70 μM; SCOV-3 cells=90 μM). A treatment of cells with 1 and 5 μM quercetin with those two cytostatics (in three concentrations marked as: K₁=(10⁻¹) × K₂; K₃=10 × K₂) caused a significant increase of sensitivity of all three cell line to paclitaxel, especially on SCOV-3 cell line which before the supplementation with quercetin was resistant to this drug. Also the sensitivity to cisplatin of OVCAR-3 and A2780P cells increased. Hence, results from this study revealed also a potential of analyzed flavonoid to overcome the chemoresistance of ovarian cancer cells to some cytostatics [53].

Catanzaro et al. [54] showed that among studied fifteen plant polyphenols, quercetin (as well as rhein and capsaicin) (0.01 μM to 100 μM) was able to cause the arrest of the human ovarian carcinoma 2008 cells and their cisplatin (CDDP)-resistant subclones-C13 cells cycle, suggesting that such cell cycle regulatory proteins are possibly involved in their intracellular mechanism of action. This effect was more potent in C13 cisplatin (CDDP)-resistant cells. Furthermore, a dose-dependent (10 μM to 50 μM) cytotoxic effects and cell cycle modulation of the quercetin in SKOV3 cancer cells and in its cisplatin (CDDP)-resistant counterpart (SKOV3/CDDP) cells was also observed in another experiment by Catanzaro et al. [55]. In this study evident changes in the distribution of cell cycle phases in the CDDP-resistant SKOV3/CDDP ovarian cell line caused by the presence of studied flavonoid and the significant decrease of the cyclin D1 expression in SKOV3 cells (probably linked with the G1/S phase alteration) were proved, but not in SKOV3/CDDP cells. Quercetin influenced also the G2/M phase of cell cycle, however without affecting the cyclin B1 levels (required for G2/M phase) which indicates the involvement of other possible mechanisms [55].

Recently, an enhancement by quercetin (50 μM, 100 μM, 200 μM) of apoptotic death of human ovarian cancer SKOV-3, OVCAR-3 and TOV-21G cells to the presence of the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) (25 ng/ml) through activation of JNK cellular signal transduction kinase, upregulation of a transcription factor CCAAT enhancer-binding protein (C/EBP) homologous protein (CHOP), induction of cell death receptor DR5, activated by TRAIL [56,57], was observed [58]. According to the authors, the combination of quercetin with TRAIL did not affected on DR5 receptor expression, ROS (important upstream signals required for the upregulation of death receptors; capable to induce CHOP expression) [59] mediated endoplasmic reticulum-stress (ERS) [58]. In this study a combined treatment of the cells with both TRAIL and quercetin showed significantly higher cell death compared to TRAIL or quercetin applied alone. The IC₅₀ values for quercetin combined with or without TRAIL in ovarian cancer cells such as SKOV-3, OVCAR-3 and TOV-21G cells were found to be 222.1 ± 5.64 μM vs. 153.3 ± 4.03 μM, 217.2 ± 4.89 μM vs. 147.4 ± 3.86 μM, and 237.6 ± 6.07 μM vs. 159.4 ± 3.64 μM, respectively. However, 200 μM quercetin did not significantly affect cell viability in normal ovarian cells (HOSE cells). Apoptosis in these three cell lines was induced at 14.85 ± 2.93%, 11.99 ± 2.33%, and 12.67 ± 2.61% by quercetin, at 9.42 ± 1.75%, 8.53 ± 1.21%, and 10.82 ± 2.01% by TRAIL, and at 36.42 ± 5.04%, 30.34 ± 4.54%, and 33.29 ± 5.28% by the combination of the two agents. In accordance

with these results, activation of caspase-3, 8, and 9 and PARP cleavage were significantly increased when the SKOV-3 cells were exposed to both, quercetin and TRAIL. These observations clearly indicated that quercetin was able to increase TRAIL-induced apoptosis in studied ovarian cancer cells. Analyzed flavonoid significantly affected mitochondrial membrane potential in SKOV-3 cells significantly increased the intracellular ROS levels and decreased the levels of DΨm in SKOV-3 cells in a dose-dependent manner. Thus, results by Yi et al. [58] strongly emphasized the role of ROS in the up-regulating of CHOP and enhancing the effects of TRAIL-induced apoptosis by quercetin. Furthermore, quercetin enhancement TRAIL mediated inhibition of tumor growth of human SKOV-3 xenograft was associated with induction of apoptosis, activation of caspase-3, CHOP and DR5. According to the authors all obtained results suggest that quercetin could be an attractive candidate for combined chemotherapy against cancer.

Results concordant to those obtained by Yi et al. [58] were observed in a study by Ren et al. [59]. In this study a time (after 24 or 48 h of treatment) and a dose-dependent manner (0, 0.12, 0.23, 0.47, 0.94, 1.88, 3.75, 7.5, 15 or 30 mg/ml) inhibition of the proliferation of SKOV-3 cancer cells by quercetin occurred. Also an *in vitro* inhibition of cell cycle progression from G0/G1 to G2/M, cell apoptosis induction (analyzed by Hoechst staining and morphologically) and reduction of survivin protein level due the presence of this flavonol was also confirmed. In this study the strongest inhibitory effect on cell growth was observed following treatment with 30 mg/ml quercetin, when the inhibitory rate of cell proliferation reached peak value 58.72%. The total apoptosis rate of the SKOV3 cells treated with 15 mg/ml quercetin was significantly higher than that of the control cells (33.62 ± 1.17 vs. $7.13 \pm 0.92\%$, $n=6$, $P<0.01$). When the cells were treated with 30 mg/ml quercetin, the total apoptosis rate was $69.12 \pm 2.97\%$, which was significantly higher than that of the low dose (15 mg/ml) of quercetin group and the control ($n=6$, $P<0.01$). Further detailed analysis revealed that following treatment with quercetin the number of cells at G0/G1 phase was significantly increased, while the number of cells at S and G2/M phases was relatively decreased compared with the control group. It suggested therefore that quercetin can cause cell cycle arrest at the G1 phase for ovarian cancer SKOV3 cells, may prevent the cell cycle progression from G1 to S phase and can induce cell apoptosis. Thus, these results clearly indicated that cell cycle arrest may be one of the important mechanisms underlying the quercetin-induced inhibition of ovarian cancer SKOV3 cell growth, as well as quercetin-induced cell apoptosis [59].

Results from another study by Yang et al. [60], similar to those obtained by Maciejczyk et al. [53] or Yi et al. [58] showed that quercetin pretreatment at a low dose may augment drug-resistant ovarian cancer cells for cisplatin chemotherapy by involving the endoplasmic reticulum-stress (ERS) proces via alternation of STAT3 kinase signaling (phosphorylation), a key player involved in ovarian cancer [61,62], modulated by ERS [60]. In this study quercetin decreased the viability of both the C13⁺ and P-ris cells in a dose-dependent manner, with an IC50 value of approximately 100 μM at 48 h; the growth of both cell types could only be significantly inhibited with quercetin concentrations >40 μM, with a negligible cytotoxicity in both cell types at a dose 20 μM. Furthermore, cisplatin treatment with quercetin pretreatment had the greatest inhibitory effect on cancer cell growth. Such pretreatment markedly potentiated the cytotoxicity of cisplatin in studied ovarian cancer cells, and the apoptotic behavior was due to the activation of caspase-9 and caspase-3 of the intrinsic pathway-it was observed that quercetin pretreatment rather than

cisplatin alone significantly increased the levels of cleaved caspase-9, cleaved caspase-3 and PARP proteins. Studied plant metabolite at a dose higher than 20 μM had a greater impact on cisplatin cytotoxicity and this influence was dose dependent [60].

In a ovarian cancer xenograft mice quercetin (40 mg/kg; i.p.) enhanced the antitumor effect of cisplatin (3 mg/kg; i.p.). Tumors from mice treated with cisplatin in combination with quercetin pretreatment had repressed STAT3 kinase phosphorylation, lower BCL-2 protein and higher apoptosis levels. The weekly intraperitoneal administration of cisplatin for 4 weeks only modestly reduced the tumor growth by 44.2%, while its weekly treatment day after the 40 mg/kg quercetin treatment remarkably decreased tumor growth by 89.6%. Summarizing, Yang et al. [60] revealed a hitherto undescribed cellular response demonstrating that the quercetin pretreatment, involving the ERS process, may be a promising way to solve the systemic side effects and high incidence of resistance to cisplatin in ovarian cancer pharmacotherapy.

Although previous results indicating pro-apoptotic effect of mostly relatively high concentrations of quercetin (40 μM to 100 μM), *in vitro* (in human ovarian cancer C13⁺ and SKOV3 cells) and *in vivo* results by Li et al. [63] have demonstrated that quercetin even at low concentrations may cause the attenuation of cisplatin and other anti-neoplastic agents (taxol, pirarubicin and 5-fluorouracil) therapeutic effects in ovarian cancer cells by reducing ROS damage. In this study the IC50 value in cisplatin-treated C13⁺ cells was approximately 80 μM (IC50=78.8 μM, 95% CI: 72.9 μM to 85.1 μM). It was shown that low concentrations of analyzed flavonoid (20 μM), not high concentrations (80 μM), antagonized the cytotoxic effects of cisplatin in C13⁺ cells. It was done by increasing cell resistance to cisplatin in varying degrees; decreasing in numbers of apoptotic cells treated with cisplatin and 20 μM of quercetin than with cisplatin alone. High concentrations of quercetin had an additive effect with cisplatin. Similar to those results, treatment using other neoplastic drug taxol (3 μM), pirarubicin (3 nM) and 5-Fu (5 μM) in combination with quercetin (in a series of increasing doses) resulted in more cell resistance than treatment with three drugs alone. Quercetin at the 20 μM concentration showed anti-apoptotic effects against these three drugs. Studied flavonol showed also a low-concentration-specific protecting effect to ovarian cancer cells treated with 5-Fu, similar to the results obtained with cisplatin. In combination with taxol or pirarubicin, however, it maintained the effect of promoting cells survival against anticancer drugs even at relatively high concentrations (80, 100 μM). Further detailed analysis of quercetin influence on the oxidative injury of ovarian cancer cells caused by cisplatin revealed that low concentrations of quercetin were observed to suppress ROS-induced injury, reduce intracellular ROS level. It was shown, for example, that the quantities of both ROS: cH2AX (a common marker of DNA damage) and 8-hydroxydeoxyguanosine (8-OHdG; a marker of oxidative DNA stress) were much lower in the treatment group of 80 μM cisplatin combined with the low concentration of quercetin (20 μM) than the group of 80 μM cytostatic alone. Contrary to this, 80 μM cisplatin with high concentration (60 μM; 100 μM) of analyzed plant metabolite increased the intensity of fluorescence. Combination treatment with quercetin (20 μM) had a more obvious decrease of 8-OHdG than that of cH2AX, compared to cisplatin treatment alone (36.40% and 19.33%, respectively). Moreover, comparing to cells treated with vehicle control or cisplatin alone, treatment with an additional 20 μM quercetin lead to a reduction in intracellular levels of ROS. Furthermore, low quercetin concentration caused increased expression of endogenous antioxidant enzymes transcripts (superoxide dismutase 1-SOD1,

endonuclease G-ENDOG, cytochrome c-cyto-c, glutathione peroxidase-GPx, catalase-CAT and uncoupling protein 2-UCP2). Summarizing, obtained results suggested a ROS-mediated mechanism of action of quercetin attenuating anti-neoplastic drugs effects. Surprisingly, in an applied xenogeneic model (a nude mouse xenograft model injected with C13⁺ cells), studied flavonol (40 mg/kg; i.p.; daily) led to a substantial reduction of therapeutic efficacy of cisplatin. For example, it was found out that tumors from mice treated with cisplatin in combination with quercetin were approximately 1.8 times larger at day 30 than those treated with cisplatin alone ($P < 0.001$). Even more, tumors treated with combination of quercetin and cisplatin (72.53 ± 7.33 mg) were significantly heavier than that treated with cytostatic alone (41.47 ± 6.72 mg) ($P < 0.001$). With this effect also an enhancement of the endogenous SOD1 enzyme expression and reduction of the ROS-induced damage in xenograft tumor tissue were observed. The SOD1 protein was highly overexpressed in tumors treated with quercetin or cisplatin with quercetin compared to tumors treated with vehicle or cisplatin alone [63]. Overall, data obtained by Li et al. indicated that administration of quercetin at low concentrations may antagonize the cytotoxic effects of anti-neoplastic drugs in studied ovarian cancer cells, and that this plant metabolite is able to decrease the levels of oxidative injury caused by cisplatin [63].

An induction of apoptosis of human ovarian carcinoma SKOV-3 and A2780 cells through activation of the extrinsic death receptors and intrinsic mitochondrial apoptotic pathways via modulation of expression levels of cleaved caspase-3 (as was shown in experiments by Yi et al. [58] or Yang et al. [60]) and induction of overexpression of miR-145 by quercetin was also confirmed by Zhou et al. [64]. These results are with the trend of studies indicating that some microRNAs, including the miR-145, are downregulated in ovarian cancer [65,66], and that cancer cells growth inhibition caused by quercetin may be correlated with the modulation of several miRs expression of [60, 63, 67,68].

Quercetin, among other eight studied compounds, was also able to inhibit OVCAR5, OVCAR3, TOV112D, CAO3 ovarian cancer cells growth via induction of apoptosis and inhibition of the glycolytic pathway-by blocking five glycolysis pathway molecules-(glucose transporter 1 (GLUT1), hexokinase II (HKII), PFKFB3 (an isozyme; a component of fructose-2, 6-bisphosphate (Fru-2, 6-BP), an allosteric activator of 6-phosphofructo-1-kinase (PFK1) [69]), pyruvate dehydrogenase kinase 1 (PDHK1) and lactate dehydrogenase (LDH)) via an increased extracellular glucose and decreased lactate production, which was especially seen at low oxygen conditions. Variation in oxygen levels between 0.5% and 7% had a relatively small effect on the efficacy of the studied inhibitors. The IC₅₀ values of quercetin for OVCAR5, TOV112D, OVCAR3, CAO3 cells were 154, 94, 21, 240 μ M, respectively [70]. The choice of glycolytic pathway enzymes analyzed in this study was due to the fact, that they are well substantiated and considered a 'hallmark' of advanced cancers [71]. The fact that cancer cells reduce their dependence on mitochondrial oxidative phosphorylation and are more reliant on glycolysis provides a wide range of potential targets for therapy. Targeting aerobic glycolysis is a promising strategy to preferentially kill cancer cells which are dependent on this pathway and in recent years multiple glycolytic inhibitors have been developed [72,73].

The above-described results were related so far to quercetin being a non-physically component of the plant extract. Wang et al. [74] demonstrated results of the influence of quercetin aglycone (and seven other flavonols: myricetin-3-galactoside, quercetin-3-galactoside,

quercetin-3-glucoside, quercetin-3-xylopyranoside, quercetin-3-arabinopyranoside, quercetin-3-arabinofuranoside, quercetin-3-rhamnopyranoside) as well as eleven member of A-type proanthocyanidins isolated from *Vaccinium macrocarpon* (American cranberry) against SKOV-3 and OVCAR-8 cancer cells. Two of them, quercetin aglycone and PAC DP-9, showed promising *in vitro* cytotoxic and anti-proliferative properties. Compared to quercetin glycosides, quercetin aglycone exhibited higher cytotoxicity against these cancer cell lines (IC₅₀=83 and 61 μ g/ml for SKOV-3 and OVCAR-8 cells, respectively).

Both compounds strongly induced apoptosis led to caspase-3 activation and PARP deactivation, and increased sensitivity to cisplatin (concentrations of: quercetin aglycone 10 μ g/ml, cisplatin=15 μ g/ml or 60 μ g/ml; measurement after 12 h of cisplatin treatment). In addition, quercetin aglycone (and PAC DP-9) deactivated the MAPK-ERK kinases pathway, induced downregulation of: cyclin D1 kinase, DNA-dependent protein kinase (DNA-PK) and the phospho-histone H3 - proteins - often overexpressed in ovarian cancer cells.

Also this aglycone caused an upregulation of p21 protein, and the cell cycle progression arrest (G1/S-phase arrest in OVCAR-8 cells after the treatment for 24 and 48 h). In conclusion, according to Wang et al. the integration of quercetin (and/or PAC DP-9) in ovarian cancer chemotherapy may improve outcomes. Moreover, among studied compounds quercetin aglycone induced cellular apoptotic events including cell cycle arrest and suppression of DNA repair pathways highlighted their potential as dietarily available therapeutic agents [74]. The quercetin-induced apoptotic cell death correlated with several kinases phosphorylation was also proved in the study by Kim et al. [75] but in a different cell line model. In this study the presence of quercetin, detected in *Rubus coreanus* Miquel (RCM) extract, or other phenolic compound - an ellagic acid, caused an apoptosis of the doxorubicin-resistant NCI/ADRRES ovarian cancer cells induced the phosphorylation of JNK and AKT kinase. The same effect was observed in the case of RCM extract, however, without the synergism with doxorubicin in reduction of cells viability [75].

An inhibition of proliferation of ovarian cancer cells by quercetin (0, 2.5, 5 and 10 μ g/ml; after 72 hours) was also observed by Choi et al. [76]. This study was performed not only on paclitaxel sensitive (SKOV-3) but also on SKOV-3/PAX (paclitaxel resistant) ovarian cancer cells as well. The action was compared to 80% methanolic extract of *Rhus verniciflua* Stokes (syn. *Toxicodendron vernicifluum* Stokes) (RVS) (50, 100, 200 and 500 μ g/ml) in which quercetin was present. In this study quercetin dose-dependently (more potent than fisetin alone) reduced cell growth. Other activities of quercetin, observed in the case of another analyzed flavonoid-butein, such as strong activation of cleavage of caspase-9, -8, -3, and PARP, reduction of AKT phosphorylation or SKOV-3/PAX cells arrest in sub-G1 phase, were not further examined [76].

Recent study by Ediriweera et al. [77] demonstrated also a cytotoxic potential of quercetin (and other three compounds) isolated from the chloroform extract of *Mangifera zeylanica* Hook.f. bark in ovarian epithelial cancer (SKOV-3), as well as in three other cancer cell lines - triple negative breast cancer (MDA-MB-231), estrogen receptor positive breast cancer (MCF-7), and normal mammary epithelial (MCF-10A) cells. Quercetin was found to be cytotoxic to all three cancer cell lines examined in a dose dependent manner. IC₅₀ values for quercetin at 24 h and 48 h for SKOV-3 cells were 170.18 ± 3.92 , 109.9 ± 2.64 mM, respectively. Also a DPPH free radical scavenging activity (EC₅₀= 33.1 ± 0.59 mM) of quercetin was shown [77].

Studies by Liu et al. [78] on human ovarian cancer (OC) CAOV3 cells and on primary OC cells provided evidences for a novel role of endoplasmic reticulum (ER-responsible for protein translocation, protein folding, and protein post-translational modifications) stress (perturbations in ER function triggering the unfolded protein response (UPR), a tightly orchestrated collection of intracellular signal transduction reactions designed to restore protein homeostasis; triggering the apoptotic machinery ultimately leading to cell death under severe or chronic stress conditions) as, as authors described, a "double edge sword" participating in quercetin-induced apoptosis [78]. In this study quercetin (40 μ M; 80 μ M for 48 h) irreversibly evoked ER stress to involve in mitochondria apoptosis pathway via the p-STAT3/Bcl-2 axis in OC cell lines and in primary OC cells. The quercetin-induced ER stress activated protective autophagy concomitantly by activating the p-STAT3/Bcl-2 axis in this process. Moreover, the autophagy scavenger-3-methyladenine (3MA) was shown to enhance quercetin's anticancer effects in an ovarian cancer mice xenograft model (5×10^{-6}) of human ovarian cancer CAOV3 cells in 100 μ l volume of PBS injected s.c. into the right supra scapula region of mice). For this purpose following *in vivo* experimental conditions were carried out: 5 days after tumor implantation the mice were treated i.p. with 80 mg/kg of quercetin twice a week; 30 mg/kg 3MA twice a week; a combination of 30 mg/kg 3 MA and 80 mg/kg of quercetin twice a week; or a vehicle control injected with the same volume of saline for 4 weeks [78]. The results of this study are consistent with the trend of predecessor studies [56,60,77] showing that quercetin has the potential to induce protective autophagy in cancer cells through AKT-mTOR and hypoxia-induced factor 1 α signaling [79,80] and in this way to induce autophagy in ovarian cancer cells [81].

There is also a growing number of studies justifying the use of plant-derived bio-active metabolites, especially polyphenols, in cancerogenesis prevention/inhibition by applying them in combinations with other micronutrients for achieving pleiotropic effects [34,82-89]. This is especially interesting when one considers the fact of their poor bioavailability in the human body. In addition, their interactions with other natural compounds in a diet may hinder or complicate consistency of their efficacy [90]. According to the supporters of this strategy these compounds, which are abundant from dietary sources, show great promise in cancer treatment, especially considering their potential safe use. Due to their ability to modulate multiple biological mechanisms involved in cancer initiation/progression, they may offer more comprehensive therapeutic effects than single drugs [34]. It is believed that the bioavailability, as well as chemopreventive or even therapeutic properties of these nutrients, may be enhanced and expanded in combination therapies that include natural compounds of the same or different chemical class. Thus, a combinations of i.e. polyphenols with micronutrients essential for maintaining integrity and stability of extracellular matrix and therefore provides the opportunity to expand the preventive/therapeutic effect of their anti-cancer action. They include targeting complementary metabolic pathways important in curtailing cancer invasion and metastasis. Therefore, specifically designed combinations of several bio-active compounds, especially polyphenols or combinations of polyphenols with other natural agents aimed at defined biological targets will expand metabolic effects of constituents of such mixtures in controlled and reproducible ways. In addition, proper combinations of micronutrients enable use of lower doses of individual components without compromising their efficacy, rather than expanding the scope of cellular mechanisms affected. According to researchers this

approach may open up a possibility of developing more effective anti-cancer strategies [34].

In the context of the above information such anti-cancer potential of quercetin with EGCG (at concentrations between 0 and 1000 μ g/ml) and in combination with the micronutrient mixture against ovarian cancer using two different cell lines (ES-2 or A-2780) or in athymic female mice inoculated with ES-2 cells was demonstrated by Roomi et al. [82,91-93]. Studied micronutrient mixture EPQ (containing the following nutrients in the relative amounts indicated: vitamin C (as ascorbic acid and as Mg, Ca ascorbates, and ascorbyl palmitate) 700 mg; L-lysine 1000 mg; L-proline 750 mg; L-arginine 500 mg; N-acetyl cysteine 200 mg; standardized green tea extract (80% polyphenol) 1000 mg; quercetin as quercetin dihydrate, from *Saphora japonica* 50 mg; selenium 30 g; copper 2 mg; manganese 1 mg) was investigated and the proposed mechanism of action was via modulation of expression of two metalloproteinases (MMP-2 and MMP-9) (these MMPs are prognostic for survival and metastatic potential in ovarian cancer [94-96]. Athymic female mice inoculated with ES-2 ovarian cancer cells showed that dietary intake of EPQ (0.5%, w/w) inhibited weight and burden of tumors by 59.2% ($p < 0.0001$) and 59.7% ($p < 0.0001$), respectively [91]. In a study with mice injected (i.p.) with A-2780 cancer cells, control mice developed large ovarian tumors, whereas five out of six mice in the EPQ group developed no tumors, and one, only a small tumor-tumor growth was suppressed by EPQ by 87% ($p < 0.0001$) [92]. *In vitro* analyzes proved also the antiproliferative potential of EPQ. EPQ exhibited 35% toxicity over the control in ES-2 cells [91] and 80% in A-2780 cells [92] at 1000 g/mL concentration. ES-2 cells demonstrated only MMP-2, with and without PMA, which was inhibited by EPQ in a dose dependent fashion, with near total inhibition at 1000 g/ml [91]. A-2780 cells demonstrated only MMP-9 expression, which EPQ inhibited in a dose dependent fashion, with virtual total block at 250 g/mL concentration [92], while the migration of ES-2 cells were inhibited in a dose dependent manner with total block of invasion and migration at 500 g/ml [91]. However, it is difficult to indicate which compound of nutrient mixture examined the higher anticancer activity, a 1000 mg quercetin dihydrate concentration may suggest its crucial role in the EPQ action, however such hypothesis along with their putative action in a synergistic manner requires further evaluation [91,93]. Nevertheless, these studies clearly give credit for the usage of natural health compounds widely presented in human diet to prevent or inhibit ovaries carcinogenesis [91-93].

Studies on curcumin alone or with quercetin and other compounds in ovarian cancer cells

A potential of curcumin and quercetin for enhancing cisplatin sensitivity in two ovarian cancer cell lines: SKOV3 and CAOV3 (less susceptible to cisplatin, with a higher IL-6 protein production) was observed by Chan et al. [51]. Both compounds inhibited the growth of SKOV3 and CAOV3 cells in a dose-dependent and similar manner (quercetin at 10 μ M to 20 μ M; curcumin at 5 μ M to 15 μ M). Such inhibition was attained by day 2-3 after drug treatment and persisted thereafter (in max. 5 days experiment duration). They increased the sensitivity of CAOV3 and SKOV3 cells to cisplatin (2 μ g/ml) (the concentrations of quercetin were as follows: 0; 5; 10; 15; 20 μ M, while curcumin were as follows: 0; 2.5; 5; 10; 15 μ M). This effect of sensitization was obtained when the compounds were added 24 h before or simultaneously with cisplatin. In an applied experimental model the high IL-6 protein producing CAOV3 cells were less

susceptible to cisplatin-induced cytotoxicity than the low producer, SKOV3 cell line. The survival rate of CAOV3 cells at 2 days after treatment with 1 mg/ml of cisplatin was 87%, whereas that of SKOV3 was at 45% (IC50 values for the two cell lines were 6.1 mg/ml and 0.9 mg/ml, respectively). According to authors these difference resembles the levels of resistance seen in clinics (2-4-fold) and are more closely than laboratory-induced cisplatin resistant cell lines (which are in the order of 10-1000-fold) [97]. In this experiment curcumin inhibited the production of interleukin IL-6 in CAOV3 cells suggesting that one of the possible mechanisms for synergy between cisplatin and curcumin was related to decrease autologous production of IL-6. However, such synergy was also observed in the SKOV3 cell line (exerting low IL-6 expression) indicating that this action was most probably a result of multiple targeting [51].

A complex study by Montopoli et al. [98] confirmed curcumin cytotoxic activity in ovarian cancer cells and strongly revealed its synergistic action to cisplatin and a third generation platinum derivative drug, oxaliplatin-1R, 2R-diamnocylohexanoxalateplatinum (II) leading to an increase of cisplatin's and oxaliplatin's cytotoxicity in wild type human ovarian cancer cell line and C13 cisplatin-resistant ones when they were combined treated. It was shown that curcumin caused a concentration- and time-dependent inhibition of ovarian cancer cell viability. Its synergism with cisplatin and oxaliplatin in such action also occurred. In this study all compounds caused concentration-dependent inhibition (0.01 μM to 100 μM) of cell viability. The IC50 values for curcumin were 14.92 μM ; 7.87 μM ; 6.36 μM after 24 h, 48 h and 72 h, respectively; for cisplatin were as follows: 1.35 μM ; 0.96 μM ; 0.94 μM after 24 h, 48 h and 72 h, respectively. While for oxaliplatin following values were obtained in above-mentioned time periods: 5.62 μM ; 2.52 μM ; 3.58 μM , respectively. Montopoli et al. showed that curcumin was more effective in cisplatin-resistant than in cisplatin-sensitive ovarian cancer cells. Moreover, a synergistic inhibitory effect on cell viability of curcumin (0.1 μM to 1 μM) with platinum drugs (all at concentrations: 0.1 μM to 5 μM) in both, in wild type 2008 and C13 cells, was clearly visible. A curcumin alone (0.5 μM , 1.0 μM , 5.0 μM) significantly reduced the percentage of wild type 2008 cells in G2/M phase in a concentration-dependent fashion and caused apoptosis (sub-G0), whereas in C13 cells it significantly increased numbers of cells in G0/G1 phase and reduced cells in S and G2/M, in comparison with untreated cells (with similar results after all three studied experimental periods: 24 h, 48 h, 72 h). Compared to single-drug treatment, curcumin combined with cisplatin or oxaliplatin, at concentrations lower than IC50 (0.5 μM for all three), caused the best dose- and time-dependent increases in cell-cycle arrest and apoptosis. According to Montopoli et al. [98] these results suggested a crucial role for curcumin in alteration of cell-cycle parameters, when combined with platinum drugs. It was clearly indicated that concurrent treatment of curcumin with platinum drugs makes cisplatin-resistant ovarian carcinoma cells more susceptible to the action of the platinum drugs. It was also shown that in cisplatin-resistant cells (compared to cisplatin-sensitive cells), higher glutathione content may be parallel to lower ROS level (used as a markers of curcumin's antioxidative potential), and that curcumin is able to cause the concentration-dependent changes of ROS or glutathione after different time of incubation (an increase of ROS after 2 h incubation, but increase in glutathione after 24 h). Similarly, curcumin's early pro-oxidative effect (increased ROS and reduced glutathione) and late antioxidant effect (increased glutathione) have been shown by Weir et al. [99] in ovarian cancer cells and i.e. in human myeloid leukaemia cells [100]. Therefore, study by Montopoli et al.

provided an evidence that curcumin is able to increase the glutathione level in both, cisplatin-sensitive and cisplatin-resistant, ovarian cancer cells, suggesting that the increased susceptibility of ovary cancer cell to combined treatment of curcumin with platinum drugs is not related to a glutathione elimination [98].

A synergism of actions between platinum drugs-cisplatin and oxaliplatin, applied in binary combination with the curcumin and anethole were also observed in three ovarian cancer cells: A2780 (wild - type), A2780 (cisR) (cisplatin-resistant) and A2780 (ZD0473R) (ZD0473 - resistant) in another experiment by Nessa et al. [101]. Both plant metabolites were able to increase intracellular levels of glutathione and glutathione-S-transferase, hence their possessed an antioxidative activity [102-104]. Although, in this study, oxaliplatin was found to be the most active and analyzed plant compound-anethole-had the lowest activity at all, it had a greater activity in the resistant cells (A2780 (cisR) and A2780 (ZD0473R)) than other compounds. The obtained results indicated that anethole was better able to induce programmed cell death i.e. apoptosis of the resistant cell lines than of the parent cell line. Moreover, applied 2/0 combinations of anethole with both cisplatin and oxaliplatin were found to be highly synergistic, indicating that pretreatment of ovarian cancer cells with the phytochemical serves to sensitize them to platinum (cisplatin and oxaliplatin) action. Similar effects were observed in the case of curcumin. The role of NF- κ B as a potential molecular target in the action of studied plant-origin compounds was strongly postulated by authors and others [105]. The enhanced cell kill resulting from treatment of cancer cells with these two phytochemicals (applied 2/0 sequence of administration) may be related to the reduced expression of NF- κ B and down-regulation of AKT kinase and cyclooxygenase-2 (COX2) protein pathways. It was therefore stated that bolus addition of platinum drugs and the selected phytochemicals leading to change from agonistic to antagonistic in action may suggest that concurrent administration of these two compounds in some cases may cause failure of sensitize the ovarian cancer cells to platinum action, perhaps due to less significant reduction in the expression of NF- κ B [50]. Thus, the prior treatment of ovarian cancer cells with anethole and curcumin appeared to be the critical determinant in lowering the expression of NF- κ B [101]. The observed action of anethole promoting cell survival at a lower concentration (0.2 μM) and promoting cell death at higher concentrations (25 μM) seemed to support such thesis [101].

Conclusions

Upon basic studies in this paper we have summarized available evidences of inhibitory activities of several often studied plant-origin bio-active compounds (mostly quercetin and curcumin) of ovarian cancer cells proliferation, their mechanisms of action (relying i.e. on suppression of signal transduction activity of several cellular kinases [37,47-49,60,75], induction of apoptosis [52,58], cell cycle arrest [37,55,59]) induction of ER stress activating protective autophagy via the p-STAT3/Bcl-2 axis [78], as well as their strong potential to sensitization of ovarian cancer cells to the presence of several platinum-based cytostatics (cisplatin and oxaliplatin) [75] and others.

Up to date several dietary, clinical (cohort and case-control) studies evaluating the association of some flavonoids (mostly nonisoflavones) and its subgroup components consumption and ovarian cancer risk were already performed (Mohammadi et al. [32], Parvaresh et al. [33], Hua et al. [6]). According to the researchers, there has been no association between ovarian cancer risk and total nonisoflavone flavonoids intake [32]. Although, some studies reported an inverse

association between certain nonisoflavone flavonoids subclasses (flavonols) [106,107] or other individual flavonoids (i.e. kaempferol and luteolin [107] and apigenin [108]) and ovarian cancer risk, there is a still an insufficient amount of data designed to explain the effect of quercetin or curcumin (alone or together) on ovarian cancer development and/or its chemotherapy. According to Mohammadi et al. [32] these results provide limited support for an association between nonisoflavone flavonoids intake and ovarian cancer risk; therefore there is a need for further and more accurate researches to be confirmed. Thus, we are of the opinion that this paper will contribute to a better understanding of the molecular basis for positive interactions between concomitant usage of quercetin or curcumin with above-mentioned cytostatics and other bio-active agents. This work may also contribute to an increase in the number of preclinical studies or other clinical, dietary trials using these or other phenolic/alkaloid, plant-origin constituents in order to investigate efficiency and safety of pharmacotherapy of ovarian cancer patients. We hope that such point of view, presented in this paper, extended by actual state of knowledge will be helpful to find other connections between active substances like quercetin and curcumin and cytostatics depending on dose.

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