Synergistic Interactions Between Anticancer Chemotherapeutics and Phenolic Compounds and Anticancer Synergy Between Polyphenols*

Summary

Chemoprevention has recently gained a new dimension due to the possibility of studying the mechanisms of action of chemopreventive agents at the molecular level. Many compounds have been proved to inhibit early stages of carcinogenesis in experimental models. These compounds include both recognized drugs (such as tamoxifen and nonsteroidal anti-inflammatory drugs) and natural constituents of edible and therapeutic plants, particularly polyphenols. Phenolics are characterized by high structural diversity and, consequently, a very broad spectrum of biological activities. They are increasingly looked upon as a valuable alternative or a support for synthetic drugs, as evidenced by a growing number of clinical trials regarding the use of phenolic compounds and polyphenol-rich extracts in chemoprevention and therapy. In the present work, we discuss the effectiveness of natural polyphenols as cancer preventive and therapeutic agents resulting from their synergy with synthetic or semisynthetic anticancer drugs as well as with other phenolic compounds of plant origin.

Key words: polyphenols • synthetic drugs • chemoprevention • polyphenol-drug interactions • synergism

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Introduction

Numerous research groups have focused on anticancer therapies for many years; the progress in techniques of imaging and therapy has also been impressive. Nevertheless, cancer mortality rates are still on the rise. The highest number of cancer-related deaths among women are caused by cancers of the breast, lungs, stomach, colon and/or rectum, and cervix, while among men cancers of the lung, stomach, liver, colon and/or rectum, esophagus, and prostate result in the highest mortality [33, 87]. The increase in industrialization and environmental pollution (resulting in food contamination), together with life style (smoking, excessive consumption of highly processed food, long-term stress) are all looked upon as the causes of this phenomenon. Many chemical compounds present in air, water, food, synthetic materials, and other products act as carcinogens [28, 47, 64]. In spite of the huge progress in understanding the molecular pathogenesis of tumors, surgery combined with chemotherapies and radiotherapy still remains the most effective therapy. To date, gene therapies have not fulfilled the hopes associated with their application.

An alternative approach is chemoprevention, which consists in using synthetic, semisynthetic or natural agents to inhibit or reverse the process of carcinogenesis, particularly in individuals with a high risk of developing cancer. Epidemiological studies indicate that in Asia the incidence of some cancers (including cancers of the breast, colon, prostate, and lung) is lower than, for instance, in Europe and USA [33, 45, 82]. This phenomenon may result from the fact that an Asiatic diet is markedly richer in health beneficial plant-derived polyphenols than European or American diets. It may be a consequence of drinking high amounts of green tea (with epigallocatechin gallate/EGCG as the main phenolic constituent) as well as of consuming large amounts of soy products that contain genistein [36, 54]. The results of recent studies indicate that natural compounds originating from edible and therapeutic plants could be used in both prevention and therapy of cancer, as they act pleiotropically on cancer cells. Through modulation of some signaling pathways, these compounds can exhibit a variety of biological activities, including antiproliferative [11], proapoptotic [24, 25], and anti-angiogenic activities [43, 46]. Accordingly, they can inhibit carcinogenesis by various mechanisms, which renders it possible to choose a suitable agent.

There is a growing body of evidence on stronger chemopreventive activities of combinations of phytochemicals than in the case of individual compounds, which is referred to as a synergistic effect [14]. Some combinations of compounds exhibit biological activity that is not detected when its individual constituents are tested separately. Moreover, polyphenols interact additively, synergistically, or antagonistically not only with other phenolics but also with other food components as well as with drugs of natural or synthetic origin [14, 45]. The above-mentioned interactions are looked upon as the reasons for chemopreventive effectiveness of a diet rich in fruits, vegetables, and whole grain cereals against civilization diseases, including cancer [14, 15, 45].

Synergism of Chemotherapeutics and Phenolic Compounds

Chemotherapy, despite many side effects, is still the most popular way of treating cancer [37]. Polyphenolic compounds give hope for an improvement of chemotherapeutic efficacy as well as reduction of side effects. For instance, cisplatin (cis-diamminedichloroplatinum) is a cytostatic drug widely used in the treatment of different types of cancer; however, its application is limited because of increasing resistance and many undesirable side effects in humans. Olas et al. [59] investigated in vitro synergistic action of cisplatin and trans-3,3',5,5'-tetrahydroxy-4'-methoxystilbene on blood cells. The combination of the inorganic platinum derivative with the polyphenolic compound significantly reduced DNA damage in lymphocytes and lipid peroxidation, protein carboxylation, and thiol group oxidation in blood platelets. Other examples of anticancer synergy between chemotherapeutic drugs and phenolic compounds or polyphenol-rich extracts are presented in Table 1 and discussed below.

Genistein

Genistein, a polyphenolic compound occurring in soya seeds (Glycine soja), combined with cisplatin significantly reduced proliferation of BxPC-3 pancreatic carcinoma cells and induced their apoptosis. A reduction of tumor growth was also observed in vivo, in a murine xenograft model of BxPC-3 cells [54]. Moreover, the regression of cancer was observed in C57Bl6 mice with Lewis lung carcinoma cells after administration of a single dose of combinations of intraperitoneal cisplatin, 5-fluorouracil, and genistein [51]. It is worth mentioning that in this experiment pharmacotherapy was combined with radiotherapy.

The results of many experiments indicate that overexpression and activation of nuclear factor-κB (NF-κB) reduces the efficacy of chemotherapeutics via inhibition of apoptosis [79]. However, polyphenolic compounds can alter this action. Mohammad et al. [54] showed that genistein caused a decrease in expression of antiapoptotic proteins Bcl-2 and BclXL and down-regulated NF-κB in xenografts of pancreatic carcinoma cells in animals fed with the addition of this isoflavone. In in vitro studies,

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pretreatment of pancreatic carcinoma cells COLO 357 and L3.6pl with genistein before incubation with cisplatin inhibited their proliferation and triggered apoptosis by down-regulation of NF-κB [6].

Similar results were obtained by Ali et al. [1] in experiments carried out on squamous cell carcinoma ME-180PT and UMSCC-5 cell lines using a mixture of isoflavones containing genistein and daidzein combined with cisplatin.

### Table 1. Examples of synergy between phenolic compounds and anticancer chemotherapeutics. Animal models are in bold.

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<th>Anticancer chemotherapeutics</th>
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<td>genistein</td>
<td>combination of cisplatin and S-fluorouracil</td>
<td>CS781/6 mice implanted with Lewis lung carcinoma cells</td>
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<td></td>
<td>S-fluorouracil</td>
<td>human colon cancer cell line (HT-29)</td>
<td>Hwang et al., 2005 [29]</td>
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<td></td>
<td>arsenic trioxide</td>
<td>human hepatocellular carcinoma cell lines (HepG2, Hep3B, and SK-Hep-1), HepG2 xenografts in BALB/c nude mice</td>
<td>Jiang et al., 2010 [34]</td>
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<tr>
<td></td>
<td>doxorubicin</td>
<td>hormone-independent human breast cancer cell line (MDA-MB-231)</td>
<td>Lim et al., 2006 [44]</td>
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<td></td>
<td>gemcitabine</td>
<td>murine xenografts of human pancreatic carcinoma cells (COLO 357 and L3.6pl)</td>
<td>Banerjee et al., 2005 [5]</td>
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<td></td>
<td>camptothecin</td>
<td>human cervical cancer cell line (HeLa), human ovarian carcinoma line (OAW-42)</td>
<td>Papazisis et al., 2006 [62]</td>
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<td></td>
<td>hydroxycamptothecin</td>
<td>SCID mice xenografts of human bladder carcinoma TCC-SUP cells</td>
<td>Wang et al., 2013 [90]</td>
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<td></td>
<td>combination of genistein and daidzein</td>
<td>cisplatin human squamous cell carcinoma cell lines (ME-180PT and UMSCC-5)</td>
<td>Ali et al., 2009 [1]</td>
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<tr>
<td>curcumin</td>
<td>S-fluorouracil, combination of S-fluorouracil and oxaliplatin</td>
<td>human colon cancer cell line (HT-29)</td>
<td>Du et al., 2006 [17]</td>
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<td></td>
<td>cisplatin, oxaliplatin</td>
<td>human ovarian carcinoma cell lines (2008 and C13)</td>
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<td></td>
<td>cisplatin, etoposide, camptothecin, doxorubicin</td>
<td>human and rat glioblastoma cell lines</td>
<td>Dhandapani et al., 2007 [15]</td>
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<td>(-)-epigallocatechin 3-gallate (EGCG)</td>
<td>doxorubicin</td>
<td>murine xenografts of human carcinoma DOX-resistant cells (KB-A-1)</td>
<td>Zhang et al., 2004 [95]</td>
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<tr>
<td></td>
<td>gemcitabine</td>
<td>human cholangiocarcinoma cell line (Mz-ChA-1), murine xenografts of Mz-ChA-1 cells</td>
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<td></td>
<td>cisplatin</td>
<td>human ovarian cancer cell lines (SKOV3, CAOV3, and C200)</td>
<td>Chan et al., 2006 [12]</td>
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<td>quercetin</td>
<td>doxorubicin</td>
<td>neuroblastoma and Ewing's sarcoma cell lines</td>
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<td></td>
<td>doxorubicin</td>
<td>murine xenografts of 4T1 (murine mammary carcinoma) cells</td>
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<td></td>
<td>cisplatin</td>
<td>human laryngeal carcinoma cell line (Hep-2)</td>
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<td></td>
<td>arsenic trioxide</td>
<td>human leukemia cell lines (U937 and HL60)</td>
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<td></td>
<td>temozolomide</td>
<td>human astrocytoma cell line</td>
<td>Jakubowicz-Gil et al., 2010 [31]</td>
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<td>resveratrol</td>
<td>cisplatin</td>
<td>human acute myeloid leukemia cell lines (ML-2/DX30, AML-2/DX100, and AML-2/DX300)</td>
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</tr>
<tr>
<td></td>
<td>doxorubicin</td>
<td>Wistar rats</td>
<td>Do Amaral et al., 2008 [16]</td>
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<td>Wang et al., 2009 [89]</td>
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- Sharma et al., 2007 [79]
- Banerjee et al., 2007 [6]
- McDonnell et al., 2004 [51]
- Hwang et al., 2005 [29]
- Sharma et al., 2007 [79]
- Banerjee et al., 2007 [6]
- Ali et al., 2009 [1]
- Wistar rats | Do Amaral et al., 2008 [16] | Wang et al., 2009 [89] | Kweon et al., 2010 [40] |
tin. In vivo studies on COLO 357 confirmed that genistein is a repressor of NF-kB expression [6]. In another study, Hwang et al. [29] showed that genistein and 5-fluorouracil synergism induced p53, p21, and Bax expression in colon cancer HT-29 cells. The induction of these genes was probably caused by genistein. A decrease in cyclooxygenase 2 (COX-2) and prostaglandin secretion caused by 5-fluorouracil was also observed. Furthermore, the combination of arsenic trioxide with genistein increased apoptosis (cytochrome c release and caspase-3 activation) and generated reactive oxygen forms in human leukemia U937 cells [72]. In hepatocellular carcinoma cell lines (HepG2, Hep3B, and SK-Hep-1), a reduction in cell viability and apoptosis stimulation were observed as a result of a synergistic interaction between arsenic trioxide and genistein. Moreover, this synergism was confirmed in HepG2 xenografts in BALB/c nude mice [34].

Studies indicate that genistein might act selectively on hormone-independent breast cancer cells (such as MDA-MB-231 cell line). Genistein sensitized MDA-MB-231 cells to doxorubicin (an anthracylane antibiotic) whereas it had no effect on the hormone-dependent MCF-7 cell line. Probably, genistein induced expression of a gene involved in maintaining cell viability, namely, glucose-regulated protein 78 (GRP78) as well as suppressing glucose uptake in both cell lines [44].

In female nude mice implanted with human pancreatic carcinoma cell lines (COLO 357 and L3.6pl), a combination of genistein and gemcitabine (a nucleoside analog used in chemotherapy) was much more effective as an antitumor agent compared with either agent alone [5]. It was also reported that this phenolic compound and camptothecins synergistically decreased proliferation of HeLa (cervical cancer) and OAW-42 (ovarian cancer) cell lines. Pretreatment of HeLa cells with irinotecan (a semisynthetic analogue of camptothecin) before incubation with genistein inhibited CDK1 phosphorylation, causing abrogation of G2/M checkpoint control and thus enhancing apoptosis (as evidenced by an increase in PARP cleavage) [63]. Recently, Wang et al. [90] reported significant and dose-dependent sensitization of bladder cancer cell lines and bladder epithelial cells to hydroxyacamptothecin-triggered apoptosis by genistein both in vitro and in vivo (in a bladder cancer xenograft model). The sensitization caused double-stranded DNA breaks leading to synergistic activation of ataxia telangiectasia mutated (ATM) kinase, which resulted in a delay in DNA damage repair, reduction in NEMO/NF-κB/IKK/caspase signal transduction and induction of apoptosis [90]. The isoflavone was previously shown to sensitize various cancer cell lines (including prostate and cervical cancer) to hydroxyacamptothecin, which has been used in bladder cancer chemotherapy for nearly 40 years.

Additionally, genistein may exhibit protective properties. Studies revealed that this isoflavone protected murine bone marrow cells against cisplatin-induced clastogenesis and apoptosis [2].

Curcumin
Curcumin, a phenolic compound obtained from the rhizome of Curcuma longa L., can also synergize with chemotherapeutics. Importantly, curcumin and other curcuminoids are among the most promising polyphenols for possible future use in co-medication with standard anticancer drugs [88]. Du et al. [17] revealed that curcumin in combination with 5-fluorouracil effectively inhibited the growth of colon cancer HT-29 cells and reduced COX-2 expression in those cells. Moreover, curcumin, 5-fluorouracil, and oxaliplatin synergism resulted in inhibition of HT-29 cell proliferation and induction of their apoptosis. The combination of the three above-mentioned compounds was more effective than curcumin alone or curcumin combined with 5-fluorouracil. On one hand, expression of epidermal growth factor receptor (EGFR), human epidermal growth factor receptor (HER)-2, HER-3, and insulin-like growth factor 1 receptor (IGF-1R) was downregulated. However, on the other hand, an increment in insulin-like growth factor binding protein 3 (IGFBP-3) expression was observed and led to inhibition of the interaction of IGF-1 with the receptor, thus hindering its activation [55].

Furthermore, curcumin combined with cisplatin or with oxaliplatin caused cell cycle arrest in human ovarian carcinoma 2008 and C13 cells and induced their apoptosis. Interestingly, the combination of those compounds was much more effective than when they were used individually [55]. In vitro studies, curcumin sensitized human and rat glioblastoma cells to cisplatin, etoposide, camptothecin, and doxorubicin. The results revealed downregulation of Bcl-2 and of the enzymes taking part in DNA repair [15].

Epigallocatechin gallate
Results of numerous studies indicate that tea polyphenols increase susceptibility of cancer cells to chemotherapeutics; as far as synergy with standard anticancer drugs is concerned, gallatechins of green tea are among the most promising polyphenols (beside curcuminoids) [88]. For instance, (-)-epigallocatechin 3-gallate (EGCG), a compound isolated from tea (Camellia sinensis) leaves, intensified doxorubicin action in human carcinoma KB-A-1 xenografts in mice [95]. EGCG increased doxorubicin concentration in xenografts by 51% and stimulated apoptosis in the tumors. Probably, EGCG inhibited efflux of the drug from cells by altering ATP hydrolysis in P-glycoprotein and cytochrome c release into the cytosol which triggered apoptosis. In vivo studies, EGCG also decreased the growth of Mz-ChA-1 cell xenografts in nude mice and increased their sensitivity to gemcitabine. Combination of EGCG with cisplatin intensified the drug’s action,
thus causing oxidative stress in ovarian cancer cell lines (SKOV3, CAOV3, and C200) [12]. Moreover, EGCG sensitized glioma cells to cisplatin and tamoxifen by inhibiting telomerase expression [80].

Molecular mechanisms underlying the synergistic induction of apoptosis of human cancer cells, inhibition of tumor formation in mice, and enhanced suppression of tumor growth in xenograft murine models by combinations of anticancer drugs with EGCG or other green tea catechins were reviewed by Suganuma et al. [84] and Fujiki and Suganuma [22].

Quercetin

Quercetin is a flavonol commonly found in plants, including onion [91]. According to Zanini et al. [93], this phenolic compound might intensify the action of doxorubicin in neuroblastoma cells and, to a smaller extent, in Ewing’s sarcoma cells via inhibition of heat shock protein expression. Quercetin combined with cisplatin exhibited a proapoptotic effect toward human laryngeal carcinoma cells [39]. Similar results were obtained by Ramos and Aller [71], who treated human leukemia cells U937 and HL60 with a combination of quercetin and arsenic trioxide.

Furthermore, Du et al. [18,19] investigated synergism between quercetin and doxorubicin. Injection of a combination of the above-mentioned flavonol and chemotherapy drug into 4T1 breast tumors implanted subcutaneously in mice revealed inhibition of tumor growth and reduction of doxorubicin side effects, leading to long-term tumor-free survival of the mice. It is known that cytokines secreted by Th1 and Th2 lymphocytes have an influence on the immune response. Quercetin combined with doxorubicin caused an increase in the concentrations of IL-2 and IFN-γ (cytokines inhibiting tumor growth) and a decrease in the concentrations of IL-4 and IL-10 (cytokines promoting tumor growth) in the serum at the same time [19].

Temozolomide is an alkylating chemotherapeutic agent, and quercetin was reported to act synergistically with this autophagy-inducing drug by causing severe necrosis in a human astrocytoma cell line [31]. Interestingly, the observed effect was biphasic: at a low (5 µM) temozolomide concentration quercetin potentiated its proautophagic effect, while at a higher drug concentration (50 µM) one form of programmed cell death (i.e. autophagy) switched to another (apoptosis). The migratory phenotype of the glioma cells was also suppressed by the combination of the drug and the flavonol. Moreover, temozolomide attenuated the toxic effect of quercetin. The cytotoxicity of the flavonol (when used alone), the protective effect of the drug, and the concentration dependence of the triggered type of programmed cell death (autophagy vs. apoptosis) were confirmed in a further study by Jakubowicz-Gil et al. [30] on the same cell line. Interestingly, the sequence of administration of temozolomide and quercetin was also important, with the highest number of dead cells observed after simultaneous administration of both agents or after pre-incubation with temozolomide followed by treatment with quercetin.

Resveratrol

Resveratrol belongs to stilbenes and occurs, among others, in grapes, wine, peanuts, and soy [10]. It is able to attenuate side effects of chemotherapeutics [16,58,89,94,96]. For instance, Do Amaral et al. [16] evaluated the influence of resveratrol on cisplatin-induced renal damage. The study was carried out on male Wistar rats which were treated with resveratrol before cisplatin administration. Then, urine and blood samples were collected and kidneys were removed. Acute tubular cell necrosis was observed only in the group of rats treated with cisplatin without resveratrol pretreatment, whereas in the group treated with resveratrol before cisplatin administration lower levels of creatinine in serum and proteins in urine (markers indicating renal injuries) were reported. Additionally, in comparison with the group of rats treated only with cisplatin, resveratrol was able to decrease the degree of lipid peroxidation and increase the level of reduced glutathione in tissues.

Furthermore, resveratrol was also examined as a preventive agent against cisplatin-induced cardiotoxicity in rats [89]. It is well known that cisplatin treatment might cause cardiac function deterioration and myocardial injury. Moreover, cisplatin increased the levels of lactate dehydrogenase, creatine kinase, and malondialdehyde and decreased the levels of glutathione, glutathione peroxidase, superoxide dismutase, and catalase. Treatment of the rats with resveratrol reduced these sides effects of the chemotherapeutic agent in question.

The in vitro studies performed by Kweon et al. [40] on acute myeloid leukemia cells revealed that resveratrol might facilitate doxorubicin uptake by the cells, probably by downregulation of the expression of mrp-1 (mrp-1 belongs to ATP-binding cassette transporter family, involved in multidrug resistance). It acts as an energy-dependent efflux pump whose overexpression causes a decrease in doxorubicin concentration in the cells [40].

Polyphenol-rich extracts

In addition to individual phenolics, polyphenol-rich extracts were also reported to act synergistically with anticancer chemotherapeutics (Table 2). For instance, an evening primrose seed extract sensitized human metastatic melanoma (HTB-140) and hepatoma (HepG2) cells to vincristine, a mitotic inhibitor used in cancer chemotherapy [32]. The combined use of the extract (25 µg/mL) and the chemotherapeutic drug (1 µM) in HTB-140 and HepG2 cells resulted in more than a 4- and a 1.5-fold increase in cytotoxicity, respectively, when compared to vincristine alone. The extract is rich in prostanoids and hydrolyzable tannins (mainly pentagalloyl glucose) which could significantly contribute to the observed effect.
Different classes of compounds have been isolated so far from various parts of *Teucrium polium* L. (*Lamiaceae*), a wild-growing flowering plant, found abundantly in South-Western Asia, Europe, and North Africa and used traditionally for different pathological conditions (including gastrointestinal disorders, inflammation, diabetes, and rheumatism) [4]. The main groups are flavonoids and terpenoids [4], and the major flavonoid compounds from a methanolic extract of *T. polium* L. are rutin and apigenin [20]. Rajabalian et al. [70] reported that a methanolic extract of this plant potentiates the cytotoxic and proapoptotic effects of three anticancer drugs (vincristine, vinblastine, and doxorubicin) toward several cancer cell lines (Skmel-3, Saos-2, SW480, MCF-7, KB, EJ, and A431).

Apple procyanidins with a mean degree of polymerization equal to 4, and three lysosomotropic drugs (MDL 72527 and chloroquine) potentiates the cytotoxic and proapoptotic effects of three anticancer drugs (vincristine, vinblastine, and doxorubicin) toward several cancer cell lines (Skmel-3, Saos-2, SW480, MCF-7, KB, EJ, and A431).
Phytochemicals present in plant extracts (including phenolic compounds in polyphenol-rich extracts) can interact synergistically with one another, which results in higher anticancer activity of an extract than the sum of activities of its individual components [14]. Synergistic anticancer effects of combinations of two or more phenolic compounds or polyphenol-rich plant extracts were also observed (Table 3). For instance, OptiBerry IH141 formulation composed of six anthocyanin-rich extracts (from the fruits of wild blueberry, bilberry, cranberry, elderberry, and strawberry, and from raspberry seeds) exhibited superior anti-angiogenic activity both in vitro and in vivo compared to other combinations tested and, importantly, low toxicity [3]. In the in vitro studies, OptiBerry suppressed a key regulator of tumor angiogenesis by significantly inhibiting H2O2- and TNF-alpha-induced vascular endothelial growth factor expression by human keratinocytes. Consistently, the anthocyanin-rich formulation impaired angiogenesis in human microvascular endothelial cells, as assessed by Matrigel assay. Pretreatment of mouse hemangioendothelioma endothelial cells (EOMA cell line) with OptiBerry significantly inhibited the transcription of inducible NF-kappa-B and of basal monocyte chemotactic protein-1 (MCP-1, also referred to as chemokine (C-C motif) ligand 2 (CCL2) or small inducible cytokine A2). OptiBerry reduced the ability of the endothelioma cells to form tumors; a pronounced decrease in tumor growth (by more than 50%) was observed in a murine model of hemangioma. Furthermore, infiltration of macrophages into tumors was markedly lower in mice treated with the OptiBerry formulation than in placebo-treated controls. Extensive infiltration of tumors with macrophages was shown to correlate with cancer metastasis and poor prognosis in a variety of human carcinomas [83].

**Anticancer synergy between polyphenols**

Synergistic interactions between anticancer chemotherapeutics and polyphenol-rich extracts were also observed *in vitro* or *in vivo* for, among others, a combination of irinotecan (a semisynthetic analogue of camptothecin) and propolis extract [7,60]; doxorubicin or paclitaxel combined with silymarin (a standardized extract of *Silybum marianum* (L.) Gaertn. seeds) [13]; doxorubicin or cisplatin and polyphenol-rich extracts of *Phyllanthus emblica* and *Terminalia bellerica* [67]; cisplatin, doxorubicin, docetaxel, or 5-fluorouracil and *Solanum nigrum* leaf extract [85]; the hormonal form of vitamin D (1alpha,2,5-dihydroxyvitamin D3) and carnosic acid-rich rosemary leaf extract [76,88]; 5-fluorouracil and rosemary leaf extract [23], and doxorubicin and *Artemisia princeps* var. *orientalis* leaf extract [73].

Polyphenols are the major components of the above-mentioned extracts. For instance, among typical phenolic compounds of propolis are apigenin, chrysin, galangin, kaempferol, quercetin, naringenin, pinocembrin, pinostrobin, caffeic acid, cinnamic acid, p-coumaric acid, and ferulic acid [8]. Silymarin is a mixture of seven distinct flavonolignans (silybin A, silybin B, isosilybin A, isosilybin B, silychristin, isosilychristin, silydianin) and one flavonoid (taxifolin) isolated from the seeds of *Silybum marianum* (L.) Gaertn. [69]. A methanolic extract of *Phyllanthus emblica* fruit is rich in ellagitannins, flavonoids, and simple gallic acid derivatives [92]. A number of phenolic compounds (mainly hydrolyzable tannins, including simple gallate esters, ellagic acid derivatives, and glycosides and various el-
In a study by Mertens-Talcott et al. [53], quercetin and ellagic acid (phenolic compounds abundant in small fruits such as blackberries, strawberries, red raspberries, blueberries and grapes) were shown to synergistically reduce proliferation and viability and trigger apoptosis of human leukemia MOLT-4 cells at the concentrations of 5 and 10 µM each. Cell cycle kinetics were also considerably altered as a result of the combined treatment. In a further study by Mertens-Talcott and Percival [52], two other combinations of phenolics (ellagic acid + resveratrol and quercetin + resveratrol) were tested on the same cell line as potential antiproliferative and apoptosis-inducing agents. Both combinations showed more than additive interactions, with a slightly stronger synergistic effect for the former than for the latter (with a combination index of 0.64 vs. 0.68, respectively).

Another research group demonstrated that resveratrol and curcumin synergistically inhibited the growth

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<td>human keratinocytes, human microvascular endothelial cells, murine model of hemangioma</td>
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<td>p53-positive (wt) and p53-negative colon cancer</td>
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<td>combination of quercetin and ellagic acid</td>
<td>human leukemia cell line (MOLT-4)</td>
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<tr>
<td>combination of resveratrol and ellagic acid or quercetin</td>
<td>human leukemia cell line (MOLT-4)</td>
<td>Mertens-Talcott and Percival, 2005 [52]</td>
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<td>combinations of resveratrol and ellagic acid or grape seed extract (additional tested agent: calcium D-glucarate)</td>
<td>SENCAR mice</td>
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<td>combination of epicatechin and epigallocatechin gallate (EGCG)</td>
<td>human colon cancer cell line (HT-29)</td>
<td>Shimizu et al., 2005 [81]</td>
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<td>combination of EGCG and other green tea catechins; catechin mixtures treated with tannase</td>
<td>human cervical carcinoma cell line (HeLa)</td>
<td>Morré et al., 2003 [56]</td>
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<td>combinations of grape extracts (obtained from pulp, skins, juice, seeds, or ground freeze-dried pomace) and decaffeinated green tea extracts</td>
<td>human cervical carcinoma cell line (HeLa), murine mammary carcinoma cell line (4T1), 4T1 tumor-bearing mice</td>
<td>Morré DM and Morré DJ, 2006 [57]</td>
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<td>pomegranate fruit juice</td>
<td>human cell lines: oral cancer (KB, CAL27), colon cancer (HT-29, HCT116, SW480, SW620), and prostate cancer (RWPE-1, 22Rv1)</td>
<td>Seeram et al., 2005 [74]</td>
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<td>combined extract of clove, oregano, thyme, walnuts, and coffee</td>
<td>human monocytic leukemia cell line transfected with a DNA construct containing three NF-κB sites from the Ig κ light chain promoter coupled to the gene encoding firefly luciferase (3x-NF-κB-luc) (U937-κB), human hepatocellular carcinoma cell line (HepG2), transgenic NF-κB-luciferase reporter mice</td>
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<tr>
<td>propolis extract</td>
<td>human cell lines: breast cancer (BT-474), undifferentiated lung cancer (ChACo), hepatocellular carcinoma (HepG2), gastric carcinoma (KATO-III), colon adenocarcinoma (SW-620), and human non-transformed foreskin fibroblast cell line (HS-27)</td>
<td>Teerasripreecha et al., 2012 [86]</td>
</tr>
<tr>
<td>complex virgin olive oil phenolic extracts</td>
<td>human promyelocytic leukemia cell line (HL-60)</td>
<td>Fabiani et al., 2011 [21]</td>
</tr>
<tr>
<td>ginger extract and selected ginger phenolics (6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol)</td>
<td>human prostate cancer cell line (PC-3)</td>
<td>Brahmabhatt et al., 2013 [9]</td>
</tr>
<tr>
<td>extracts of Rosa roxburghii Tratt. and Fagopyrum cymosum</td>
<td>human cell lines: esophageal squamous carcinoma (CaEs-17), gastric carcinoma (SGC-7901), pulmonary carcinoma (A-549)</td>
<td>Liu et al., 2012 [46]</td>
</tr>
<tr>
<td>sweet potato greens extract</td>
<td>prostate tumor xenografts in nude mice</td>
<td>Gundala et al., 2013 [26]</td>
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</table>
of both p53-positive (wild type) and p53-negative human colon cancer HCT-116 cells [49]. In the in vivo studies, this combination of phenolic compounds was also more effective in suppressing the growth of HCT-116 (wt) tumors in SCID mice xenografts than either applied agent alone. Similarly, resveratrol combinations with ellagic acid, grape seed extract, and other phytochemicals were proved to effectively inhibit skin tumorigenesis in SENCAR mice (7,12-dimethylbenz[a]anthracene-induced skin carcinogenesis model) [38]. Resveratrol was applied topically, grape seed extract was either applied topically or administered in diet, and ellagic acid was administered in diet; another agent tested in the study was calcium D-glucarate (administered in diet). Importantly, all combinations including resveratrol exhibited a synergistic effect against epidermal hyperplasia and Ha-ras mutations.

Synergistic interactions were also reported for green tea catechins; for instance, a combination of ECGG (10 μg/mL) and epicatechin (1 μg/mL) synergistically inhibited the growth of human colon cancer HT-29 cells and triggered their apoptosis [81]. Furthermore, Morré et al. [56] reported that when ECGG was combined with (-)-epicatechin (EC), (-)-epigallocatechin (EGC) or (-)-epicatechin 3-gallate (ECG), a 10-fold reduction in its concentration required to suppress the growth of human cervical carcinoma HeLa cells and to inhibit the activity of a cancer-associated cell surface–located NADH oxidase (ECTO-NOX, designated tNOX) was observed. Importantly, EC, EGC, and ECG were inactive when tested alone. In further experiments, mixtures based on purified catechins and decaffeinated tea extracts were treated with tannase to reduce the content of ester bond-containing catechins. After the enzymatic treatment, the content of ECGG in the mixtures or extracts varied from 0.065 to 40%. Interestingly, their effectiveness was comparable to that of decaffeinated green tea extracts as long as ECGG was present and the ratio of total catechins to ECGG + EGC was about 1.5.

Grapes and various grape extracts (from pulp, skins, juice, seeds, or ground freeze-dried pomace) were tested and proved to be active in similar experiments, focused on NNOX and on the growth of HeLa cells (in vitro) and of murine mammary 4T1 cells (in vitro and in 4T1 tumor-bearing mice) [57]. Moreover, synergy between the grape extracts and decaffeinated green tea extracts was observed, both in the inhibition of tNOX activity and in the inhibition of cancer cell growth. In the murine model of breast cancer, the growth of 4T1 tumors was suppressed most effectively by a mixture of a green tea extract and ground freeze-dried grape pomace in the ratio of 25:1, respectively.

Synergy between the constituents of pomegranate juice was demonstrated by Seeram et al. [74]. The juice showed the strongest antiproliferative activity against two oral cancer cell lines, four colon cancer cell lines, and two prostate cancer cell lines when compared with its major polyphenol (punicalagin, an ellagitannin) and with a standardized total pomegranate tannin extract.

A combined extract of clove, oregano, thyme, walnuts, and coffee proved to be a potent modulator of NF-kappaB signaling both in vitro (in a monocytic cell line) and in vivo [65]. The inhibition of lipopolysaccharide (LPS)-induced NF-kappaB activation by the combination extract was synergistic, as evidenced by a comparison with the activities of the individual extracts. A single dose of the combined extract administered to transgenic NF-kappaB luciferase reporter mice prior to LPS treatment resulted in a decrease in whole body LPS-induced NF-kappaB activity after the first 6 hours by 35% when compared with control mice. The expression of genes related to inflammation, cell migration, and proliferation in the liver was also reduced by the combination extract.

Propolis is a honeybee product rich in flavonoids and phe-nolic acids; its polyphenol-rich dichloromethane and hexane extracts (resulting from a sequential extraction with methanol, dichloromethane, and hexane) acted as antiproliferative/cytotoxic agents toward five cell lines derived from human carcinomas of breast (BT474), lung (Chaco), liver (Hep G2), stomach (KATO-III), and colon (SW620) [86]. The half maximal inhibitory concentration (IC50) values across the above-mentioned cancer cell lines ranged from 41.3 to 52.4 μg/mL for the hexane extract and from 43.8 to 53.5 μg/mL for the dichloromethane extract. Bioactivity-guided fractionation led to isolation of two main active compounds, namely, cardanol and cardol (phenolic lipids). Their IC50 values across the five cancer cell lines and a control (human non-transformed foreskin fibroblast HS-27 cell line) ranged from 10.8 to 29.3 μg/mL and from < 3.13 to 5.97 μg/mL, respectively. Importantly, after treatment with cardanol or cardol, cytotoxicity and cell death without DNA fragmentation were observed only in cancer cells, whereas in the case of HS-27 only an antiproliferative effect was observed. However, according to the work in question, the isolated compounds did not account for the net antiproliferative/cytotoxic activity of the crude extracts. Therefore, the authors suggested that either other potent compounds are present in the extracts or synergistic interactions between their constituents are involved [86].

Taking into account that olive is one of the major components of the Mediterranean diet, Fabiani et al. [21] compared in vitro chemopreventive activities of four complex virgin olive oil phenolic extracts (derived from four Italian cultivars) in a study on the human promyelocytic leukemia HL-60 cell line. The antiproliferative and proapoptotic activities of the extracts were positively correlated with the content of secoiridoids. Representatives of this subgroup of polyphenols are hydroxytyrosol and oleuropein (elonenolic acid ester of hydroxytyrosol), the main phenolic compounds of olive oil. Interestingly, the above-mentioned anticancer activities of the tested extracts were negatively correlated with the concentration of two other subgroups of olive oil phenolic compounds, namely, phenyl alcohols and lignans. Importantly, when a complex mixture of the olive oil phenolics was tested, a more pronounced anticancer effect was observed in comparison with individual compounds, which may imply either synergistic interactions or
the presence of other unidentified extract constituent(s) exhibiting antiproliferative and/or proapoptotic activities toward HL-60 leukemia cells.

Brahmbhatt et al. [9] observed growth inhibition of prostate cancer cells and induction of their apoptosis both in vitro and in vivo as a result of treatment with a ginger extract and decided to investigate the nature of interactions between selected phenolic constituents of the extract (namely, 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol). The obtained results indicate that binary combinations of the above-mentioned ginger phenolics synergistically inhibit proliferation of human prostate cancer PC-3 cells. Furthermore, a combination of ginger extract with its constituents (particularly 6-gingerol) enhanced the extract’s antiproliferative activity.

Among traditional Chinese medicines, Rosa roxburghii Tratt. and Fagopyrum cymosum are recognized to be effective in improving immune responses and enhancing digestive ability as well as to act as antiaging agents [46]. In vitro anticancer activities of their extracts were assessed in a study on three human carcinoma cell lines (esophageal squamous carcinoma CaEs-17, gastric carcinoma SGC-7901, and pulmonary carcinoma A549). A combination of extracts of both plants synergistically inhibited cell growth and caused an increase in apoptosis.

As a result of a study demonstrating growth-inhibitory and apoptosis-inducing properties of polyphenol-rich sweet potato greens extract toward human prostate cancer PC-3 cells both in vitro and in vivo (prostate cancer xenografts in nude mice), Gundala et al. [26] carried out bioactivity-guided fractionation of the extract. A polyphenol-enriched fraction was identified with approximately 100-fold higher activity than the parent extract (as assessed on the basis of IC50 measurements in human prostate cancer cells). The fraction was characterized by a distinct phenolic profile; the authors reported approximately 2.6- and 3.6-fold enrichment of quinic acid and chlorogenic acid, respectively, and a definitive ratiometric relationship between the isochlorogenic acids (4,5-di-caf feoylquinic acid, 3,5-di-cafeoylquinic acid, and 3,4-di-cafeoylquinic acid). Importantly, its subfractionation resulted in loss of bioactivity, thus suggesting involvement of synergistic interactions among the constituent compounds. In the in vivo studies, daily oral administration of the fraction (400 mg/kg body weight) suppressed growth and progression of prostate tumor xenografts in nude mice by approximately 75%.

**CONCLUDING REMARKS**

The results of in vitro and in vivo studies reported so far in the literature and briefly discussed above imply that phenolic compounds and polyphenol-rich extracts have a high potential as chemopreventive and therapeutic agents. In the case of extracts, the potential results from the presence of various phenolics in natural proportions, from their complementary biological activities, as well as from synergistic interactions among them. The growing interest in biological activities of polyphenols is a consequence of, among others, an increasingly high incidence of civilization diseases (particularly various cancers) and a need to find safe and effective methods of prophylaxis and therapy. Literature data indicate that among numerous mechanisms underlying the anticancer activity of polyphenols there are, for instance, cell cycle arrest, inhibition of signal transduction pathways, induction of differentiation, down-regulation of oncogenes, upregulation of tumor suppressor genes, apoptosis induction, and impairment of angiogenesis. Such a variety of mechanisms of action exhibited by phenolic compounds renders it possible to choose an appropriate anticancer agent among them.

From the point of view of a potential patient, prevention is a more beneficial strategy than therapy, and the use of natural chemopreventive agents bears less risk of adverse side effects, is less expensive, and more environmentally friendly than in the case of synthetic drugs. On the other hand, it is worth emphasizing that in clinical trials relatively few cases of polyphenol overdose and the related toxicity have been reported so far (some of them were caused by intravenous administration of tested preparations) [87]. Therefore, it is essential to develop a set of appropriate biomarkers which would render it possible to precisely monitor the effects of phenolic compounds in cancer chemoprevention and therapy.

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